Molecular perspectives on the ecologically inconsistent effectiveness of the mycoherbicide *Fusarium oxysporum* f. sp. *strigae* against *Striga hermonthica*

Dissertation

submitted in fulfilment of the regulations to acquire the degree "Doktor der Agrarwissenschaften" (Dr.sc.agr. in Agricultural Sciences)

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Prof. Dr. Frank Rasche

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presented by

Williams Oyifioda Anteyi

Born in Lagos, Nigeria

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Examination committee

Chairperson of the oral examination	Prof. Dr. Jörn Bennewitz (Institute of Animal Science)
Supervisor and Reviewer	Prof. Dr. Frank Rasche (Institute of Agricultural Sciences in Tropics, Hans-Ruthenberg-Institute)
Co-Reviewer	Prof. Dr. Alan K. Watson (Department of Plant Science, Faculty of Agricultural and Environmental Sciences, McGill University, Québec, Canada)
Additional examiner	Prof. Dr. Hans-Peter Piepho (Institute of Crop Science)

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Dedication

This thesis is dedicated to my visionary grandfather Mr. Anteyi Ochola; including to my wonderful parents, Engr. (late) Benedict Audu Anteyi and Mrs. Helen Onyechi Anteyi, for their limitless love, sacrifices and prayers.

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List of Abbreviations

1,4-Nq: 1,4-naphthoquinone

ABM: Aboveground dry biomass

ANOVA: Analysis of variance

BCA: biological control agents

bp: base pairs

CIMMYT-Kenya: International Maize and Wheat Improvement Centre, Kibos research facility, Kenya

DAS: diacetoxyscirpenol

DNA: Deoxyribonucleic acid

EF1A: Eukaryotic translation elongation factor 1 alpha

Equi: equisetin

F: Forward primer

***Fos*:** *Fusarium oxysporum* f. sp. *strigae*

FuA: fusaric acid

Hym: hymeglusin

IITA-Nigeria: International Institute of Tropical Agriculture, Ibadan, Nigeria

LC-MS: Liquid chromatography-mass spectrometry

LD: Linkage disequilibrium

Mbp: mega base pairs

Neo: neosolaniol

PCR: polymerase chain reaction

PDA: Potato dextrose agar

PDB: Potato dextrose broth

PGPR: plant growth promoting rhizobacteria

qPCR: Real-time quantitative Polymerase chain reaction

R: Reverse primer

RNA: Ribonucleic acid

rRNA: Ribosomal ribonucleic acid

RT-qPCR: Quantitative reverse transcription Polymerase chain reaction

SARC-Ethiopia: Sirinka Agricultural Research Centre, Sirinka, Ethiopia

SE: Standard error

SSA: Sub-Saharan Africa

SSRs: Simple sequence repeats

T-2: T-2 toxin

TpFs: Trichothecene producing Fusarium species

UG-Sudan: University of Gezira, Wad Medani, Sudan

Chapter 1. General introduction

1.1 Global perspective of food security under the current climate change: with focus on sub-Saharan Africa

Food is the most essential requirement for the sustenance of human existence. Hence, among the immediate basic needs of man, “food” comes first (Denton 1990; Singh 2014). It is common knowledge that the importance of food in any given society cannot be overemphasized, therefore, food security related issues constitute the first 2 goals of the Sustainable Development Goals (SDGs) of the United Nations (UN) i.e., no poverty, zero hunger (UN DESA 2015). As it is projected that the world population will rise to roughly 10 billion people by 2050 (Ranganathan et al. 2018; UN DESA 2019), hence, at least a 50% increase in global food production must be achieved to meet the estimated food demand of the world’s population by 2050 (Chakraborty and Newton 2011; Valin et al. 2014). From an economic perspective, agricultural productivity contributes an average of 6.4% to the Gross Domestic Product (GDP) worldwide. However, in sub-Saharan Africa (SSA) where most countries are primarily agrarian nations, agriculture contributes an average of 23% to nations GDP. This ranges from about 1.8% in Botswana, to 60.7% in Sierra Leone (Fig. 1.1) (The World Factbook 2021). It therefore implies that economic performance of most nations in SSA is associated to the performance of the agricultural sector (Senbet and Simbanegavi 2017).

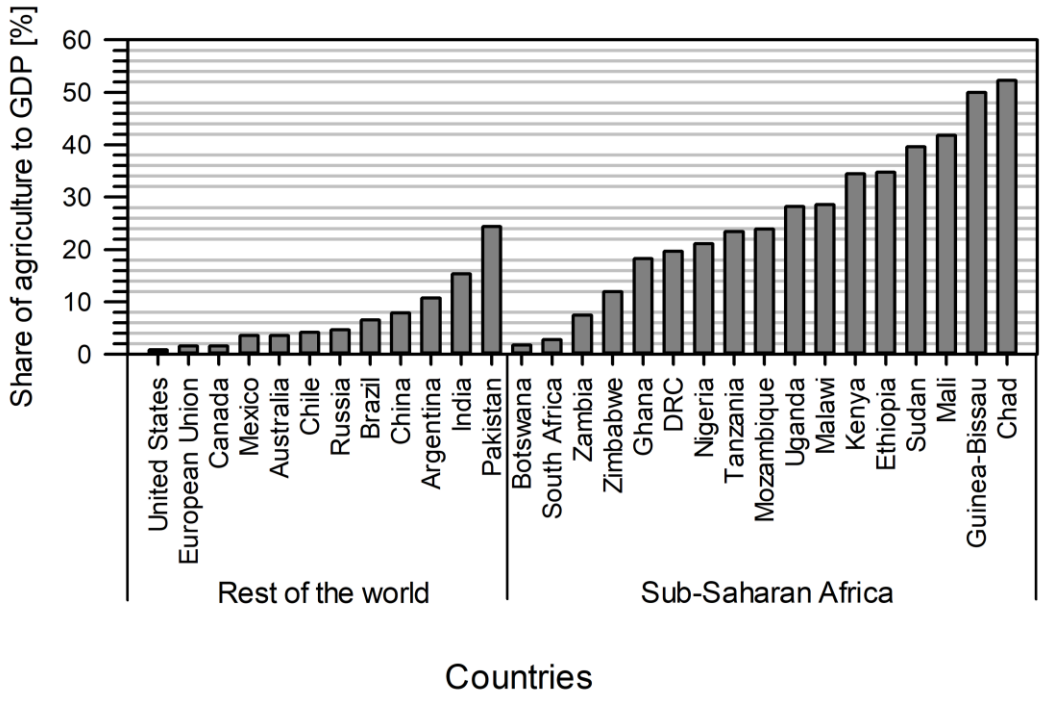

Fig. 1.1: Contribution of agriculture to total GDP (%) in some nations in 2017. DRC - Democratic Republic of the Congo. (Courtesy: The World Factbook 2021).

In SSA, smallholder farming makes up 80% of the agricultural systems, and it directly employs about 175 million people (AGRA 2014). However, despite the role of agriculture to people's livelihood and the economy in SSA, the full agricultural potential of this region remains largely not gained. For instance, a recent analysis by Goedde et al. (2019) reported that Africa could produce 2 to 3 times more cereal grains than it currently produces. Thereby, adding 20% more grains to the current worldwide output of 2.6 billion t. Unfortunately, factors such as low available resource inputs, increasing population growth rates and socio-political instability, are major constraints that hinder SSA from achieving its full agricultural potentials (OECD/FAO 2016). In addition, the existence of non-favourable agroecological factors, that encompasses the abiotic (i.e., soil physico-chemistry, water, climate) and biotic components (i.e., pests and pathogens), are important challenges to agricultural development in SSA (Gressel et al. 2004; Waddington et al. 2010; Khan et al. 2014). Furthermore, owing to the shortcomings of certain farming practices or food production systems which are widely practiced in SSA, the abiotic and biotic constraints to food production are aggravated. For example, the association of extensive food crop production systems in SSA to low yields, resource depletion and ecological imbalance, which ultimately affect food security (Reynolds et al. 2015). According to the World Food Summit (1996), that is convened by the Food and Agriculture Organization of the United Nations, "Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". Sadly, the current climate change patterns may further affect global food security. For instance, the environmental impact of climate change increase food market volatility in terms of production and supply (Wheeler and von Braun 2013; Myers et al. 2017; Firdaus et al. 2019). The negative consequences of climate change on food security emanate from the direct impact of climate change on important components of the environment, such as alteration of the diversity, abundance and distribution of pests, pathogens and diseases (Elad and Pertot 2014; Pareek et al. 2017; Velásquez et al. 2018), elevated ambient temperature and CO_2 (Hamilton et al. 2005; Deutsch et al. 2018), and changes in rainfall pattern i.e., decline in rainfall frequency, but increase in rainfall intensity. Thus, dry areas (subtropics/tropics) become drier, and wet areas (mid to high latitudes) become wetter (Trenberth 2008; Hoegh-Guldberg et al. 2018).

SSA has faced remarkable consequences of climate extremes, which have been increasing in frequency and intensity over the past decades (Taylor et al. 2017). As the situation of climate change worsens, it further heightens food insecurity in the region (Mulungu and Ng'ombe 2019). Climate change is projected to reduce the yields of cereal crops in SSA, through shortening the length of growing seasons, amplifying water stress and increasing the prevalence of diseases, pests and weeds (Niang et al. 2014). For instance, the prevalence of

the most devastating parasitic weed to cereal production in SSA i.e., *Striga* spp., is expected to accelerate with climate change. This is because, high temperatures and poor soil fertility (especially limiting soil nitrogen and phosphorus), are environmental conditions that generally favour the incidence of parasitic *Striga* spp. (Aflakpui et al. 1998a; Ejeta 2007a; Jamil et al. 2011; 2012). Hence, as the current global climate change intensifies these *Striga*-favouring environmental conditions in SSA, it therefore raises critical concerns about the proliferation and rapid spread of *Striga* in the region, which in turn could trigger food shortage (Strand 2000; Demeke and Di Marcantonio 2013; Mandumbu et al. 2017). As a further matter, it is believed that the threat of *Striga* would be more severe in Ethiopia than other regions in SSA. Because unlike other parts of SSA, where *Striga* mainly grows within isolated locations, it is ubiquitously distributed across farmlands in Ethiopia (AATF 2011).

1.2 Sorghum

Sorghum is a naturally tropical, C4 plant, that belongs to the grass family (Poaceae). The typical cultivar of the genus *Sorghum*, and which is predominantly cultivated worldwide is *Sorghum bicolor* (L.) Moench subsp. *bicolor* (Parry 1990; OECD 2017). Basically, cultivated sorghum is a diploid (2n=20), self-pollinating (geitonogamous) monocot, that has a genome size of 735 mega base pairs (Mbp) (Dillon et al. 2007; Paterson 2008; Chala 2018). Common English names of cultivated sorghum varieties include; broomcorn, broom millet, forage sorghum, grain sorghum, great millet, guinea corn, sorghum, sweet sorghum and wild grain (FAO 1995; USDA 2021). Sorghum has various uses; this mainly depends on the cultivar. Primarily, sorghum is grown for its grains, as food for humans. In addition, sorghum grains with Stover (leaves and stalks) are widely used as feed/fodder for poultry and livestock. Sorghum biomass also serves as raw materials for bioenergy production, brewing, and manufacturing of renewable building materials. Varieties such as sweet sorghums are grown for their sugar-rich stalk, which is used for the production of sweet syrup (Fadahunsi et al. 2013; Heuzé et al. 2015; United Sorghum Checkoff Program 2016a; 2016b).

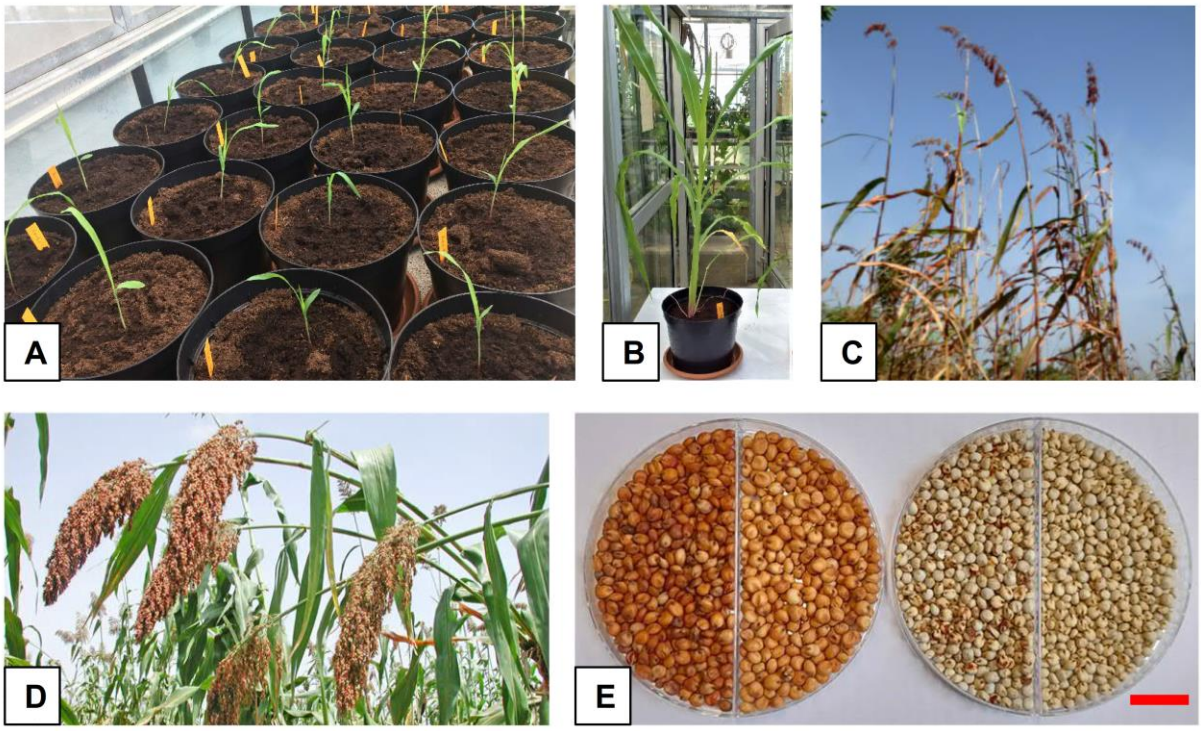

Fig. 1.2: Sorghum (*Sorghum bicolor*). **(A)** Emerged seedlings one week after sowing seeds in pots. **(B)** Plant growing at 7 weeks after sowing seed. **(C)** Panicle-bearing matured sorghum (guinea race) in field. **(D)** Close-view of guinea race sorghum panicles in field. **(E)** Seeds of diverse sorghum accessions from SSA (scale bar 2.5 cm). (Photos by: Williams O. Anteyi).

According to FAOSTAT (2021), the global sorghum production in 2019 was 57.9 million t; whereby, the top five producing nations were the United States of America (8.7 million t), Nigeria (6.7 million t), Ethiopia (5.3 million t), Mexico (4.4 million t) and Sudan (3.7 million t). Africa alone accounted for 28.6 million t of sorghum produced in 2019 i.e., about 49.4% of the global sorghum production. Thus, maximizing sorghum productivity would be a great asset for minimizing the problems associated with food insecurity, especially in the world's arid and semi-arid regions, particularly in SSA (Mwadalu and Mwangi 2013; Chepng'etich et al. 2015; Phiri et al. 2019). This is because, the high grain productivity of sorghum, including its tolerance to waterlogging, high temperature and drought, qualifies it to be among feasible staple crops for warmer climates (Maiti and Satya 2014; Kamal et al. 2019). Unfortunately, a variety of biological constraints (i.e., parasites, pests and pathogens) constitutes a serious menace that hinders sorghum production. An important biological constraint to sorghum productivity, especially in tropical and subtropical agroecosystems, is the parasitic weed *Striga* spp., particularly *S. hermonthica* (Parker and Riches 1993; Ejeta 2007b). Gressel et al. (2004) reported that out of the 26.23 million ha of all crop fields that were infested by *Striga* spp. in

SSA, sorghum fields alone accounted for roughly 20 million ha, thereby causing an estimated yield loss of 6.5 to 6.9 million t per annum. Thus, elimination of this obnoxious weed would be a significant progress towards achieving food security in SSA.

1.3 *Striga hermonthica*

The genus *Striga* belongs to the broomrape family (i.e., Orobanchaceae). This family contains parasitic weedy plants that are of paramount agricultural importance (Kokla and Melnyk 2018). Parasitic weeds belonging to Orobanchaceae include a group that lacks chlorophyll, and thus, exhibit full/complete parasitism (i.e., holoparasites) e.g., *Orobanche* spp. (the broomrapes). The family also includes a partial parasitic group that have chlorophyll, but require plant hosts for germination and development (i.e., obligate hemiparasites) e.g., *Striga* spp. (the witchweeds) (McNeal et al. 2013). Like every biotic constraint to agricultural productivity, crop yield is the most agronomically important parameter that witchweeds affect. Hence, yield loss is the most common measure for estimating the damage caused by parasitic *Striga* spp. to crop production and the economic consequence (Berner et al. 1995; Gressel et al. 2004; Yacoubou et al. 2021). In Africa, parasitic *Striga* spp. cause cereal yield losses estimated at over US$10 billion per annum, and sorghum is the most affected cereal (Ejeta 2007a). Though, out of all members of the parasitic *Striga* spp. that affects cereal crop production in SSA, *Striga hermonthica* (Delile) Benth. is the most devastating. Common English names of *S. hermonthica* include; purple witchweed, giant witchweed, witchweed and Striga (CABI 2016).

S. hermonthica is an obligate cross-pollinating (allogamous), C3, dicot plant (Safa et al. 1984; Press et al. 1987a). It is expected to be a diploid plant (n=19), with an estimated genome size of 1,801 Mbp (± 321 Mbp) (Aigbokhan et al. 1998; Yoshida et al. 2010). Though, Estep et al. (2012) later reported the genome size of *S. hermonthica* as 1425 Mbp. Some adaptative features for the survivability of *S. hermonthica* includes the ability for a single plant to produce numerous seeds (10,000 to 200,000), which are extremely small (0.2 mm width × 0.3 mm length) (Figs. 1.3A and B), light weight (4 – 5 µg), easily dispersed by animals, wind, water, or man (farming practices/tools or trading activities), and can remain in a dormant (but viable) state in the soil for several decades (Ramaiah et al. 1983; Parker and Riches 1993; Hearne 2009; Yoshida and Shirasu 2009). *S. hermonthica* particularly attacks sorghum (*Sorghum bicolor* L. Moench), maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* L. R. Br.), and rice (*Oryza sativa* L.) (Gressel et al. 2004; Parker 2009; Spallek et al. 2013). Other cereal-parasitizing members of the genus *Striga* (in order of declining economic importance) are

Striga asiatica (L.) Kuntze, *Striga aspera* (Willd.) Benth., and *Striga forbesii* Benth. (Berner et al. 1997).

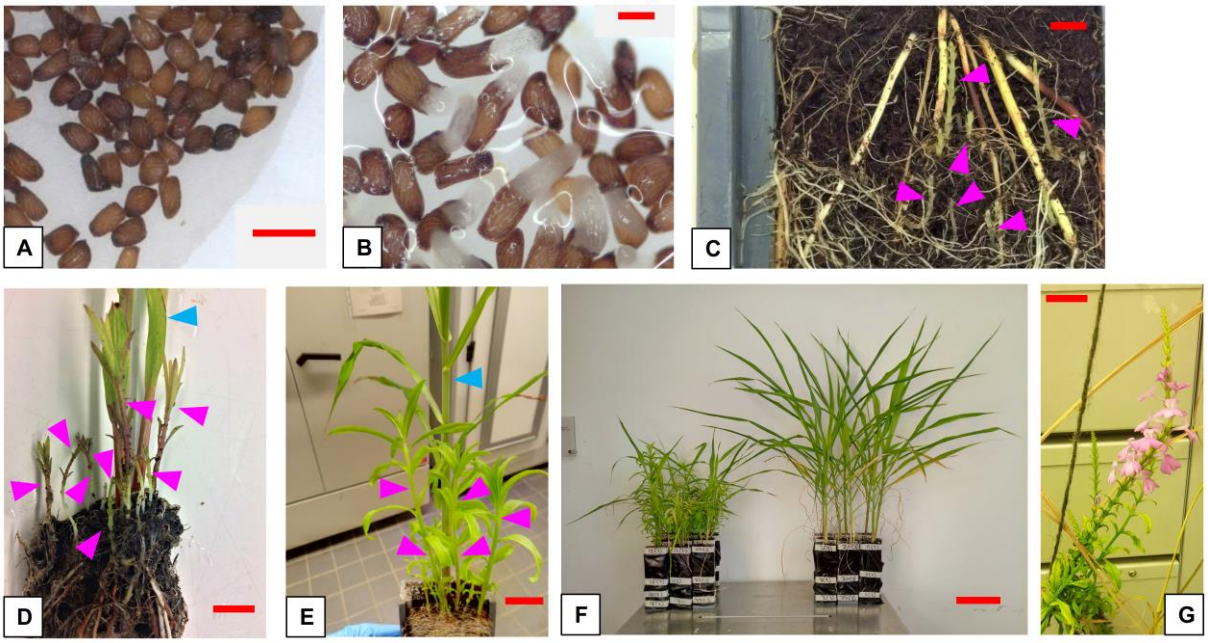

Fig. 1.3: Some photos from *Striga hermonthica* planting experiments under controlled environment. **(A)** *S. hermonthica* seeds (scale bar 0.5 mm). **(B)** Germinating *S. hermonthica* seeds (scale bar 1.5 mm). **(C)** *S. hermonthica* shoot elongation belowground (purple arrows) (scale bar 5 mm). **(D)** *S. hermonthica* shoot emerging aboveground (purple arrows - *S. hermonthica*, blue arrow – sorghum (*Sorghum bicolor*)) (scale bar 12.5 mm). **(E)** Numerous *S. hermonthica* plants parasitizing a sorghum plant (purple arrows - *S. hermonthica*, blue arrow - sorghum) (scale bar 25 mm). **(F)** Sorghum plants heavily infested with *S. hermonthica* (left) vs. *S. hermonthica*-free sorghum plants (right) (scale bar 35 mm). **(G)** *S. hermonthica* plant at flowering stage (scale bar 60 mm). (Photos by: Williams O. Anteyi).

The life cycle of *S. hermonthica* can be broadly divided into two stages (Fig. 1.4). These are highlighted as follows:

(a) Belowground stage – which is made up of
- seed germination.
- radicle growth towards host root and haustorium initiation.
- haustorium attachment/penetration of host root.
- vascular recognition (xylem connection)
- belowground shoot elongation.

(b) Aboveground stage – which consists of
- aboveground shoot emergence and vegetative growth.
- flowering and seed production/dispersal.

All events that make up the belowground stage, together with seed production, are the most crucial stages in the life cycle of *S. hermonthica* (Matusova et al. 2005).

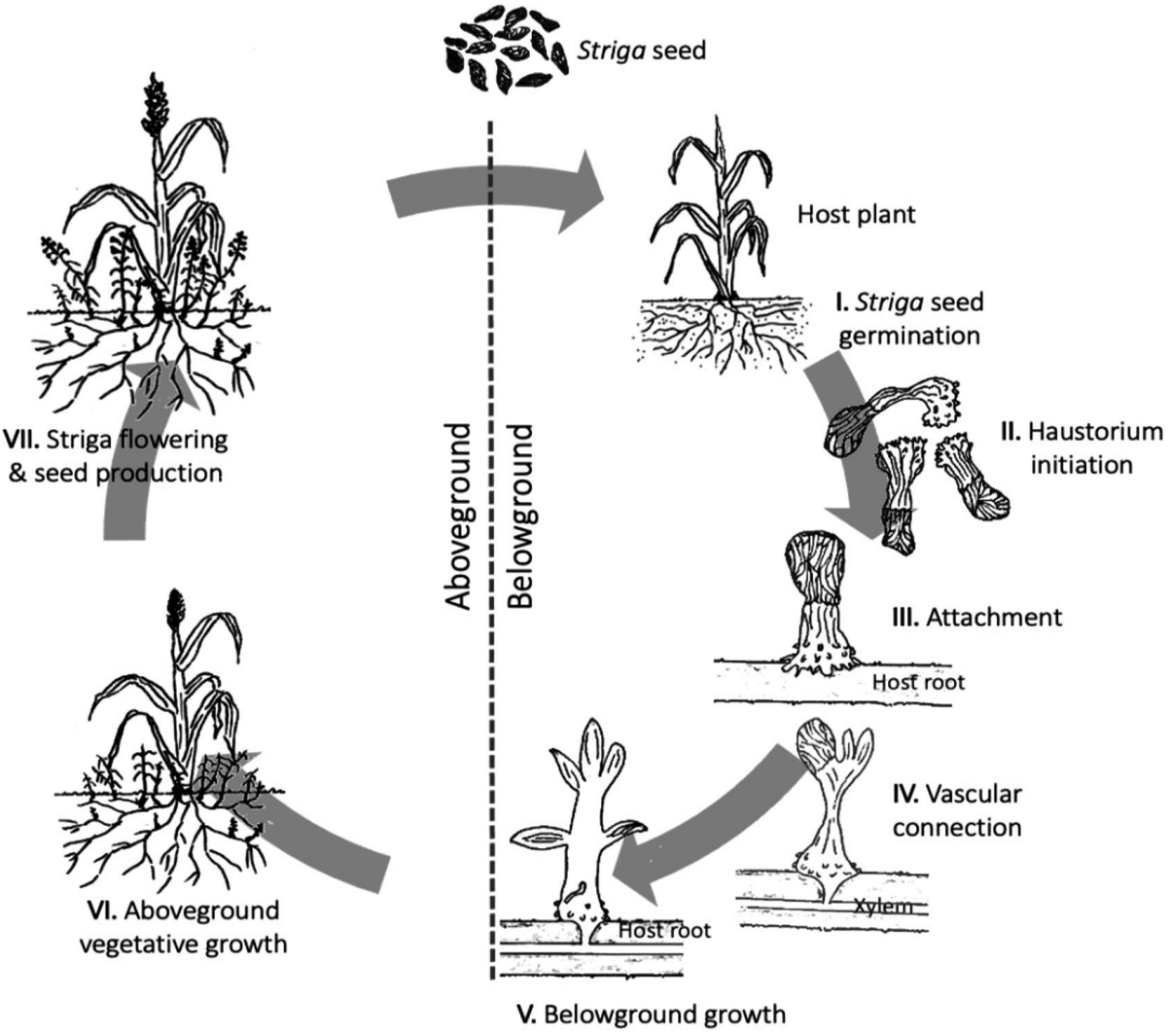

Fig. 1.4: General life cycle of parasitic *Striga* spp. (Courtesy: Mwangangi et al. 2021).

S. hermonthica seed germination, like other parasitic members of Orobanchaceae, requires a necessary induction by organic germination stimulants i.e., strigolactones (SLs), which are exuded by the roots of the host plant (Xie et al. 2010). Normally, higher plants produce SLs for regulating their architecture, and for triggering belowground symbiotic relationships. For instance, SLs and/or its derivatives occur as endogenous phytohormones for regulating shoot branching (Umehara et al. 2008), and SLs are also known to stimulate hyphal branching of arbuscular mycorrhiza fungi (Akiyama et al. 2005). However, parasitic *Striga* spp. evolved the capacity to exploit SLs as recognition factors to induce its seed germination (Yoshida and Shirasu 2009). The production and exudation of SLs by *S. hermonthica* host plant is promoted by soil nitrogen and phosphorus deficiency (Yoneyama et al. 2007 Jamil et al. 2011; 2012). The first naturally occurring SL to be isolated i.e., strigol, was from the root exudates of cotton (*Gossypium hirsutum* L.), a non-host plant for *S. hermonthica* (Cook et al. 1966). In *S. hermonthica* host plants, strigol was first isolated from the roots of maize (*Z. mays*) and proso millet (*Panicum miliaceum* L.) by Siame et al. (1993). Other naturally occurring SLs which are closely related to strigol, but more active, were also isolated from parasitic *Striga* spp. host plants in the same period. These include sorgolactone from sorghum roots (Hauck et al. 1992), and alectrol from cowpea (a host of *S. gesnerioides* (Willd.) Vatke) (Müller et al. 1992). About 25 naturally occurring SLs have been identified from the root exudates of different plant species (Xie 2016). In terms of synthetic analog of naturally occurring SLs, GR24 is the most studied and mostly used as standard for germination studies involving parasitic members of Orobanchaceae (Kramna et al. 2019). All SLs (natural or synthetic) share the same structural format i.e., a common skeleton of four rings that is made up of three ABC rings connected to a butenolide D-ring through an enol ether bridge (Fig. 1.5). The germination stimulating bioactivity of SLs resides in the CD moiety. This is the bioactiphore of the SL molecule. Thus, in absence of the A ring and/or B ring, the CD molecule alone is still active as a germination stimulant to *S. hermonthica* (Zwanenburg and Pospíšil 2013; Zwanenburg et al. 2016).

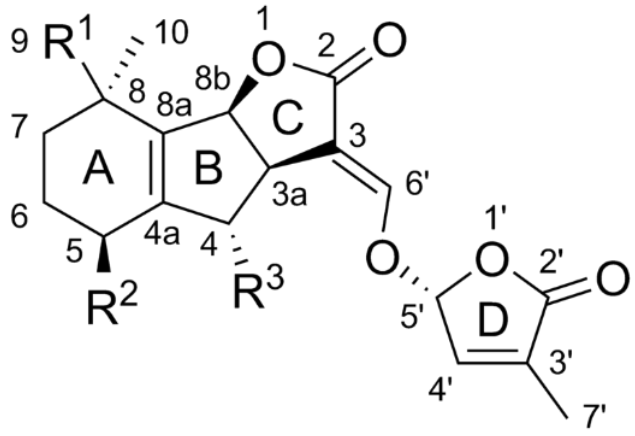

Fig. 1.5: General structure of strigolactones (Courtesy: Cacycle 2008).

In SL dependent plants, the SL signal is perceived by a receptive site which transduces (hydrolyse) the strigolactone molecule. Genetic studies with model plants revealed that SL receptors are α/β-hydrolase family proteins which is encoded by certain gene homologs and/or orthologs in different plants e.g., homologs or orthologs of *DWARF14* (*D14*) in (*OsD14*) rice and *Arabidopsis* (*AtD14*), *decreased apical dominance 2* (*DAD2*) in petunia, or *HYPOSENSITIVE TO LIGHT* (*HTL*)/*KARRIKIN INSENSITIVE2* (*KAI2*) in *Arabidopsis* (*AtHTL*) and *S. hermonthica* (*ShHTLs*). Thus, the SL signal transduction pathway from its perception, up to its resultant phenotypic responses (i.e., induction of seed germination, inhibition of shoot branching) have been modelled in SL dependent plants (Hamiaux et al. 2012; Nakamura et al. 2013; Tsuchiya et al. 2015; Brun et al. 2018). After *S. hermonthica* seed germination, the emerging radicle utilizes the seed nutrient store for growth, elongation, and development into a haustorium, which is the organ that it uses for attaching and penetrating the host plant (Fig. 1.6). Therefore, following *S. hermonthica* seed germination, the seedling must be attached to its host plant in the next 5 to 10 days, otherwise it would perish (Berner et al. 1997; Yoneyama et al. 2010; Saucet and Shirasu 2016). The elongating *S. hermonthica* radicle produces enzymes and inorganic compounds (e.g., peroxidases and H_2O_2), which oxidize the phenolic acids (i.e., syringic acid and sinapic acid) in the cell wall of the host root surface to produce quinones. These quinones acts as specific biochemical triggers, otherwise referred to as haustorium-inducing factors (HIFs), that signal haustoriogenesis of the elongating *S. hermonthica* radicle. Hence, 2,6-dimethoxy-p-benzoquinone (2,6-DMBQ) is the first identified and most-studied HIF (Chang and Lynn 1986; Kim et al. 1998a; Keyes et al. 2001).

For successful infection of host plants by *S. hermonthica*, there has to be vascular continuity between *S. hermonthica* and the host. This will ensure that the parasitic channel by which *S. hermonthica* accesses the host plant resources is maintained (Ejeta 2005). *S. hermonthica* lacks phloem vessels in its haustorium, hence, it does not establish a phloem-to-phloem contact with its host plants (Rogers and Nelson 1962; Spallek et al. 2013). Precisely, the parenchyma and xylem cells of *S. hermonthica* invade the host plant's central cylinder, to establish vascular connections (Yoshida and Shirasu 2009). After *S. hermonthica* establishes a compatible xylem-to-xylem connection with its host, the haustorial cells lose their protoplasts and changes its wall to become water conducting elements, which is continuous with the host xylem (Dörr 1997). Thus, there are four layers (levels) of incompatible interactions that *S. hermonthica* must overcome before it can successfully parasitize its host. These include *S. hermonthica* haustorial attachment/penetration of the host epidermis, penetration of the root cortex, penetration of the endodermis, and the recognition/penetration of the vessel elements after vascular connection (Yoshida and Shirasu 2009).

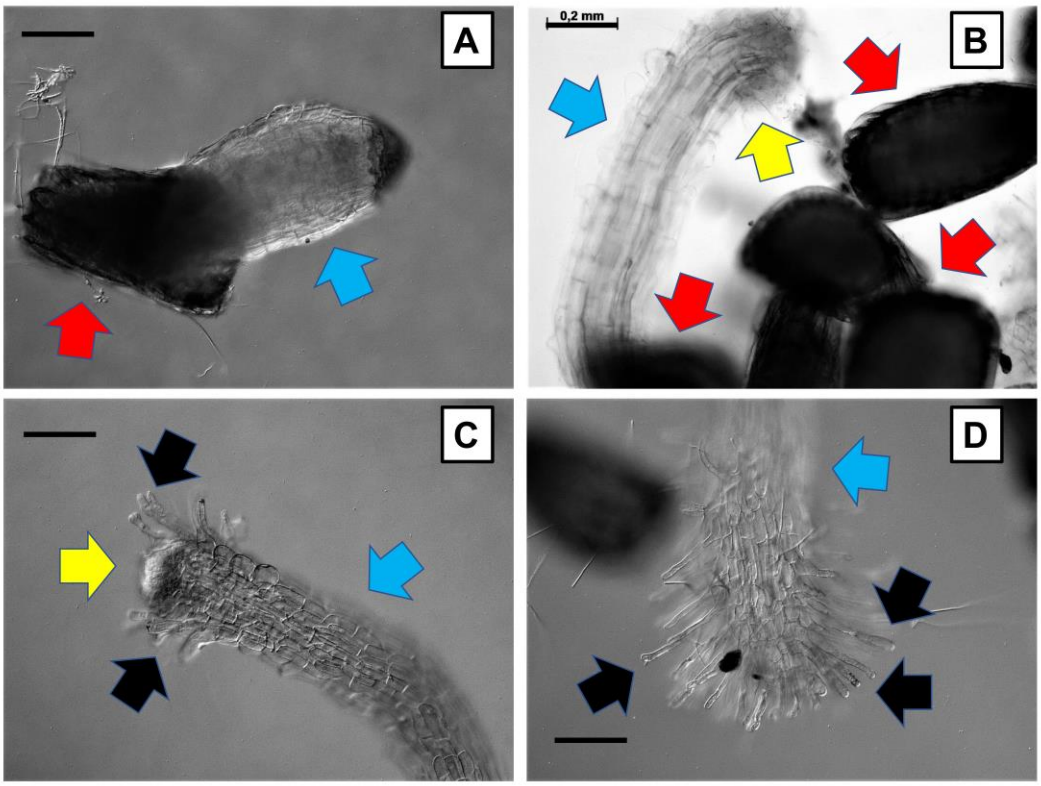

Fig. 1.6: Development of attachment organ (haustorium) in *Striga hermonthica* to facilitate its parasitic lifestyle. **(A)** Radicle emergence in germinated *S. hermonthica* seed. **(B)** Radicle elongation and swelling of the radicle tip in preparation for haustorial hairs (papillae) formation. **(C)** Initial stage of haustorial hairs proliferation at the radicle tip. **(D)** Proliferation of numerous haustorial hairs for *S. hermonthica* attachment and haustorial penetration of the host plant root. Red arrow – *S. hermonthica* seed. Blue arrow – Haustorium. Yellow arrow – Swollen radicle tip. Black arrow – Haustorial hair. Scale bars = 0.2 mm. (Photos by: Williams O. Anteyi).

1.4. *S. hermonthica* control strategies: with focus on biological control agents

Various management practices are utilized for controlling *S. hermonthica*. These range from simple cultural/traditional strategies, to sophisticated (scientific) techniques. As such, *S. hermonthica* control strategies can be broadly categorized into four approaches i.e., traditional or cultural, chemical, genetic and biological (Table 1). Unfortunately, the utilization of any single strategy for *S. hermonthica* management has not been completely reliable for controlling the parasite, due to limitations from their respective peculiar challenges. These challenges primarily centre on efficiency, consistency and sustainability of the technique. Therefore, various researchers agree on the integration of two or more control strategies for a more efficient *S. hermonthica* control (Berner et al. 1996; Schulz et al. 2003; Magani et al. 2011; Mrema et al. 2020).

Table 1: Major *S. hermonthica* management approaches (adapted from Esilaba 2006; Babiker 2007; Sibhatu 2016, and with some contributions by Williams. O. Anteyi).

***S. hermonthica*-management approach**	**Strategy**	**Major challenge(s)**
Traditional or cultural	Making use of *S. hermonthica*-free planting materials.	• Challenging for low-income farmers in heavily *S. hermonthica*-infested regions.
	Hand pulling.	• Labour intensive. • Low efficiency. • Not applicable to the belowground life-stages of *S. hermonthica.*
	Push-pull technology: • Intercropping with repellent "push" plants e.g., *Desmodium* spp. • Catch/trap crop planting with "pull" plants e.g., cotton, cowpea, soybean and groundnut to stimulate suicidal germination.	• Reduces available land for cultivating the cereal crop (*S. hermonthica* host) of interest. • Additional cost for procuring planting materials of the diverse crop species, and equipment.
	Crop rotation with *S. hermonthica* non-host plants e.g., soybean.	• Additional cost for procuring planting materials of the diverse crop species, and equipment. • Unstable levels of return.
	Soil fertility improvement e.g., N and P fertilization.	• Cost implication for low-income farmers.
Chemical	• Chemical pesticides (weedicides) e.g., Dicamba, imazapyr, pyrithiobac. • *Striga* seed suicidal germination approach e.g., pre-planting application of ethylene, ethephon, strigol and strigol analogues.	• Cost implication for low-income farmers. • Environmental hazards. • A single pesticide may not be suitable for all stages of *S. hermonthica* life-cycle.
Genetic	*S. hermonthica*-resistant crop varieties.	• Genetic improvement requires skill and technical know-how. • Time and labour tasking. • In some cases, the improved varieties are agronomically inferior.
Biological	The use of biological control agents (BCA) as bioherbicides/biopesticides: • Living organisms e.g., certain fungi, bacteria and insects. • Highly bioactive (phytotoxic) natural metabolites especially of fungal origin.	• Identifying *S. hermonthica*-specific BCA. • Consistency of action. • Adaptability and efficacy of an exotic BCA strain in a new location. • Bioherbicide formulation and standardization.

Generally, chemical weed management offers the most effective and immediate action for weed control (El-Sayed 2005). However, due to the excessive costs of developing chemical pesticides, together with recent awareness on the potential environmental hazards posed by chemical pesticides, there are growing interests among researchers to investigate alternative and safer systems of weed control, such as with biological control agents (also referred to as biocontrol agents or BCA) (Boyette et al. 1996; Duke et al. 2015; Jabran and Chauhan 2018). Biological control of weeds offers several advantages over chemical control. For example, biological control of weeds is an environmentally friendly approach that does not build up chemical residues in the environment (Prabha et al. 2016; Iqbal et al. 2018; CANNA Research 2020), it circumvents the problem of herbicidal resistance commonly developed by target weeds (Harding and Raizada 2015; Hershenhorn et al. 2016), it supports the growing market of organic agriculture thereby ensuring minimal application of synthetic chemicals in food production (Bale et al. 2008; Grzywacz et al. 2014; Cai and Gu 2016), and it is a cost-effective alternative when compared to the rising high costs of generating and registering novel, potent chemical herbicides (Charudattan 2001; Weaver et al. 2007).

Among the various groups of potential bioherbicides for weed control (i.e., viruses, bacteria, fungi, nematodes, insects and higher organisms), fungi are widely accepted as the most reliable BCA. This is because fungi often have stronger host-specificity, and they do not require a vector for dissemination (but through their hyphae), thereby resulting in their greater growth rate and spread (Boyette et al.1996; Whipps 2001; Hershenhorn et al. 2016). The detection and isolation of fungal diseases of *S. hermonthica* (in West Africa) was first reported by Zummo (1977). The diseases included leaf spot, stem lesion and vascular wilt, which were caused by *Cercospora* sp., *Phoma* sp. and *Fusarium equiseti* respectively. Accordingly, the quest to identify plant pathogenic fungi with specific and significant pathogenicity towards *S. hermonthica* later became a major interest among researchers in the following years (Musselman 1983). Survey of diseased *S. hermonthica* in Western Africa had revealed *Fusarium* spp., particularly *F. oxysporum*, as the predominant fungal species with intense pathogenicity and specificity towards *S. hermonthica* (Ciotola et al. 1995; Abbasher et al. 1995; 1998, Marley et al. 1999). Besides fungi, certain bacteria species, particularly belonging to the group of plant growth promoting rhizobacteria (PGPR) have also shown promising potentials as BCA against *S. hermonthica*. Examples of these rhizobacterial species mostly belong to the genera *Pseudomonas* sp., *Bacillus* sp., and *Azospirillum* sp. The *Striga*-attacking potentials of these PGPR is in addition to their typical ability of improving the health and/or promoting the growth of crops (Bouillant et al. 1997; Ahonsi et al. 2002a; Hassan et al. 2009b; Mounde et al. 2015). Also, several *S. hermonthica* seed germination inhibitors have been identified among the secondary metabolites produced by various microorganisms. For

instance, some *Fusarium* extracellular metabolites have demonstrated great potential in completely inhibiting *S. hermonthica* seed germination at very low concentrations (≤ 1 mM) *in vitro* (Zonno and Vurro 1999; Sugimoto et al. 2002; Vurro et al. 2009). Notwithstanding the promising bioherbicidal potentials of the various BCA types against *S. hermonthica*, a major challenge to the efficacy of BCA against their target weed (e.g., *S. hermonthica*) is the BCA inconsistent effectiveness under planting conditions, especially in natural agricultural systems. This daunting phenomenon may depend on a variety of factors, which ranges from the biotic (biotype variability, antagonists/pathogens) to abiotic components (climate and soil physico-chemical inhibitors) (Boyette et al. 1996; Ghosheh 2005; Bonaterra et al. 2012). If these factors that presumably underlie the inconsistent effectiveness of BCA against *S. hermonthica* are well-understood and tackled, remarkable progress will be made in improving the efficiency of combating *S. hermonthica* by biological control approach.

1.4.1 Fusarium oxysporum f. sp. strigae (Fos)

Fusarium oxysporum Schlecht. emend. Snyder & Hansen (Snyder and Hansen 1940), a member of the phylum Ascomycota, is a ubiquitous and well-diverse fungal species, whose predominantly plant-associating strains may occur as endophytes (endosymbionts), phytopathogens or saprophytes (Karim et al. 2016; Rana et al. 2017; de Lamo and Takken 2020). As the use of BCA, especially fungal phytopathogens, for combating *S. hermonthica* gained more attention, *F. oxysporum* was identified as a promising fungal-based bioherbicide (mycoherbicide) for effectively tackling *S. hermonthica* under both controlled and field conditions (Kroschel et al. 1996; Abbasher et al. 1998; Marley and Shebayan 2005). Hence, Elzein et al. (2008a) designated a *forma specialis* known as *Fusarium oxysporum* f. sp. *strigae* (commonly abbreviated as '*Fos'*), which encircles *F. oxysporum* isolates with specific pathogenicity towards *S. hermonthica*. This *forma specialis* was created following revelation of a similarly distinctive partial sequence in the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene. Through this finding by Elzein et al. (2008a), together with previous survey of diseased *S. hermonthica* in Western Africa which identified *F. oxysporum* as the predominant fungal species that attacks *S. hermonthica* (Ciotola et al. 1995; Abbasher et al. 1995; 1998, Marley et al. 1999), including available literatures on *S. hermonthica* biocontrol researches thus far, it therefore reveals that *Fos* is the most renowned fungal BCA for effectively antagonizing *S. hermonthica*. Several *Fos* isolates with significant pathogenicity towards *S. hermonthica* have been identified in SSA (Zimmermann et al. 2015). Notable isolates among them include; 'Foxy-2' from Ghana (Abbasher et al. 1995), 'M12-4A' from Mali (Ciotola et al. 1995), 'PSM-197' from Nigeria (Marley et al. 1999), and 'FK3' from Kenya (Beed et al. 2013). *F. oxysporum* is generally regarded as an asexual fungus, because so far, it is not known to have any sexual structure or stage (Irzykowska and Kosiada 2011; Brankovics

et al. 2017). Fungal isolates/strains which are capable of fusing asexually (i.e., hyphal anastomosis) to form stable and functional heterokaryons (i.e., cells with more than one nucleus that are genetically different) are referred to as vegetatively compatible or belonging to the same vegetative compatibility groups (VCG) (Leslie 1993; de Carvalho and Mendes-Costa 2011). Thus, VCG analysis is a useful technique for differentiating isolates within a *formae specialis* of *F. oxysporum* into genetically dissimilar (or genetically contrasting) groups. In other words, VCG analysis is useful for determining population genetic structure within a *formae specialis* of an asexual fungal species (Kistler 1997; Halpern et al. 2020). Through VCG classification, various *Fos* isolates have been differentiated into genetically similar or contrasting groups (Watson et al. 2007; De Klerk 2017). For instance, Foxy-2, M12-4A, and PSM 197 all belong to a common VCG, whereas Foxy-2 and FK3 are genetically contrasting because they belong to different VCGs (De Klerk 2017).

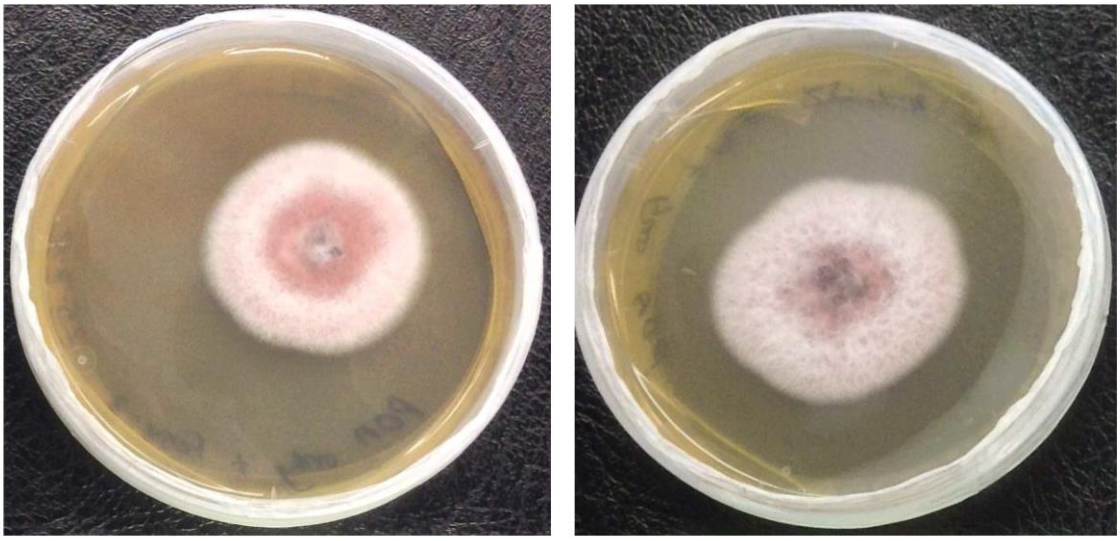

Fig. 1.7: *Fusarium oxysporum* f. sp. *strigae* (*Fos*) isolates Foxy-2 (left) and FK3 (right) growing on PDA. (Photos by: Williams O. Anteyi).

The mechanism by which *Fos* kills *S. hermonthica* is by first colonizing the *S. hermonthica* xylem vessels with its (*Fos*) hyphae, before it either digests or obstructs the xylem vessels in young or matured *S. hermonthica* plants respectively (Ndambi et al. 2011). It is noteworthy to state that in combating *S. hermonthica* with *Fos*, the fungal agent does not affect the *S. hermonthica*-host plant i.e., cereal crop. Rather, the cereal crop biomass is improved following the suppression or death of the witchweed (Ciotola et al. 1995; Kroschel et al. 1996; Abbasher et al. 1998). Thus, earlier assessments of *Fos* led to the declaration of the fungal agent as pathogenically specific to parasitic *Striga* spp., especially *S. hermonthica* and *S. asiatica*. This

assertion was also corroborated by the pathogenic specificity of *Fos* (isolate PSM-197) towards *S. hermonthica*, *S. asiatica* and *S. gesnerioides*, but not to another parasitic weed, *Alectra vogelli* Benth. (Marley et al. 2005); including *Fos* (isolate Foxy-2) pathogenic specificity towards *S. hermonthica* and *S. asiatica*, but not to 25 non-target plant species (Elzein and Kroschel 2006). However, host range studies with 26 economically important crops revealed that some tested solanaceous crops e.g., potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.) and eggplant (*S. melongena* L.), were susceptible to PSM-197 and Foxy-2, just like *S. hermonthica* (Zarafi et al. 2015). This, therefore, questioned the hitherto acclaimed 'host (*Striga*)–specific' status of *Fos*. It also suggests the need for caution when *Fos* is intended for use against *S. hermonthica* under an intercropping agricultural system. Especially when cereals are intercropped with other crops (e.g., solanaceous crops) which have been shown to be susceptible to *Fos* infection. Notwithstanding this disputation, *Fos* is still highly-valued as an effective mycoherbicide for *S. hermonthica* management. This is also because of *Fos* non-production of secondary metabolites that are of special toxicological concerns to humans and animals, therefore supporting its environmental safety when used under field conditions (Savard et al. 1997). So far, *Fos* has been reported to produce fusaric acid and dehydrofusaric acid (with their methyl esters) (Savard et al. 1997; Amalfitano et al. 2002), as well as beauvericin (Ndambi 2011), and these metabolites are characterized by low acute toxicity to humans and animals (Boonman et al. 2012; EFSA 2014).

Generally, *Fos* inoculum delivery for *S. hermonthica* control is through various methods. They include by: liquid/broth media culture (Kroschel et al. 1996, Marley et al. 1999), solid/agar media culture (Abbasher and Sauerborn 1995), dried-powdery spore culture (Rebeka 2013), cultured on plant materials e.g., cereal seeds or straw (Ciotola et al. 1995; Yonli et al. 2004; Kangethe et al. 2016), cereal seed coating (Elzein et al. 2006; Avedi et al. 2014), pesta/granular formulation (Connick et al. 1991; Elzein et al. 2004), as well as wounding or mechanical entry (Nzioki et al. 2016). Nonetheless, for field application, *Fos* inoculum delivery through seed coating or pesta/granular formulation is more beneficial especially for low-income farmers in SSA. This is because these preserved forms of *Fos* inoculum support easy transportation, storage and have longer shelf-life, which therefore minimizes costs (Elzein et al. 2006; 2008b). Notwithstanding the considerable advantages of *Fos* as a mycoherbicide against *S. hermonthica*, further research efforts are still required to improve its mycoherbicidal efficiency, in order to overcome its drawbacks. A very important drawback of *Fos*, especially in recent times, is the inconsistent effectiveness of its isolates against *S. hermonthica* under field/natural environments (Venne et al. 2009; Avedi et al. 2014). Although, early indications of this critical phenomenon have been reported from studies under controlled/artificial environments. For instance, through glasshouse trials, PSM-197 was able to effectively

suppress the incidence of *S. gesnerioides*, in addition to *S. hermonthica* and *S. asiatica* (Marley et al. 2005); whereas Foxy-2 only effectively suppressed the incidence of *S. hermonthica* and *S. asiatica*, but not *S. gesnerioides* (Elzein and Kroschel 2004a). This critical challenge of *Fos* inconsistent effectiveness affects its reliability as a dependable *Striga*-bioherbicidal agent. As such, it restricts *Fos* isolates general applicability and widespread use for *S. hermonthica* management (Avedi et al. 2014). Hence, this critical problem is the central justification for the PhD study.

1.4.2 Potentials, drawbacks and knowledge gaps of some plant growth promoting rhizobacteria (PGPR) as Striga-attacking agents

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of free-living beneficial bacteria that colonize plant root/rhizosphere, and improve the plant health and/or promote the plant growth directly or indirectly (Goswami et al. 2016; dos Santos et al. 2020). Broadly, PGPR enhance plant growth directly in two ways i.e., through the PGPR innate metabolisms or directly affecting the plant metabolism (Grichko and Glick 2001; Pérez-Montaño et al. 2014; Radhakrishnan et al. 2017; Backer et al. 2018). These are summarized as follows:

(a) Direct role of PGPR innate metabolisms in enhancing plant growth;
    - N, P and Fe biofertilization (i.e., asymbiotic atmospheric N_2 fixation, phytate degradation by extracellular phytase activity, siderophore secretion to sequester and solubilize Fe).
    - Rhizoremediation e.g., environmental pollutant degradation and/or heavy metal uptake (Cu, Zn, Cd, Cr Ni, and Pb).
    - Synthesis of certain gene products that contribute to abiotic stress control e.g., ACC deaminase for plant tolerance against flooding, and spermidine (a polyamine), which is a precursor of spermine and thermospermine for plant tolerance against salinity and drought.

(b) Direct impact of PGPR on plant metabolism;
    - Phytostimulation, through synthesis of important plant hormones e.g., auxin, gibberellins and cytokinin.

Indirect enhancement of plant health and/or growth by PGPR is mainly by elimination (i.e., biological control) of plant pathogens/pests e.g., through secretion of antimicrobial or biopesticidal agents, induction of systemic resistance, or competitive colonization for nutrients or niche (Lugtenberg and Kamilova 2009; Mariutto and Ongena 2015; Jiao et al. 2021). PGPR belong to diverse bacterial genera, which mainly include; *Acetobacter* sp.*, Azospirillum* sp.*, Azotobacter* sp.*, Bacillus* sp.*, Burkholderia* sp.*, Klebsiella* sp.*, Pseudomonas* sp., *Enterobacter*

sp., *Alcaligenes* sp., *Arthrobacter* sp. and *Serratia* sp. (Verma et al. 2019; Mohanty et al. 2021). *Pseudomonas* sp. and *Bacillus* sp., however, are the predominant PGPR genera (Adesemoye 2008; Radhakrishnan et al. 2017).

Apart from the numerous highly-admirable plant growth promoting abilities of PGPR, some PGPR strains, especially belonging to *Pseudomonas* sp., *Bacillus* sp., and *Azospirillum* sp., have been reported as potential BCA against *S. hermonthica*. These *Striga*-attacking PGPR suppress *S. hermonthica* by inhibiting the seed germination, prevent haustorial development/attachment, as well as delay aboveground emergence (Bouillant et al. 1997; Ahonsi et al. 2002a; Hassan et al. 2009; Mounde et al. 2015). Notwithstanding these unusual bioherbicidal advantages of these particular PGPR strains against *S. hermonthica*, it was, however, observed that in some cases, the *S. hermonthica* seed germination inhibiting potential of the PGPR strains (e.g., *Azospirillum* sp.) diminished with time, thereby eventually favouring *S. hermonthica* germination. This disadvantage was attributed to the instability of the *S. hermonthica* germination inhibitor(s) produced by PGPR (Miché et al. 2000). Also, though, Mounde et al. (2015) detailed the highly promising potential of *Bacillus* spp. in supressing *S. hermonthica* development and promoting the biomass of *S. hermonthica*-free sorghum plants, unfortunately, the PGPR strains were unable to improve the biomass of *S. hermonthica*-infected sorghum plants in a *S. hermonthica*-sorghum pathosystem. These drawbacks, amongst others, are important limitations against utilizing PGPR for *S. hermonthica* control. Hence, as part of measures to improve the efficiency of *S. hermonthica* biocontrol in terms of an integrated biocontrol system, it was assumed that a combined treatment application (i.e., bioherbicide cocktail) of *Fos* and PGPR into a *S. hermonthica*-cereal plant parasitic system could synergistically optimize *S. hermonthica* biocontrol i.e., simultaneous *S. hermonthica* suppression with enhancement of the *Striga*-infected cereal crop biomass. Unfortunately, knowledge of the microbe (*Fos*)–microbe (PGPR) interaction, their localization and ecological niche, in achieving this expected outcome from their co-inoculation in a *S. hermonthica*-cereal plant parasitic system was unknown.

1.4.3 Potentials, drawback and knowledge gaps of some Fusarium extracellular metabolites (exometabolites) as Striga-germination inhibitors

Secondary metabolites, also referred to as specialised metabolites, toxins, secondary products or natural products; are low-molecular weight, usually bioactive organic compounds, which are produced by different organisms, but are mostly not essential for life of the producers (Luckner 1990; Pusztahelyi et al. 2015, Erb and Kliebenstein 2020). Although, bacteria, fungi and plants are the most notable producers of secondary metabolites because of their well-developed secondary metabolism, however, fungi are foremost in this regard

owing to its highly diverse and versatile secondary metabolites that are of great importance to health, industrial and agricultural sectors worldwide. For instance, the significance of fungal secondary metabolites in the production of drugs and agrochemicals, as well as its notoriety for causing diseases to crops and mycotoxicosis to humans/animals (Keller et al. 2005; Scharf et al. 2014; Bills and Gloer 2016). In spite of the non-essentiality of secondary metabolites for fungal growth, they still play vital roles in the survival of fungi e.g., the ability of fungi to metabolize diverse nitrogen sources through its secondary metabolites enables it to colonize different ecological niches and survive under nutrient limitations (Tudzynski 2014; Boruta 2018). Also, some fungal secondary metabolites act as sporogenic factors for inducing asexual spore production, and are required for spore viability (Calvo et al. 2002). Based on origin (location), microbial metabolites are broadly classified as endogenous/intracellular (originating from within a cell), or exogenous/extracellular (arising from outside the cell) (Vaidyanathan 2005; Horak et al. 2019). Of these two classes extracellular metabolites (exometabolites) are globally paramount, due to their importance (beneficial or hazardous) in medicine, manufacturing, agriculture, including environmental protection (especially considering the growing interest of fungal exometabolites as environmentally-friendly alternative for the biological control of pathogens, pests and weeds) (Schueffler and Anke 2014; Harding and Raizada 2015; Mérillon and Ramawat 2017; Hyde et al. 2019). Many studies have reported the ability of several highly bioactive fungal exometabolites, especially from *Fusarium*, to obstruct early developmental stages (germination, haustorial development/attachment), and later stages (post-attachment/aboveground development) in the life cycle of parasitic Orobanchaceae (*Orobanche* sp., and *Striga* sp). This has led to their suggestion as potential BCA for controlling the parasitic weeds (Vurro et al. 2009; Cimmino et al. 2015; Triolet et al. 2020).

With regard to *S. hermonthica*, some specific *Fusarium* exometabolites have been reported to completely inhibit the seed germination *in vitro*, at very low concentrations (≤ 1 mM) e.g., T-2 toxin (Zonno and Vurro 1999), neosolaniol and its mono- or di- acetates (Sugimoto et al. 2002), and fusaric acid (Idris et al. 2003). So far, apart from the laboriousness and relatively high costs of isolating/purifying (or procuring) sufficient quantities of these exometabolites from their fungal producers, no major drawback has been linked with the strategy of using *Fusarium* exometabolites (at very low concentrations) for *S. hermonthica* control through laboratory experiments. However, in terms of knowledge gaps, it was unknown if these highly active *Fusarium* exometabolites will consistently prevent the incidence of *S. hermonthica* under planting conditions (controlled or field), without affecting the *S. hermonthica*-host cereal crop. It was also unknown if efficient *Fusarium* toxins for achieving maximum *S. hermonthica* biocontrol can be sourced from *Fos*. Thus, in making progress with the use of fungal

specialised metabolites for efficient *S. hermonthica* management, it is imperative to verify through experimental surveys that the exometabolite potency against *S. hermonthica* is not impaired *in planta*.

1.5 Problems (Research justification)

The mycoherbicidal effectiveness of *Fos* in controlling *S. hermonthica* under natural ecologies have been demonstrated through various field trials in several locations of SSA. For instance (but not limited to), the effectiveness of Foxy-2 against *S. hermonthica* in Western Africa e.g., in Nigeria (Schaub et al. 2006), Benin (Venne et al. 2009) and Burkina Faso (Yonli et al. 2004; Venne et al. 2009). On the contrary, field trials in Kenya (Eastern Africa) did not validate Foxy-2 effectiveness against *S. hermonthica* (Avedi et al. 2014). Instead, FK3 has shown to be effective against *S. hermonthica* in the East African country (Beed et al. 2013; Kangethe et al. 2016; Oula et al. 2020). Generally, the inconsistency of a BCA in suppressing its target organism (pathogen, parasite or pest) is a critical limitation that deters the general acceptance, widespread use or commercial development of the BCA product (Meyer and Roberts 2002; El-Sayed 2005; Harding and Raizada 2015).

From a broad perspective, this inconsistent effectiveness of a particular *Fos* isolate against *S. hermonthica* in different locations could be attributed to the influence of the differing site-specific factors that is peculiar to the given ecosystem. They include abiotic factors such as: climate (i.e., precipitation/moisture and temperature) (Kempenaar and Scheepens 1999; Venne et al. 2009; Zimmermann et al. 2016a) and/or soil physico-chemistry (Zimmermann et al. 2016a); as well as biotic factors such as the: diversity and interaction of the plant microbiome (i.e., phyllosphere or rhizosphere microbiota) (Auld and Morin 1995; Kremer 2005; Trognitz et al. 2016), specificity/mode of action of the BCA (Hasan and Ayres 1990; Boyette et al. 1996; Ghosheh 2005), genetic diversity among isolates of the BCA taxon (Watson et al. 2007; De Klerk 2017), genetic diversity among the weed species (Auld and Morin 1995; Nissen et al. 1995; Amsellem et al. 1999), regional genetic variation of both the BCA and weed species (Kangethe et al. 2016), and the biological/biochemical interactions that occur between the biotic components in the BCA–target weed pathosystem (Hoagland 1996; Trognitz et al. 2016; Petit et al. 2018). Thus, with regard to *S. hermonthica* genetic diversity (as a biotic component), genomic variation of individuals or overall susceptibility response unique to a sampling zone (i.e., regional genetic variation), were the two major forces suspected. However, the important determiner out of these two forces was unknown. These abovementioned abiotic and biotic components, indisputably, interact through ecologically complex networks of bipartite, tripartite or multipartite associations that occur both within and

between trophic and non-trophic interspecific levels, which may eventually lower or enhance a mycoherbicidal efficacy against its target weed (Olff et al. 2009; Knudsen and Dandurand 2014). Though, these aforementioned suppositions emphasizing the significance of abiotic or biotic factors in a BCA–target weed pathosystem, were not specifically studied in the context of *Fos* isolates inconsistent mycoherbicidal effectiveness against *S. hermonthica*. A thorough investigation of the roles of various abiotic or biotic components will give an in-depth understanding of specific ecological forces that influence *Fos* efficacy against *S. hermonthica*. Thus, in the context of genetic diversity in *S. hermonthica*, the PhD study focused on gaining (molecular) insights into the inconsistent effectiveness of *Fos* against *S. hermonthica*. This knowledge is among crucial steps towards developing workable solutions for overcoming this critical limitation facing the biological control of *S. hermonthica* by *Fos*. Another limitation in the use of *Fos* for *S. hermonthica* control is the largely irreversible physiological damage that *S. hermonthica* initiates to an infested cereal crop, despite the *Fos*-induced suppression/death of *S. hermonthica* (Beed et al. 2007; Rumsey 2012). Hence, as part of efforts to improve the efficiency of *S. hermonthica* biocontrol by *Fos*, the PhD study additionally investigated the integration of other non-*Fos* inoculum BCA (i.e., a bioherbicide cocktail of *Fos* and *S. hermonthica*-attacking PGPR, or utilizing *S. hermonthica* seed germination inhibiting *Fusarium* exometabolites) for *S. hermonthica* biocontrol. In a wider sense, these strategies were expected to serve as means for circumventing the main problem of the inconsistent effectiveness of *Fos* isolates against *S. hermonthica*, and the physiological consequences of *S. hermonthica* on infested host cereal crop.

1.6 Research hypotheses

The research works presented in this thesis were based on the following hypotheses:

- The inconsistent effectiveness of a *Fos* isolate against differing *S. hermonthica* populations is better explained by genomic variation in *S. hermonthica*, rather than by the *S. hermonthica* sampling zone/origin (i.e., East Africa and West Africa).
- Coinoculation of *Fos* and *Bacillus subtilis* (a PGPR) into a *S. hermonthica*-sorghum parasitic system will synergistically optimize *S. hermonthica* management, whereby *Fos* will tackle *S. hermonthica* incidence, while *B. subtilis* will improve (or at least adequately compensate) the biomass/yield of *S. hermonthica*-infested sorghum.
- Among a selection of highly phytotoxic *Fusarium* exometabolites, the most potent/efficient candidate (i.e., having the greatest effect at minimal concentration) to antagonize *S. hermonthica* is part of the *Fos* exometabolome composition which it uses for attacking *S. hermonthica*.

1.7 Study objectives

The objectives of this PhD study were to:

(a) Examine the molecular genetic basis, underlying the variable susceptibility of *S. hermonthica* populations sampled from differing zones of SSA (West Africa, East Africa) to contrasting *Fos* isolates (Foxy-2, FK3).
(b) Investigate the impact of co-inoculating *Fos* isolates (Foxy-2, FK3) and a PGPR (*B. subtilis* isolate GB03) on *S. hermonthica* incidence and sorghum aboveground biomass in a *S. hermonthica*-sorghum parasitic system, including the localization of the coinoculants in infected *S. hermonthica* plants.
(c) Screen the performance of a set of highly phytotoxic *Fusarium* exometabolites against *S. hermonthica* seed germination (*in vitro*) and incidence (*in planta*), towards identifying the most potent/efficient candidate exometabolite that antagonizes *S. hermonthica*, and determine if *Fos* is a producer of the candidate exometabolite.

1.8 Brief review of major molecular biological/biochemical methods used for the study objectives

1.8.1 Genomic basis for the variable susceptibility of S. hermonthica to Foxy-2 and FK3

The first research examined the molecular genetic basis for the variable susceptibility of differing *S. hermonthica* populations to contrasting *Fos* isolates (Foxy-2, FK3). For this, after the sampled *S. hermonthica* populations (from Western Africa and Eastern Africa) were phenotyped for their susceptibility response to Foxy-2 and FK3 (through climate chamber planting experiments), molecular genetic studies were performed through the following major methodological approaches: *S. hermonthica* genotyping with simple sequence repeats (SSRs) markers, population genetic analysis, targeted genome sequencing and bioinformatics. Molecular genetic markers, especially SSRs and SNPs (single-nucleotide polymorphisms), are powerful genomics analysis tools for associating heritable phenotypic traits with the underlying genomic variation (Duran et al. 2009; Tsykun et al. 2017). SSRs (also known as microsatellites or short tandem repeats (STRs)) is a tract of tandem repetitive DNA, in which certain DNA motifs (of one to six or more nucleotide bp) are repeated usually five to fifty times (Field and Wills 1996; Richard et al. 2008). SSRs are widely used for molecular genetic investigations because of their multiallelic and highly polymorphic nature, co-dominant inheritance, reproducibility, versatility, and genome abundance (Kalia et al. 2011; Nadeem et al. 2018). Hence, the study utilized 22 SSR markers (which consisted of 12 genomic SSR markers by Estep et al. (2010) and 10 expressed sequence tag (EST)-SSR markers by

Yoshida et al. (2010)), for SSR genotyping by PCR. This was followed by automated capillary electrophoresis to obtain microsatellite genotype data of the amplified DNA fragments lengths, and the dataset was used for downstream population genetic analysis (Delmotte et al. 2001; Mason 2015).

As *S. hermonthica* is an obligate outcrossing species, it exhibits a high rate of interbreeding, which thereby result to high genetic diversity of individuals and population genetic structure (Musselman and Parker 1983; Aigbokhan et al. 1998; 2000; Estep et al. 2011). Population genetic structure of a species is the presence of a systematic difference in the allele frequencies between subpopulations/subgroups of a population, or between case and control groups, which is attributable to background population diversity and/or non-random mating between individuals of the background population (Cardon and Palmer 2003; Sebro et al. 2010). Knowledge of population genetic structure of a species is crucial for understanding important phenotypic attributes associated with differing populations of the species (Chakraborty 1993; Agre et al. 2019), such as the variable susceptibility of differing *S. hermonthica* populations to contrasting *Fos* isolates (e.g., Foxy-2, FK3). Hence, analyses of population genetic structure in the sampled *S. hermonthica* (based on susceptibility response to Foxy-2 and FK3), genetic differentiation between structured *S. hermonthica* groups, and the flow of genetic variation (gene flow) between structured *S. hermonthica* groups, were determined by analysis of molecular variance (AMOVA) (Excoffier et al. 1992), fixative index or F_{ST} (Wright 1965), and effective number of migrants (*Nm*) exchanged between the groups per generation (Wright 1969), respectively. AMOVA and F_{ST} are important foundational concepts for determining if phenotypic differences (e.g., susceptibility pattern), shown by subpopulations of a given species (e.g., *S. hermonthica*) to certain treatments (e.g., Foxy-2 and FK3), has an underlying genetic cause or not. It takes at least two populations to determine genetic differentiation by F_{ST}, and statistically test the genetic differentiation by AMOVA (Peakall and Smouse 2006).

Marker-trait association of genetically structured *S. hermonthica* groups was revealed by the exact test of Hardy-Weinberg Equilibrium (HWE), precisely by the Markov chain Monte Carlo procedure. The exact test of HWE is known to be valid for any sample size and minor (rare) allele frequency (Wang and Shete 2012). Through this, alleles that are non-randomly associated at different SSR loci of a subpopulation i.e., linkage disequilibrium (LD), was determined. A pair of loci are said to be in LD when the frequency of association of their different alleles is higher or lower than would be expected if the loci were independent and randomly associated. Therefore, a decay of LD implies tendency towards independent assortment or random association of the alleles (Flint-Garcia et al. 2003; Slatkin 2008). Targeted (Sanger) sequencing was used for determining the nucleotide sequence of amplified

regions of *S. hermonthica* DNA which are associated with the phenotypic (susceptibility) responses. Hence, variation (mutational differences) in the nucleotide and translated protein sequences between the *S. hermonthica* populations were revealed after the sequences were aligned by the multiple sequence comparison by log-expectation (MUSCLE) algorithm (Edgar 2004).

1.8.2 Localization of Fos and B. subtilis in infected S. hermonthica plants

In addition to investigating the impact of *Fos* and *B. subtilis* coinoculation on *S. hermonthica* incidence and sorghum aboveground biomass in a *S. hermonthica*-sorghum pathosystem, the second research also examined the localization of *Fos* and *B. subtilis* in infected *S. hermonthica* plants. This aim was achieved by preliminarily transforming both *Fos* and *B. subtilis* with a vector (plasmid DNA) each that both carry similar antibiotic resistance genes as selectable markers, but contain contrasting fluorescent reporter genes (i.e., red and cyan fluorescent proteins). Then, the transformants were monitored in infected *S. hermonthica* by *in vivo* imaging, using a laser scanning microscope. Fluorescent reporter genes are robust signaling and biomarker tools for monitoring biological pathway activities, gene expression and histomorphology in living systems i.e., cells, tissues, organs or whole organisms. To this end, the reporter gene encodes a protein that expresses a characteristic fluorescence emission spectrum when it is excited with light at a specific wavelength (Chudakov et al. 2010; Martin et al. 2019). This technology serves as a live-imaging, real-time monitoring technique, that does not necessarily require the rigours of purifying, tagging, fixation with toxic compounds (e.g., aldehydes), chemical dyeing/isotopic labelling of cellular proteins, or production of specific antibodies against antigens (Giepmans et al. 2006; Chudakov et al. 2010; Piston et al. 2021).

In the study, *Fos* was transformed by a polyethylene glycol (PEG) mediated procedure (Zhang et al. 2014), and *B. subtilis* was transformed by a “mixed salts” mediated procedure (iGEM 2014). The PEG–mediated transformation is a simple, inexpensive, and efficient form of chemical transformation, which produces a transformed cell population of high survival and division rate. The procedure is also easily adaptable and it helps to overcome the challenges of other transformation systems such as biolistics (gene gun) and *Agrobacterium*–mediated transformation (Mathur and Koncz 1998; Liu and Friesen 2012). The mixed salts mediated transformation procedure that was utilized is also a simple, inexpensive, and efficient form of chemical transformation, through which competent bacterial cells (i.e., prepared cells that are ready/able to take up foreign or extracellular DNA e.g., plasmid DNA, from its surrounding environment) are first produced using a mixture that is rich in K, P, and Na inorganic salts.

Then the competent cells are transformed with plasmid DNA using amino acids rich mixtures (iGEM 2014, Martin 2021).

In addition to the ability of both *Fos* and *B. subtilis* transformants to grow on antibiotic-selective media and also express characteristic fluorescence under fluorescent microscope, PCR of unique nucleotide sequences within the contrasting fluorescent gene inserts of the two plasmids verified the successful integration of the different fluorescent genes into *Fos* and *B. subtilis* transformants, respectively. This novel confirmatory strategy is a more reliable means for verifying the successful transformation of an organism. It is especially important for circumventing a major limitation of the common strategy of performing PCR of nucleotide sequence within the plasmid antibiotic resistance genes. This is because other environmental microbes (or contaminants) may also carry one or more of these antibiotic resistance genes (Smalla et al. 2015; Müller et al. 2016). Besides, since the study utilized two different plasmids that carry the same antibiotic resistance genes (which confer resistances to chloramphenicol, kanamycin and hygromycin), their contrasting fluorescence reporter genes (which expresses red fluorescence for *Fos* and cyan fluorescence for *B. subtilis*) was a dependable distinguishing factor during *in vivo* imaging of *Fos* and *B. subtilis* within infected *S. hermonthica*.

1.8.3 Biosynthesis ability of a candidate S. hermonthica-antagonizing Fusarium exometabolite by Fos, and the exometabolite degradability in soil substrate

In the third research, following the identification of a candidate exometabolite (out of a set of highly phytotoxic *Fusarium* exometabolites) which was most potent/efficient (i.e., having the greatest effect at minimal concentration) for completely antagonizing *S. hermonthica* seed germination (*in vitro*) and incidence (*in planta*), the ability of *Fos* to produce the candidate exometabolite was also investigated. This was aimed at uncovering specific metabolites that are part of the *Fos* metabolomic composition for attacking *S. hermonthica*. Hence, exometabolomics study was performed with Foxy-2 and FK3 liquid cultures. Exometabolomics (also known as metabolic footprinting) is a subfield of metabolomics that studies exogenous metabolites produced by an organism, using spectral analytical approaches e.g., liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC–MS), or nuclear magnetic resonance (NMR). Through this, specific exometabolites that are produced by organisms and released into the immediate environment are detected (Mapelli et al. 2008; Silva and Northen 2015).

Also, the research further examined the molecular genetic basis for *Fos* production (or non-production) of the candidate exometabolite through the expression of specific genes (gene expression) that underlie the molecular biosynthetic pathway of the exometabolite. Gene

expression is the process by which genetic information (DNA) directs the synthesis of a functional gene product i.e., protein or non-coding RNA (Doug Chung and Le Roch 2013; JoVE 2021). This is the central dogma of molecular biology (Crick 1970), whereby the process by which a genotype (genetic information) gives rise to the phenotype (observable traits e.g., the organism's structure, development or specific metabolic pathways) is investigated (Holmes and Jobling 1996; Chen 2020).

Additionally, preliminary examinations of the environmental impact/safety of the candidate exometabolite were performed. Firstly, the impact of the candidate exometabolite on soil microbiota was investigated through absolute quantification of gene copy numbers of the major soil microbial groups i.e., bacteria and fungi, through the 16S rRNA (16S ribosomal RNA) and 18S rRNA (18S ribosomal RNA), respectively (Lane 1991, Muyzer et al. 1993; Vainio and Hantula 2000). 16S rRNA (about 1500 bp) is a component of the prokaryotic ribosome 30S subunit; while 18S rRNA (approximately 1800 bp) is a component of the small eukaryotic ribosomal subunit 40S, which constitute eukaryotic ribosomes together with 60S. Comparably, 16S rRNA is highly conserved, specific, and exists in the genome of all bacteria, and 18S rRNA is highly conserved intra-eukaryotic species (according to the variable regions for different taxonomic resolutions e.g., fungi) (Buse et al. 2014; Wu et al. 2015; CD Genomics Blog 2018a; 2018b). Due to their highly conserved and specific regions among prokaryotic and eukaryotic microbial groups, determination of 16S rRNA or 18S rRNA gene copy numbers are very useful for environmental biodiversity screening, and are the most common approach for estimating the abundance and/or profiling bacterial and fungal communities in environmental samples (CD Genomics Blog 2018a; 2018b; Tkacz et al. 2018; Jurburg et al. 2021). Then, in a second step, the degradability of the candidate exometabolite in soil substrates was studied through metabolite target analysis (i.e., targeted metabolomics). Targeted metabolomics is the quantitative (or semi-quantitative) measurement of one, a few, or defined groups of chemically characterized and biochemically annotated metabolite(s) of interest, in a given sample (Mapelli et al. 2008; Roberts et al. 2012; Ribbenstedt et al. 2018). Thus, because the metabolite of interest (i.e., candidate exometabolite to be investigated for its degradability in the soil substrate) would have been pre-defined from germination assays and planting experiments, therefore targeted metabolomics was clearly suitable for achieving this aim, rather than non-targeted metabolomics approach (Roberts et al. 2012). These preliminary examinations of the environmental impact/safety of the candidate exometabolite in soil substrates serve as pilot ecological indicators of the candidate exometabolite safety when it is used for *S. hermonthica* management under natural ecosystems.

1.9 Outline of the thesis

This PhD thesis focused on understanding the genomic basis for the inconsistent effectiveness of *Fos* isolates (Foxy-2, FK3) against differing *S. hermonthica* populations from SSA, in the context of genetic diversity in *S. hermonthica*. Also, as part of efforts to overcome this critical problem, the PhD thesis further examined different biocontrol strategies for improving the efficiency of *S. hermonthica* biocontrol by *Fos*. For this purpose, this PhD thesis has been written as a cumulative thesis that comprises one review article (chapter 2) and three original research articles (chapters 3, 4 and 5). All the articles presented in the thesis (i.e., chapters 2, 3, 4 and 5) have been published in peer-reviewed journals. Chapter 1 presents a general introduction on the global perspective of food security under the current climate change (with focus on sub-Saharan Africa). This chapter also presents a brief introduction on sorghum, *S. hermonthica*, *S. hermonthica* control strategies (with focus on biological control agents), *Fusarium oxysporum* f. sp. *strigae* (*Fos*), as well as the potentials, drawbacks and knowledge gaps of some plant growth promoting rhizobacteria (PGPR) and some *Fusarium* extracellular metabolites (exometabolites) as *Striga*-attacking agents and *Striga*-germination inhibitors respectively. Chapter 1 concludes with the problems (research justification), research hypotheses, study objectives, and a brief review of major molecular methods used for the study objectives. Chapter 2 presents a review of the tripartite biotic interaction in a pathosystem of *Striga* spp.–cereals–plant root-associated microorganisms. This is because the biotic interactions that occur in a bioherbicide–parasitic weed–host plant (crop) pathosystem play major roles in determining the bioherbicide specific efficacy against the target parasitic weed, but not to the host crop. In chapter 3, the role of underlying genomic variation in explaining the variable susceptibility of differing *S. hermonthica* populations to Foxy-2 and FK3 was investigated. In examining different biocontrol strategies for overcoming the critical challenge of the inconsistent effectiveness of *Fos* isolates against *S. hermonthica*, chapter 4 assessed the role/impact of coinoculating *Fos* isolates (Foxy-2, FK3) and *B. subtilis* (a PGPR) on *S. hermonthica* incidence and sorghum aboveground biomass in a *S. hermonthica*-sorghum pathosystem, including their localization within infected *S. hermonthica* plants. In contrast, chapter 5 examined the performance of a set of highly phytotoxic *Fusarium* exometabolites against *S. hermonthica* seed germination (*in vitro*) and incidence (*in planta*), towards identifying the most potent/efficient candidate out of the tested exometabolites. Chapter 5 also examined the ability of *Fos* to produce the candidate exometabolite. Lastly, chapter 6 presents the general discussion and conclusions from the findings of the PhD study, including the study limitations, recommendations and future research directions.

Chapter 2. Tripartite interaction between *Striga* spp., cereals, and plant root-associated microorganisms: a review

Lenard G. Mounde[1], Williams O. Anteyi[2] and Frank Rasche[2]

[1]Pwani University, Mombasa-Malindi Highway, P.O Box 195-80108 Kilifi, Kenya.

[2]Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), University of Hohenheim, 70593 Stuttgart, Germany.

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2.1 Abstract

Striga spp. is a major threat to cereal and legume production, putting the food security and economy of smallholder farmers in sub-Saharan Africa at severe risk. This is manifested in the fact that *Striga* spp. infestation can result in up to 90% loss of both cereal and legume production. A consensus exists that there is yet no single measure to efficiently control *Striga* spp. This is mainly because of the limited fundamental knowledge of the genetics and ecology of *Striga* spp. and its interaction with its hosts and host root-associated microorganisms, including plant growth-promoting rhizobacteria (PGPR). Since *Striga* spp. is a root parasite, it is speculated that PGPR play a key role in controlling the emergence and development of *Striga* spp. At the same time, PGPR may exhibit beneficial effects on growth promotion of the host to strengthen its tolerance against *Striga* spp. attacks, while on the other hand, it may also induce, similar to biocontrol agents, direct suicidal effects on *Striga* spp. Such hypothesized associations between *Striga* spp., crops (e.g., cereals such as sorghum and maize), and PGPR remain largely unknown, and the central question remains if PGPR play an important role in the *Striga*-crop pathosystem. This knowledge gap is the central impetus of this review. It will elaborate the complex and fascinating tripartite ecological system of cereals, *Striga* spp. and root-associated microorganisms. In a first step, the review will provide a comprehensive summary of the pairwise interactions between *Striga* spp. and cereals, cereals and PGPR, as well as *Striga* spp. and PGPR. This summary will then merge into the discussion about the yet limited knowledge of the tripartite interaction between cereals, *Striga* spp., and PGPR. This specifically includes the exploration of recent discoveries related to population genetics and the life cycle of *Striga* spp, host (cereal) defence responses to and mechanisms of *Striga* spp. infestation, as well as the inhibitory and stimulatory role of PGPR on *Striga* spp. seed germination. In a concluding section, remaining research gaps are identified and necessary research perspectives are provided to direct prospective research toward further understanding the relationship between the three biological components paving the avenue to develop biological and environmentally friendly measures to fight off the everlasting threat of *Striga* spp.

Keywords: *Striga* spp., cereals, rhizosphere, plant growth-promoting rhizobacteria (PGPR), tripartite interaction, biological control.

2.2 Introduction

2.2.1 Daunting challenge of Striga spp. infestation in sub-Saharan Africa

Several flowering plants have developed parasitic associations with other members of the plant kingdom. Watling and Press (2001) as well as Runo et al. (2012) estimated that about 4,000 parasitic plant species exist, which are grouped in 13 families. Parasitic plants colonize the tissue of their hosts, where they demonstrate a remarkable efficiency in obtaining water as well as organic and inorganic resources (Sauerborn et al. 2007; Atera et al. 2011). Parasitic plants of major agronomic significance belong to the genera *Cuscuta* spp., *Alectra* spp., *Orobanche* spp., and *Striga* spp. (Sauerborn et al. 2007). Currently, approximately 90 genera and 2,000 species of *Striga* spp. and *Orobanche* spp. are known. Both genera are grouped in the Orobanchaceae family, indicating a close phylogenetic relationship (Westwood 2013). *Orobanche* spp. and *Striga gesnerioides* are holoparasites as they lack chlorophyll; hence, they depend fully on the host plants (i.e., dicots) for carbon, water, and nutrients (Musselman 1980; Farquhar et al. 1982; Estabrook and Yoder 1998). *Striga* spp., on the other hand, is an obligate hemiparasite with photosynthetic abilities. It derives only a part of its carbon requirement from its host (e.g., mainly cereals) but depends fully on its host for water and nutrients (Watling and Press 2001; Eplee and Westbrooks 1990; Mohamed and Musselman 2008). In sub-Saharan Africa, the agronomically most important *Striga* spp. are *Striga hermonthica*, *Striga asiatica*, *Striga aspera*, and *Striga forbesii*. They parasitize a range of cereal crops, including sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* L.), finger millet (*Eleusine coracana* L.), and rice (*Oryza spp*. L.). *Striga gesnerioides* parasitizes dicots, including cowpea (*Vigna unguiculata* L.), Bambara groundnut (*Vigna subterranea* L.), tobacco (*Nicotiana tabacum* L.), and sweet potato (*Ipomoea batatas* L.) (Elzein and Kroschel 2004b; Scholes and Press 2008). Ejeta and Butler (1993) ranked *Striga* spp. as the leading biotic constraint to cereal production in sub-Saharan Africa, with considerable losses in yield quality and quantity ranging from 30% to 90% (Musyoki et al. 2015). Besides having a huge impact on agricultural commodities (Parker 2009), *Striga* spp. impacts have been estimated by Ejeta (2007b) as well as Scholes and Press (2008) to lead to about 100 million people facing food insecurity, causing an economic damage equivalent to approximately 1 billion $US annually (Labrada 2008; Waruru 2013).

2.2.2 Current control options of Striga spp.

Major efforts have been carried out to understand potential measures to control *Striga* spp. affecting various cereal and leguminous crops. Yet, no single control method with sufficient efficacy has been established (Parker 2009; Hearne 2009; Atera et al. 2012; Rubiales and Fernández-Aparicio 2012). Several methods with inconsistent success and potential for

adoption are anchored on cultural, mechanical, chemical, or biological means, including but not limited to hand weeding, crop rotation, trap cropping, deep or no tillage, use of herbicides and resistance breeding (Ndung'u 2009; Lagoke and Isah 2010; Jamil et al. 2011). Alternatively, biological control of *Striga* spp. has been introduced as a promising, environmentally friendly technique (Charudattan 2001; Musyoki et al. 2015; Zimmermann et al. 2015). There has been a great focus on the use of fungal microorganisms, where *Fusarium oxysporum* f.sp. *strigae* (*Fos*) strain "Foxy-2" proved valuable to control *Striga* spp. emergence by up to 95%, while increasing sorghum yields by almost 50% (Elzein et al. 2006; Franke et al. 2006; Venne et al. 2009). In Western Africa, the control efficacy was increased by combined use of "Foxy-2" and resistant varieties of affected cereals (Beed et al. 2007; Venne et al. 2009; Rebeka et al. 2013). Avedi et al. (2014) in Eastern Africa (Kenya), however, contradicted the effectiveness of "Foxy-2." Instead, "FK3", a different *Fos* isolate from Kenya, was effective in this case (Kangethe et al. 2016). The disparity in the effectiveness of both *Fos* isolates against *Striga hermonthica* in Western Africa and Eastern Africa is yet to be understood. It was assumed that the inconsistent effectiveness of a specific *Fos* isolate against *Striga hermonthica* may be attributed to biotic factors such as the biocontrol isolate specificity (Ghosheh 2005), susceptibility pattern of weed genotypes (Nissen et al. 1995), soil microbiota (Kremer and Kennedy 1996), including abiotic factors such as soil physicochemical properties (Zimmermann et al. 2016b) and climate (Kempenaar and Scheepens 1999; Venne et al. 2009). Hence, a fundamental understanding of the inconsistent effectiveness of "Foxy-2" and "FK3" recorded across agroecologies is yet to be given.

2.2.3 Knowledge gaps and review objectives

In any ecosystem, plants interact both mutually and antagonistically with other organisms, forming complex ecological associations (Herrera et al. 2002; Thompson and Fernandez 2006). For a long time, many ecological studies focused largely on bipartite interactions between species (e.g., plant host-parasite). Over the last few years, this paradigm has shifted. An increasing number of studies have highlighted the importance of linking multiple interactions, including those connecting above- and below-ground biota, to understand ecological and evolutionary processes in nature (Gehring and Bennett 2009; Lugtenberg and Kamilova 2009). This includes the recognition of essential interactions between plant roots and microorganisms (Bardgett et al. 1998; van der Heijden et al. 2008; Mendes et al. 2013). In this respect, plant growth-promoting rhizobacteria (PGPR) and also arbuscular mycorrhizal fungi (AMF) have a variety of essential functions ranging from improved uptake of immobile nutrients, protection of host from pathogens, and soil aggregation to promote plant growth (van der Heijden et al. 2008; Pérez-García et al. 2011; Venkateshwaran et al. 2013). There is

ample information providing clear evidence for the positive role of soil biotic interactions in crop growth and *Striga* spp. management:

- A significant annual death rate of *Striga* spp. seeds in the field, as linked to microbial decay (Gbèhounou et al. 1996).
- Increased *Striga hermonthica* attachment to host crops in unpasteurized soils compared to pasteurized soils (Miché et al. 2000).
- Pathogenic effects of bacteria (Ahmed et al. 2001) and fungi (Samaké 2003) against *Striga* spp. seed germination.
- A reduced *Striga* spp. incidence with increased soil suppressiveness (Berner et al. 1996).
- Microorganisms benefit plant health and crop productivity, while in other cases, root exudates can prevent growth of harmful microorganisms (Chaparro et al. 2012; Janani et al. 2013; Li et al. 2013; Neondo 2017).
- Soil microorganisms can cause decay of *Striga* spp. seeds through enzymatic and antibiotic activities (Bais et al. 2006).

Given that plant root-associated microorganisms play key ecological roles in nature, they might as well affect *Striga* spp. parasitism on cereals. Possible associations between *Striga* spp., cereals, and root-associated microorganisms remain, however, largely unknown, and the question whether these microorganisms play a role within the *Striga*-cereal pathosystem has not been answered completely. This knowledge gap is the central impetus of this review, which elaborates the complex and fascinating tripartite ecological system of cereals, *Striga* spp., and root-associated microorganisms. In a first step, a comprehensive review of existing literature was undertaken on pairwise interactions between *Striga* spp. and cereals, cereals and root-associated microorganisms, as well as *Striga* spp. and root-associated microorganisms. In a second step, we emphasized the current knowledge of the tripartite interaction between cereals, *Striga* spp., and root-associated microorganisms. We highlighted recent discoveries related to population genetics and life cycle of *Striga* spp., host (cereal) defence responses and mechanisms to *Striga* spp. infestation, as well as the inhibitory and stimulatory role of the rhizosphere microbiome in *Striga* spp. seed germination. We concluded this review with novel approaches to study the complexity of the cereal-*Striga* spp. rhizosphere, which will translate into emerging opportunities to direct future research. Such improved understanding of tripartite interactions will be paramount in developing appropriate strategies for *Striga* spp. management with high level of efficacy.

2.3 Bipartite interaction between *Striga* spp. and cereals

2.3.1 Genetic structure in Striga spp.

Striga spp. is a genus that is made up of over 25 hemiparasitic member species that exhibit diversity in various forms, including but not limited to morphology, host, virulence, and mating systems (Spallek et al. 2013). The mating system in *Striga* spp. can serve as basis for understanding the diversity patterns, gene flow, and genetic population structure with respect to a peculiar *Striga* species (Hamrick 1982; Safa et al. 1984). For instance, the two most economically important *Striga* species (namely *Striga hermonthica* and *Striga asiatica*) exhibit contrasting mating systems, which in turn determine the flow of genetic variation from one population to another. *Striga hermonthica* is an obligate allogamous (outcrossing) species (Musselman et al. 1983), while *Striga asiatica* is primarily autogamous (selfing) (Nickrent et al. 1979). In genetically structured populations, outcrossing species exhibit higher genetic variability within than among subpopulations. Conversely in selfing species, greater genetic variability is expected among than within subpopulations (Loveless and Hamrick 1984).

There is a general agreement on the influence of geography as the primary determinant of population structure in *Striga hermonthica*, through the support of higher gene flow between closer (i.e., within a structured subpopulation) than between distant (i.e., between subpopulations) populations, but host specificity is an unimportant basis for population structure. This phenomenon has been presented using different genetic marker systems, as in the case with enzyme electrophoresis (Olivier et al. 1998), amplified fragment length polymorphism (AFLP) (Welsh and Mohamed 2011), simple sequence repeat (SSR) (Estep et al. 2011), single nucleotide polymorphism (SNP) (Unachukwu et al. 2017), and expressed sequence tag-simple sequence repeats (EST-SSR) (Joel et al. 2018). Contrarily, AFLP studies with *Striga asiatica* populations from Benin revealed a higher genetic variability within subpopulations, including a high extent of host specificity (Botanga et al. 2002). However, AFLP marker-based genetic diversity studies with Kenyan *Striga asiatica* populations did not show evidence of a subpopulation structure (Gethi et al. 2005). This disparity in genetic structure in both cases of *Striga asiatica* is due to the high diversity in the sampled *Striga asiatica* from Benin, which also exhibited a strong correlation between the geographic distance and the genetic distance between the subpopulations (Botanga et al. 2002). The Kenyan *Striga asiatica* collection established by Gethi et al. (2005), on the other hand, manifested low genetic diversity with no relationship between genetic and geographic distance of the sampling locations. Gethi et al. (2005) attributed the non-robustness of their markers to cover genomic regions containing virulence or pathogenicity genes as possible explanation for the low genetic diversity. This contradictory knowledge substantiates that the genetic diversity in *Striga* spp.

is key to understand important phenotypic attributes associated with possible ecotypes of *Striga* spp., such as the contrasting response of *Striga hermonthica* to different *Fos* isolates (e.g., Foxy-2, FK3) in different geographic locations (Avedi et al. 2014; Kangethe et al. 2016).

2.3.2 Life cycle of Striga spp.

The life cycle of *Striga* spp. is harmonized with that of the host, starting from germination to maturity (Joel et al. 1995). At early developmental stages, however, *Striga* spp. does not require the presence of a host. Accordingly, Joel et al. (1995) divided the life cycle of *Striga* spp. into two phases: the independent, nonparasitic phase and the parasitic, host-dependent phase.

2.3.2.1 Independent, nonparasitic phase

The independent, nonparasitic phase is initialized when a *Striga* spp. seed germinates, culminates, and its radicle (modified as haustorium) attaches to the roots of its host. The host crop triggers *Striga* spp. germination and attachment through exudation of organic stimulants, including sesquiterpene lactones (SLs) (= strigolactones) (Cook et al. 1996; Akiyama et al. 2005; Matusova et al. 2005; Bouwmeester et al. 2007). Sesquiterpene lactones (SLs) are derived from carotenoids, found in the rhizosphere and secreted by plant roots in very small amounts (Awad et al. 2006; Alder et al. 2012). Many of these chemicals have been isolated and identified as a variety of SL-type compounds. They include strigol (Parker and Riches 1993; Yoneyama et al. 2004; Sato et al. 2005), sorgolactone (Parker and Riches 1993; Hauck et al. 1992), sorgomol (Jamil et al. 2013), as well as alectrol and orobanchol (Yokota et al. 1998). SLs facilitate the establishment of symbiotic and parasitic interactions (Soto et al. 2010; Zwanenburg and Pospíšil 2013). It is worthwhile noting that a considerable number of uncharacterized SLs exists, as was reported for sorghum and tomato (Yoneyama et al. 2004). By evolution, *Striga* spp. use strigolactones as a sensor to trigger the germination of its seeds, followed by attack of the host (Conn et al. 2015; Xiong et al. 2016). However, the mechanism by which the SLs induce *Striga* spp. seed germination is still a subject of scientific debate. Xie et al. (2010) proposed that SLs specificity and biological activities are influenced by their interaction with other molecules in the rhizosphere. The number of SLs secreted by plants of the same species and variety depends very much on the nutritional status of the host, and is inversely correlated to soil fertility. Ayongwa et al. (2006), Yoneyama et al. (2007), and Jamil et al. (2012) demonstrated that host plants growing in less fertile soils with deficiency in mineral nutrients (e.g., nitrogen and phosphorus) promoted the secretion of SLs compared to well-nourished plants. SLs secretion attracts AMF to plants roots (Yoder and Scholes 2010), by which a symbiotic relationship is established (Akiyama and Hayashi 2006; Smith and Read 2008), AMF spore germination is stimulated (Gomez-Roldan et al. 2008; Kountche et al.

2018), and hyphal branching in AMF through mitochondrial and mitotic activation induced (Yoneyama et al. 2008; Czaja et al. 2012; Zwanenburg and Pospíšil 2013). As a response, AMF secrete N-acetylglucosamine and lipochitin-oligosaccharides (Maillet et al. 2011; Bücking et al. 2012; Gutjahr and Parniske 2013), which activate a signaling pathway in the roots of the host. A symbiotic nutrient exchange follows after a successful communication network has been established (Besserer et al. 2006).

2.3.2.2 Parasitic, host-dependent phase

Striga spp. must attach to a suitable host within a short time of 5–7 days. Otherwise, *Striga* spp. radicles exhaust their resource reserves stored in the seeds and die (Chang and Lynn 1987; Press et al. 1987a). This resource demand forces them to shift from the independent to the host-dependent, hence, parasitic phase. After attachment, host-derived secondary metabolites like flavonoids and quinones stimulate the formation of the haustorium, which is the physiological connection between the parasite and vascular vessels of the host plants (Riopel and Timko 1995). Some of these compounds are phenolic in nature (e.g., 2,6-dimethyl-p-benzoquinone (DMBQ) (Kim et al. 1998b), and are released from host cell walls after stimulation by the *Striga* spp. radicle. Using a semagenetic strategy, *Striga* spp. provokes the host to synthesize signals necessary for its own development (Keyes et al. 2007). DMBQ initiates the transition from vegetative growth to haustorial development (Lynn and Chang 1990; Smith et al. 1990). Induction consists of rapid cell cycle arrest, redirection of cellular expansion from longitudinal to radial dimensions, and, ultimately, the formation of haustorial hairs on the periphery of the swelling root tip (Smith et al. 1995). Up-regulation of genes encoding for expansins (i.e., saExp1, saExp2, and SaExp3) in *Striga* spp. are responsible for the haustorium formation (O'Malley and Lynn 2000). Expansins enable cell expansion of root cells in *Striga* spp. haustoria by cleaving the hydrogen bonds within cells (McQueen-Mason and Cosgrove 1994; Losner-Goshen et al. 1998).

After successful connection of *Striga* spp. to the host, a parasitic relationship is established, where the parasite becomes a sink for metabolites and water from the host (Joel et al. 2007). The retrieval of resources is maintained by two distinct modes. The first strategy is its unique ability to maintain a high stomatal conductance (open stomata) at all times (Press 1989; Jiang et al. 2003). Press et al. (1987) and Seel et al. (1992) reported that the stomatal conductance of a parasitic angiosperm is generally higher than that of its host. This circumstance reflects the phenomenon leading to a reduced stomatal conductance in *Striga* spp.-infested plants. It results in stomatal closure of the host, allowing the diversion of more water and nutrients in host plants, as was shown for sorghum (Press et al. 1987a) and maize (Taylor et al. 1996). *Striga* spp. lacks coupling of stomatal conductance to environmental conditions, a water-

conserving strategy often employed by plants for growth during dry seasons (Press et al. 1987a). The second strategy of *Striga* spp. is the accumulation of high amounts of osmotically active compounds comprising of mineral ions (e.g., potassium), sugars, and alcohols like mannitol (Smith and Stewart 1990; Westwood 2013; Irving and Cameron 2009). In this case, open stomata elevate transpiration of the parasite that creates a high demand for water from the host. This is achieved by tapping the xylem or apoplast of the host. Osmotically active substances create a high negative water potential enabling the flow of solutes from the host to the parasite (Fer et al. 1993). The impact of water loss on host plants is accelerated if the host is growing under water stress. Shah et al. (1987) found stomata of *Striga hermonthica* and *Striga asiatica* to remain open until the relative water content of the parasite leaves is reduced to about 70%. These processes are particularly important for *Striga* spp. during its emergence phase, when its seedlings depend totally on their hosts for carbon, because of their inability to access light below ground (Graves et al. 1990). For maize, Aflakpui et al. (2005) found that *Striga* spp. derived all its carbon requirements and about 60% of nitrogen from the host crop prior to emergence. Other studies demonstrated that host-derived carbon accounts for up to 65% of *Striga* spp. in leaves of mature *Striga hermonthica* parasitizing sorghum (Graves et al. 1989; Cechin and Press 1993; Pageau et al. 1998). The amount of withdrawn carbon decreases drastically when the parasite matures and becomes photosynthetically active.

It has been estimated that 20–80% of the parasite biomass is built at the expense of the hosts (Graves et al. 1990; Tennakoon and Pate 1996; Těšitel et al. 2010). The resource withdrawal has a direct impact on crop performance and yield. Sorghum shoot yield reduction has been found to vary between 77% and 86%, depending on the infesting *Striga* spp. species (Press et al. 1987a), while carbon withdrawal ranged between 28% and 35% (Press et al. 1987b). This indicates that host yield reduction cannot only be explained by the carbon withdrawn by the parasite. This argument is corroborated by the fact that host biomass is not proportional to that of the parasite and amount of resources deprived. *Striga hermonthica* and *Striga asiatica* parasitizing grasses have been found to elicit a higher influence on their hosts causing a shoot-to-root ratio of 18% compared to *Striga gesnerioides* and *Orobanche* spp., having a much smaller influence on their broad-leaved hosts with a shoot-to-root ratio between 63% and 90% (Graves 1995; Press 1995). Graves et al. (1989) reported that competition between sorghum and *Striga hermonthica* for organic solutes may account for 20% reduction in host biomass. This loss of crop biomass is thus a clear indicator that not all resources derived from the host are consumed by the parasite (Parker et al. 1984). It could be deduced that the parasite has by far a more detrimental effect on their hosts, in addition to simple draining of resources (Press et al. 1996).

Besides such source-and-sink relationships, the loss of host crop biomass inflicted by *Striga* spp. infestation is also driven by disruption of photosynthesis and metabolism, hormonal imbalances, and toxins, where nitrogen levels have been found to be twice as high in *Striga* spp. than in its host, thus affecting host physiology including lower rates of photosynthesis (Spallek et al. 2013). In this case, Rodenburg et al. (2008) showed reduced electron transport rates through photosystem II and photochemical quenching. Deterred photosynthesis is directly related to reduced stomatal conductance, a result of elevated levels of abscisic acid (ABA) in the cell sap (Drennan and El-Hiweris 1979; Gurney et al. 1995; Frost et al. 1997). High levels of ABA inhibit leaf expansion and shoot growth and promote more resource allocation to the roots at the expense of shoots of crops (Trewavas and Jones 1991). Disruption of the hormone stability of the host has been proposed as another cause of *Striga* spp. damage to hosts, especially cereals (Drennan and El-Hiweris 1979). The disruption is generally set very early during the infestation process with decreased levels of auxin, cytokinin, and gibberellin production, while that of ABA is increased (Drennan and El-Hiweris 1979; Press et al. 1999). Conversely, host plants might be affected by toxins produced by *Striga* spp. Host damage (i.e., stunting, chlorosis, wilting, etc.) by *Striga* spp. has been observed before the emergence of the *Striga* plants. Injecting uncharacterized crude *Striga* spp. extracts on young maize seedlings, Efron et al. (1986) noted necrotic lesions at a distance from the site of injection. Additionally, injection of crude extracts from leaves and stems of *Striga* spp. was found to induce loss of chlorophyll and wilting in susceptible sorghum (Ejeta and Butler 1993). Although these experiments provide evidence that *Striga* spp. produce injurious toxins, these toxic chemicals remain to be characterized.

Although *Striga* spp. can fix carbon ((Press et al. 1987a; Press 1989; Cameron et al. 2008), its high respiratory rates enable the depletion of more carbon than it actually synthesizes. This fact reduces the net carbon gain, creating a huge demand for the resource from their hosts. Gurney et al. (1995) determined lower photosynthetic rates in leaves of Striga spp. infested sorghum and maize plants, resulting in smaller leaf sizes of the hosts (Press and Stewart 1987; Press et al. 1998; Press 1989). A study conducted by Inoue et al. (2013) indicated a higher transpiration rate of Striga hermonthica than the host crop sorghum, even when water stress was achieved through higher stomatal density. The study concluded that severe damage to the host under drought may have been caused by increased stomatal opening, leading to enhanced water and nutrient withdrawals from the crop by the parasite. It has been estimated that more than 80% of sorghum growth reduction is due to the effect of *Striga* spp. on host photosynthesis (Graves et al. 1989) and reduced leaf expansion (Gurney et al. 1995; Aflakpui et al. 1998b; 2002). Watling and Press (2001) have classified these as effects having direct influence on photosynthetic metabolism and indirect influence on host architecture by

enhancing or reducing whole-plant carbon gain, reducing light capture, or altering the balance between photosynthetic and non-photosynthetic tissues. These deleterious effects of *Striga* spp. on host photosynthesis can be categorized in terms of direct resources abstraction (source-sink interactions) and indirect non-source-sink interactions (Watling and Press 2001; Cameron et al. 2005).

2.3.3 Host defence response and mechanisms

Host resistance is regarded as key to combating *Striga* spp. attack. This may occur at all development stages of the host (Estabrook and Yoder 1998; Cameron et al. 2006; Rodenburg et al. 2010). However, utilization of host resistance is limited due to a lack of knowledge of the underlying genetic and phenotypic basis of the adaptation of *Striga* populations to new host resistance phenotypes (Scholes and Press 2008). Advanced knowledge would enable an anticipation of *Striga* spp. responses to selection imposed by resistant host crops, allowing host resistance phenotypes to be combined and integrated optimally in agroecosystems (Huang et al. 2012). According to Joel et al. (2007), the respective resistance mechanisms of the host include alteration of the host sap chemical composition to limit the water and assimilate flow or uptake by the parasite, hormonal imbalance, or toxicity to the parasite. Pérez-de-Luque et al. (2008) classified host resistance responses into three mechanisms: pre-attachment, pre-haustorial and post-haustorial mechanisms.

2.3.3.1 Pre-attachment mechanisms

Pre-attachment resistance of the host appears during parasite germination and differentiation of the radicle into the haustorium, which takes place before the haustorium attaches to the host. The resistance mechanisms include absent or reduced production of germination stimulants (e.g., strigolactones) through carotenoid-pathway inhibitors in a resistant crop variety (Reda et al. 1994; Jamil et al. 2011). This mechanism has been adopted for resistance breeding of sorghum against *Striga* spp. (Haussmann et al. 2000; Ejeta 2007b; Mohamed et al. 2010b). Similar results were observed in several wild varieties of sorghum (Hess et al. 1992; Rich et al. 2004). Low production of germination stimulants or haustorium-inducing factors, such as DMBQ released by sorghum, revealed a high potential to inhibit haustorium growth (Chang and Lynn 1986; Yoder and Scholes 2010). *Striga* spp. germination and attachment may take place, but seedlings may fail to form a haustorium. Therefore, seeds of *Striga* spp. can exhaust their resource reserves at early stages and die shortly afterward (Reda et al. 1994). This low haustorial initiation may be attributed to the synthesis of inhibitors (e.g., phenolic acids) produced by the host (Rich et al. 2004) or low quantities of haustorium-inducing substances (e.g., strigolactones) (Gurney et al. 2003; Haussmann et al. 2004; Yoder and Scholes 2010). Some resistant sorghum cultivars have demonstrated a hypersensitive

reaction (HR) characterized by necrosis at the infestation (Mohamed et al. 2010a). Agrios (1998) described HR with necrotic zones around the site of attempted infestation, which discourage further penetration into host roots. This leads to unsuccessful establishment and ultimate demise of the parasite (Ejeta 2007b; Haussmann et al. 2000).

Moreover, plants reduce the level of infestation by minimizing their contact to parasitic seeds (van Ast et al. 2005). This is enabled by reduced root formation or deeper root systems since most parasitic seeds, including those of *Striga* spp., are found predominantly in the top layers of the soil (Pérez-de-Luque et al. 2005; Rubiales et al. 2006). It has been revealed that reduced root growth may not necessarily lead to increased resistance against parasites (Olivier and Leroux 1992). Low production of germination stimulants could explain partly the resistance as demonstrated by resistant varieties, although other so far unknown compounds may have been responsible for the inhibition of *Striga* spp. germination (Weerasuriya et al. 1993). Mohamed et al. (2010a) showed successful inhibition of *Striga* spp. germination in sorghum, although the causal agent for inhibition was not determined. Some germination inhibitors of *Orobanche* spp. operating in the rhizosphere of non-cereal crops level include trigoxazonane (Evidente et al. 2007), Trichothecenes (Evidente et al. 2005), 7-hydroxylated coumarins as well as naringenin and gallic acid (Serghini et al. 2001; Mabrouk et al. 2007). However, inhibitors operating in the *Striga*-cereal crop rhizosphere are yet to be discovered.

2.3.3.2 Pre-haustorial mechanisms

Host plants can cease the vascular connection with the parasite through physical or chemical prevention of parasite penetration (Mohamed et al. 2003; Echevarría-Zomeño et al. 2006). One of these strategies is manifested through the death of haustorial cells before connecting to the host vascular tissues (Arnaud et al. 1998). Reduced nutrient flow to the haustorium or accumulation of phytotoxic compounds (e.g., phenolics and phytoalexins) and physical barriers to parasite penetration at the sites of infestation were found to inhibit haustorial development (Arnaud et al. 1999; Mohamed et al. 2010a; Timko and Scholes 2013). Inhibition of haustorium induction was caused by the presence of auxin (Keyes et al. 2000). In addition, structural changes on host cells suppress the level of infestation. These include cell wall thickening with structural carbohydrates of xylem vessels (Labrousse et al. 2001), protein cross-linking and suberization of cell walls, as was shown in *Orobanche* spp. (Mohamed et al. 2003; Pérez-de-Luque et al. 2008; Elzein et al. 2010). Moreover, several *Orobanche* spp. hosts, such as tomato, produce high amounts of phenolic compounds to boost resistance and to minimize the incidence of infestation (Goldwasser et al. 1999; Pérez-de-Luque et al. 2005; Al-Wakeel et al. 2013). It needs to be emphasized that none of these chemical and structural

responses have been documented so far in *Striga* spp.-infested cereals, offering an important field of fundamental research.

2.3.3.3 Post-haustorial mechanisms

Plants employ post-haustorial mechanisms after the parasite has established the vascular connection with the host. Death of parasite tubercles is the main indicator of these strategies. This happens either at the root cortex or endodermis, where further parasite development is hindered. Interruption of water and nutrient flow to the parasite happens when the resistant host vascular tissues are blocked by mucilage, causing tubercle death in *Orobanche* spp. (Labrousse et al. 2001; Pérez-de-Luque et al. 2005; 2006). This strategy is similar to that of pathogens causing vascular wilts in plants. However, Pérez-de-Luque et al. (2009) proposed the need for further investigation to clarify these hypothesized relationships between genes responsible for wilt resistance to those offering resistance to plant parasites. Little is known about the mechanisms by which monocots respond to *Striga* spp. attacks (Mutuku et al. 2015). It was suggested that host crops show reduced production of unidentified stimulants different from sorgoleone (Hess et al. 1992), activation of salicyclic acid and jasmonic acid, as well as electron transport signaling molecules (Tsuda et al. 2009). Endodermal thickening, pericycle lignification, and silica crystal deposition have been observed in post-attachment resistance to *Striga hermonthica* in sorghum cultivars (El Hiweris et al. 1987). Moreover, Neumann et al. (1999) revealed cell wall modifications such as collapsed and necrotic host cells appearing at the lateral site of the invading *Striga* spp. haustorium.

2.4 Bipartite interaction between cereals and plant growth-promoting rhizobacteria

In close association with plants, diverse bacterial and fungal genera provide a vital component of crop health and productivity. The narrow zone of soil surrounding closely the root system of a plant is referred to as the “rhizosphere,” a hot-spot of microbial activity. There, the composition of the microbiome and its functional potential are strongly regulated by the constant release of plant-derived rhizodeposits. These include root exudates (e.g., amino acids, organic acids, carbohydrates, and sugars) and root debris (Bardgett et al. 1998; Hinsinger et al. 2009; Jones et al. 2009). The plant itself manipulates the rhizosphere according to its physiological requirements. Such cell-to-cell communication via quorum sensing regulates the root colonization by microorganisms (Schenk et al. 2012). Quorum sensing comprises intercellular signaling mechanisms that coordinate microbial behavior (i.e., density, activity, etc.) during host colonization (Schenk et al. 2012; Bulgarelli et al. 2013).

Plant-associated microorganisms, on the other hand, employ the quorum-sensing mechanism to modulate and coordinate their individual interaction with plants. There has been a widespread recognition of many bacteria living in the root environment being capable of promoting plant health and growth (Bardgett et al. 1998). These root-associated bacteria are defined as plant growth-promoting rhizobacteria (PGPR). Well-studied examples refer to diverse bacterial genera, including *Azospirillum* sp., *Gluconacetobacter* sp., *Pseudomonas* sp., and *Rhizobium* sp., as well as some gram-positive genera, including *Bacillus* sp. and *Paenibacillus* sp. (Arkhipova et al. 2005).

PGPR directly control plant growth through auxins, gibberellins, indole-3-acetic acid (IAA), and cytokinin phytohormone synthesis (Arkhipova et al. 2005; Hassan et al. 2009a; Patel and Saraf 2017). Specifically, IAA is important for shoot and root development as well as plant vigour (Oberson et al. 2013; Vacheron et al. 2013). PGPR increase soil mineral bioavailability through diazotrophic (nonsymbiotic) atmospheric N_2 fixation (Stacey et al. 1992; Danhorn and Fuqua 2007; Pupathy and Radziah 2015) and phytate degradation to solubilize phosphate (Halder et al. 1990; Rodríguez and Fraga 1999). An indirect effect of PGPR in promoting plant growth is the synthesis of ACC (1-aminocyclopropane-1-carboxylic acid) deaminase (EC 4.1.99.4), an enzyme that degrades ACC into α-Ketobutyric acid and ammonia (Arshad et al. 2007; Saleem et al. 2007). ACC is the precursor of the plant stress hormone ethylene (Yang and Hoffman 1984; Kende 1993; Glick 2014), which is produced especially under abiotic stress conditions (Gamalero and Glick 2012; Shrivastava and Kumar 2015). The harmful effect of ethylene in plants is reflected in chlorosis, wilting, leaf senescence, and abscission (Burg 1968; Edelman 2013). A significant amount of plant ACC might be released *in planta* or from the plant roots and subsequently taken up by plant-associated micro-organisms. Bal et al. (2013), for example, demonstrated the effectiveness of plant-associated bacteria exhibiting ACC deaminase activity, such as *Alcaligenes* sp., *Bacillus* sp., and *Ochrobactrum* sp., in inducing salt stress tolerance in cereal crops, including but not limited to rice. Comparable findings were provided by Ali et al. (2014), who showed a high level of salt stress tolerance of tomato plants after treatment with ACC deaminase-active *Pseudomonas fluorescens*, while Zahir et al. (2008) proved *Pseudomonas* sp. to partially mitigate the effect of drought stress on legume growth via enhanced ACC deaminase activity.

Other indirect mechanisms of PGPR in supporting cereal growth involve the secretion of siderophores to sequester and solubilize iron (Kloepper et al. 1980; Masalha et al. 2000), including the synthesis of polyamines (e.g., spermidine), which are important for regulating the transport/exchange of bioactive ions essential for cell survival (e.g., Ca^{2+}, Na^{+}, and K^{+}) needed for maintaining membrane potential and controlling intracellular pH, and modulating ATPase that metabolizes ATP into ADP (Pottosin et al. 2014). It should be noted that

spermidine is also the precursor of spermine and thermospermine, which both contribute to tolerance against drought and salinity in plants (Singh and Jha 2017; Compant et al. 2005; Xie et al. 2014). Furthermore, another striking indirect effect of PGPR is the support of plant growth via production of extracellular hydrolytic enzymes (e.g., chitinase, glucanase, proteinase, and cellulose) that can degrade the cell wall of phytopathogens (Kim et al. 2003; Bal et al. 2013; Goswami et al. 2016). Similarly, the biological control potential of PGPR to fight off crop pests and diseases was approved by PGPR-derived antibiotics and antifungal metabolites acting as biopesticides and bioherbicides (Pereg and McMillan 2015; Goswami et al. 2016). Among cereal crops, bacteria with effective disease control properties were identified in the rhizospheres of sorghum and maize (Kundan et al. 2015).

2.5 Bipartite interaction between *Striga* spp. and PGPR

The interaction between PGPR and *Striga* spp. occurs at any stage of its life cycle, ranging from germination and haustorium establishment to maturity. Identification of bacteria inducing parasitic weed germination or inducing suicidal effects in the absence of the host has gained a huge research interest. In this respect, two major scenarios are expected when *Striga* spp. seeds interact with PGPR: their germination could be (1) enhanced or (2) inhibited.

2.5.1 Enhanced germination

During *Striga* spp. germination, strigolactones act as elicitors of ethylene biosynthesis, leading to subsequent seed germination (Logan and Stewart 1991). Ethylene produced by *Pseudomonas syringae* and *Klebsiella* sp. was found to stimulate *Striga* spp. germination (Berner et al. 1999; Frankenberger and Muhammad 1995; Ahonsi et al. 2002a), where the former bacterium was also shown to induce suicidal germination of *Striga* spp. seeds. Gibberellic acid primes *Striga* spp. seeds prior to germination (Hsiao et al. 1988) and subsequent germination (Joel et al. 1991). Studies have demonstrated that *Striga* spp. parasitism can be prevented through application of gibberellin synthesis inhibitors into the soil (Joel 2000). Evidence was further given by Zehhar et al. (2002) who reported that treatment of *Orobanche ramosa* preconditioned seeds with inhibitors of gibberellins or ethylene biosynthesis resulted in inhibition of seed germination in the presence of the germination stimulant GR24. Further research will be needed to explore additional molecules with comparable antagonism toward *Striga* germination.

2.5.2 Inhibited germination

PGPR can inhibit *Striga* spp. seed germination and attachment to the host. Hassan et al. (2009a) has noted that low levels of germination, haustorium initiation, and attachment are

important factors that may lead to reduced or delayed emergence of *Striga* spp., when exposed to PGPR. Various modes of action have been suggested. These include interruption of germination, radicle growth, and haustorium-inducing signals, together with disorientation of the radicle from the host root or reduced attachment (Dadon et al. 2004). The key stages in the life cycle of *Striga* spp. are controlled by hormones and, therefore, several PGPR were shown to affect early stages of parasitic growth Hassan et al. (2009a). There is scarce information on the causes of bacteria-induced germination inhibition in *Striga* spp. Phytohormones and lipophilic compounds released by *Azospirillum brasilense* are known to cause reductions in germination, radicle growth, and cell differentiation (Ahmed et al. 2001). Phytotoxic substances (characterized as a complex of peptides, fatty acid esters, and lipopolysaccharides) are other biochemicals produced by PGPR that inhibit seed germination and radicle elongation of weeds (Ahonsi et al. 2003). Inhibition of *Striga* app. seed germination and radicle elongation have been also attributed to breakdown and chemical alteration of the germination stimulant GR24, including the production of strigolactone-like hormones that inhibit radicle elongation, or produce GR24 inhibitors (Dadon et al. 2004). Enhanced control of *Striga* spp. can be expected if appropriate identification of the factors that are associated with bacterial inhibition of radicle elongation will be conducted. This will provide the necessary baseline to facilitate the selection of PGPR isolates with potential to control the *Striga* spp.

Some PGPR have been reported to produce yet-to-be identified substances that inhibit germination or suppress radicle development in parasitic weeds, including broomrape (*Orobanche aegyptiaca* and *Orobanche cernua*). Examples are *Pseudomonas aeruginosa* QUBC1, *Pseudomonas fluorescens* QUBC3, *Bacillus atrophaeus* QUBC16, and *Bacillus subtilis* QUBC18 (Dadon et al. 2004). Radicle growth inhibition in broomrape was also shown by *Azospirillum brasilense* (Barghouthi and Salman 2010), while ethylene-mediated suicidal germination in *Striga* spp. was verified by ethylene-producing *Pseudomonas syringae* pv. *glycinea* (Fulchieri et al. 1993; Ahonsi et al. 2002a). In addition, germination suppression in *Striga hermonthica* was approved for sorghum root inoculated with *Bacillus subtilis* GB03, *Bacillus amyloliquefaciens* FZB42, and *Burkholderia phytofirmans* PsJN (Mounde et al. 2015). Other mechanisms include growth hormones detrimental to *Striga* spp. development. For instance, *Azospirillum* sp. caused hormonal imbalance in the parasitic weed, leading to poor radicle development. Some of these hormones include IAA (Levanony and Bashan 1989; Fulchieri et al. 1993). Likewise, PGPR-derived auxin was shown to cause strong inhibition to attachment and haustorium development. This was related to their antagonistic nature with cytokinins and benzoquinone, both of which favour the two processes (Keyes et al. 2000). It could be speculated that PGPR such as *Azospirillum brasilense*, *Azotobacter* sp., *Pseudomonas putida*, and *Klebsiella* sp., which are known as auxin producers (Frankenberger

and Muhammad 1995), may be used as inoculants to efficiently inhibit the attachment process of *Striga* spp.

2.6 Tripartite interaction between cereals, *Striga* spp., and root-associated microorganisms

Though exclusively bipartite interactions between the three biological components have been discussed with regard to the control of *Striga* spp., less attention has been given to the tripartite interaction between cereals (e.g., sorghum and maize), *Striga* spp., and root-associated microorganisms, including PGPR and AMF. This extended ecological understanding will provide relevant avenues to develop applicable control measures for *Striga* spp. One of the best examples studied so far is the close interaction of AMF with a cereal host crop and *Striga* spp. Studies by Gworgwor and Weber (1992) as well as Lendzemo (2004) indicated reduced *Striga* spp. germination and emergence in consequence of AMF treatment on sorghum cultivated. The low *Striga* spp. germination resulted from reduced strigolactone production as well as structural and chemical changes on AMF-colonized plants roots. Lendzemo (2004) suggested that AMF inoculation on sorghum roots might have interfered with the exudation patterns of strigolactones. In turn, this led to poor stimulation of *Striga* spp. seed germination (see section “Independent, nonparasitic phase”) (Yoder and Scholes 2010).

It has been acknowledged that sufficient crop nutrition (specifically phosphorus) reduces strigolactone exudation, resulting in minimal *Striga* spp. germination and parasitism. Moreover, AMF generally improve phosphorus uptake by crop with positive feedback on growth and health, compensating partially the effects of *Striga* spp. attack (Chimmalagi et al. 2018). Likewise, AMF mitigate hormones secreted by *Striga* spp., which are detrimental to the crop, including auxins (Joel et al. 1995) and ABA (Drennan and El-Hiweris 1979; Frost et al. 1997). Root parasitic weeds have likely evolved a mechanism to hijack this communication signal and turn it into a germination-inducing signal to respond in the presence of a suitable host. Chimmalagi et al. (2018) reported improved chlorophyll content in AMF-inoculated plants. The physiological parameters such as photosynthetic rate and stomatal conductivity of sugarcane have been enhanced and *Striga* spp. biomass reduced following AMF inoculation Walter et al. (2010). This was attributed to the conversion of strigolactones to mycorradicin in AMF-colonized host plant. Mycorradicin does not support *Striga* spp. germination and emergence Walter et al. (2010). Ahonsi et al. (2002b) indicated the ability of *Pseudomonas fluorescens* and *Pseudomonas putida* in reducing *Striga* spp. emergence in the presence of maize hosts. This was corroborated by Ahonsi et al. (2003) who observed in cowpea and soybean rhizospheres that certain *Pseudomonas* sp. minimize *Striga* spp. infestation, either

individually or in combination with N_2-fixing *Bradyrhizobium japonicum*. Miché et al. (2003) revealed the inhibition of *Striga* spp. germination after *Azospirillum brasilense* treatment, while the growth of the host crop (i.e., sorghum) was promoted. Similar findings were given by Babalola (2011) confirming beneficial effects of selected PGPR (e.g., *Bacillus* sp. and *Pseudomonas putida*) against *Striga* spp., while promoting sorghum growth. Suppressed *Striga* spp. emergence and haustorium development were attributed to PGPR-derived auxin and auxin-like compounds (Frankenberger and Muhammad 1995; Keyes et al. 2000). Crops infested by *Striga* spp. generally, show low IAA levels (Press et al. 1999). To counteract such low IAA levels, Hassan et al. (2009a) showed for both resistant and susceptible sorghum varieties that inoculation of PGPR (e.g., *Pseudomonas putida*, *Azospirillum brasilense*, *Azospirillum amazonas*, and *Bradyrhizobium japonicum*) delayed and reduced *Striga* spp. incidence. Mounde et al. (2015) identified *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* FZB42 to promote sorghum growth, while inhibiting *Striga hermonthica* germination. Tubercle formation was significantly suppressed in comparison to non-inoculated controls, confirming the bioherbicidal potential of the two PGPR. It has been proposed that seed germination inhibition was initialized by certain unidentified metabolites, which may compete for binding sites with the germination stimulants (e.g., strigolactone) (Dadon et al. 2004). Strigolactone is produced by the host primarily for regulating its above-ground architecture (shoot branching) (Gomez-Roldan et al. 2008) and to stimulate the branching and root attachment of AMF (Besserer et al. 2006).

In strigolactone-dependent plants, the receptive site DWARF14 hydrolyses the strigolactone molecule. Strigolactone induces the interaction between DWARF14 and a repressor DELLA protein (i.e., DWARF53). This repressor protein is degraded in a strigolactone/DWARF14/DWARF53/Skp1-Cullin-F-box protein (SCF) complex through the 26S proteasome, ultimately resulting in the germination of *Striga* spp. (Nakamura et al. 2013; Marzec 2017). The disruption of DWARF14 protein by mutation or other means (e.g., PGPR-derived inhibitors) results in the inability of the receptor to transduce the strigolactone signal. Yoshimura et al. (2018) identified a novel compound DL1, which is a potent inhibitor of the strigolactone-receptive site DWARF14. Knowledge of the identity of peculiar metabolic compounds produced by PGPR, which can effectively block DWARF14, will improve the efficiency in controlling *Striga* spp. by the suggested PGPR metabolic bioherbicidal approach.

2.7 Concluding remarks and future direction

This review has presented the current knowledge on the multiple interactions that occur in the rhizosphere of cereal crops (e.g., sorghum and maize) and its close associations with *Striga*

spp. and root-associated microorganisms (e.g., bacteria and fungi). From an ecological viewpoint, the discussion about the reviewed tripartite interactions has to be extended to quadripartite interactions, since AMF as critical biological agents in the plant rhizosphere play, besides PGPR, a unique role in suppressing the emergence of *Striga* spp. and in promoting the growth of affected crops (Lendzemo 2004; López-Ráez et al. 2011).The full understanding of these complex interactions remains incomplete due to the difficulty of studying below-ground processes under controlled yet realistic conditions. For instance, while the structural chemistry of strigolactones is known, the mechanisms underlying its low production under enhanced host mineral nutrition, particularly nitrogen and phosphorus, remains elusive. In this regard, this review stressed the relationship between carbon and nitrogen accumulation by *Striga* spp. at different growth stages. The amount of carbon withdrawn depends heavily on the concentration of nitrogen in the soil. No explanation is available yet on the inverse relationship between nitrogen amount in soils and carbon withdrawal from the host by *Striga* spp. Certainly, a scientific explanation through research is needed.

It was further proposed that low haustorial initiation is a result of the host that produces inhibitors (Rich et al. 2004) or low amounts of haustorium-inducing factors (Gurney et al. 2003; Haussmann et al. 2004; Yoder and Scholes 2010). In addition, the reduced translocation of organic resources from the host to the parasite was discussed, although the factors causing the reduced translocation remain elusive. Further research is needed to better understand the cause-effect of reduced translocation of assimilates in *Striga* spp.-infested plants. In this respect, phytotoxic substances are increasingly recognized to be responsible for low parasitic seed germination and radicle elongation. These substances must be characterized and their inhibitory mechanisms on germination, radicle elongation, and haustorial initiation further understood. Plants or microbiota can alter their behaviour in response to other organisms at different omics-levels. Advanced approaches such as genomics, transcriptomics, proteomics, metabolomics, and epigenomics will reveal more information about the ecology of rhizospheres and the principal compounds, genes, functions, and mechanisms that inform the discussed tri- and quadripartite interaction and demonstrate their usefulness under diverse environments (De-la-Peña and Loyola-Vargas 2014; Radhakrishnan et al. 2017). Thus, native AMF species and PGPR associated with plant roots could form an efficient *Striga* spp. control system to be integrated with other *Striga* spp. management strategies. This integrated research strategy and knowledge enhancement will contribute greatly to the development of biological and environmentally friendly measures to fight off the everlasting threat of *Striga* spp.

Chapter 3. Population genetic structure and marker-trait associations in East and West African *Striga hermonthica* with varying phenotypic response to *Fusarium oxysporum* f. sp. *strigae* isolates Foxy-2 and FK3

Williams O. Anteyi[1] and Frank Rasche[1]

[1]Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), University of Hohenheim, 70593 Stuttgart, Germany.

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3.1 Summary

To examine the genetic basis for the variable susceptibility of *Striga hermonthica* from differing zones of sub-Saharan Africa to *Fusarium oxysporum* f. sp. *strigae* (*Fos*) isolates Foxy-2 and FK3, 10 *S. hermonthica* populations from Eastern and Western Africa were phenotyped for their susceptibility response to Foxy-2 and FK3, and then genotyped with 22 simple sequence repeat (SSR) markers. There is low genetic differentiation between East African and West African *S. hermonthica* populations (i.e., the proportion of the total genetic variance contained in the subpopulation relative to the total genetic variance, $F_{ST} = 0.012$, $P < 0.05$), but intermediate genetic differentiation ($F_{ST} = 0.143$, $P < 0.01$) underlies the *S. hermonthica* groups that are differentiated by their phenotypic responses to *Fos* isolates. An expressed sequence tag SSR (EST-SSR) marker Y53 ($P < 0.01$) and a genomic SSR marker E1009 ($P < 0.05$) were associated with the *S. hermonthica* class susceptible to Foxy-2 and FK3 (group A). A divergent *S. hermonthica* class, consisting of groups with intermediate susceptibility to Foxy-2 (group B) and susceptibility to either FK3 (group C) or Foxy-2 (group D), showed no marker–trait association, instead demonstrated linkage disequilibrium decay. Owing to point substitutions and insertion–deletion mutations, the unique, protein-coding nucleotide sequence at the E1009 locus in group A was partly dissimilar to group B, but was totally distinct from groups C and D. These findings implied that the inconsistent effectiveness of a *Fos* isolate is better explained by genomic variation in *S. hermonthica*, rather than by *S. hermonthica* sampling zones.

Keywords: *Striga hermonthica*, *Fusarium oxysporum* f. sp. *strigae*, Foxy-2, FK3, population genetic structure, marker–trait association.

3.2 Introduction

The genus *Striga* consists of more than 30 obligate hemiparasitic species. *Striga hermonthica* (Delile) Benth. followed by *Striga asiatica* (L.) Kuntze are the most devastating members of this genus with regards to cereal production in sub-Saharan Africa (SSA), particularly with respect to *Sorghum bicolor* (L.) Moench (sorghum), *Zea mays* L. (maize), *Pennisetum glaucum* (L.) R. Br. (pearl millet) and *Oryza sativa* L. (rice) (Oswald 2005; Parker 2009; Spallek et al. 2013). With the aim of combating *Striga* spp. using natural biological control agents, fungal pathogens belonging to the genus *Fusarium* have been reported as broadly successful mycoherbicides against *Striga* spp., by inhibiting *Striga* germination and emergence (Zummo 1977; Abbasher and Sauerborn 1992; Abbasher et al. 1995). Through molecular characterization of the internal transcribed spacer (ITS) sequence of the ribosomal RNA gene,

Elzein et al. (2008a) designated a forma specialis to *Fusarium oxysporum*, known as *Fusarium oxysporum* f. sp. *strigae* (*Fos*). This taxonomic category encompasses *F. oxysporum* isolates with specific pathogenicity towards *Striga* spp. *Fos* kills *S. hermonthica* by hyphal colonization, followed by the obstruction or digestion of xylem vessels (Ndambi et al. 2011). On the other hand, host (cereal) plants of *Striga* spp. employ various strategies in modifying their cell wall, e.g., endodermal/pericyclic thickening, silica deposition and lignification, as mechanisms to prevent *Striga* haustorial penetration and infection of their root cell wall (Maiti et al. 1984; Mutuku et al. 2019). Various *Fos* isolates with specific and significant pathogenicity to *S. hermonthica* have been identified, e.g., the Ghanaian isolate Foxy-2 (Abbasher et al. 1995), the Malian isolate M12-4A (Ciotola et al. 1995), the Nigerian isolate PSM-197 (Marley et al. 1999) and the Kenyan isolate FK3 (Kangethe et al. 2016). Efficacy assessments from field surveys in Western Africa showed that Foxy-2 had the ability to control *S. hermonthica*, e.g., in Nigeria (Schaub et al. 2006), and also in Benin and Burkina Faso (Venne et al. 2009). In contrast, field surveys in Kenya (East Africa) did not validate the effectiveness of Foxy-2 against *S. hermonthica* (Avedi et al. 2014); rather, FK3 proved to be effective there (Beed et al. 2013; Kangethe et al. 2016). Inconsistency of a biocontrol agent in suppressing its host range is a crucial constraint that discourages its general acceptance and use (Ghosheh 2005; Velivelli et al. 2014). It is unclear whether the inconsistent efficacy of *Fos* isolates against *S. hermonthica* is the result of local abiotic or biotic factors, a mode of action peculiar to a *Fos* isolate or the genetic attributes of *Fos* and *S. hermonthica*. Thus, with regards to *S. hermonthica*, genetic variation in various populations with differing susceptibilities to *Fos* isolates must be disentangled in order to gain insights into the genetic basis for the susceptibility of *S. hermonthica* to *Fos* isolates.

Unlike *S. asiatica*, which is a selfing species (Nickrent and Musselman 1979; Botanga et al. 2002), *S. hermonthica* is an obligate outcrossing species (Musselman and Parker 1983; Safa et al. 1984). Generally, in outcrossing species, population genetic structure demonstrates higher genetic variation within structured populations than between structured populations (Loveless and Hamrick 1984). Population genetic structure owing to host specificity in *S. hermonthica* has not been established. There is scientific consensus regarding geography acting as a major determinant of population genetic structure in *S. hermonthica*, by influencing a higher flow of genetic variation within structured subpopulations than between structured subpopulations. This perspective is shared by the authors of several *S. hermonthica* genetic diversity studies involving various molecular genetic markers, such as enzyme electrophoresis (Bharathalakshmi and Musselman 1990; Olivier et al. 1998), random amplification of polymorphic DNA (RAPD) and isozyme markers (Koyama 2000), amplified fragment length polymorphism (AFLP) (Welsh and Mohamed 2011), simple sequence repeats (SSRs) (Estep

et al. 2011), SSRs together with expressed sequence tags (EST-SSR) (Bozkurt et al. 2015; Joel et al. 2018), and single-nucleotide polymorphisms (SNPs) (Unachukwu et al. 2017). Molecular genetic markers, especially SSRs and SNPs, are robust genomic analysis tools for linking heritable phenotypic traits with the underlying genomic variation (Duran et al. 2009). SSR markers are widely used for genetic investigation owing to their codominant inheritance, high polymorphic nature, high reproducibility, high versatility and great genome abundance (Liu et al. 2013; Nadeem et al. 2018).

Despite the completion of several population-structure studies in *S. hermonthica*, knowledge of associations between genetically structured *S. hermonthica* groups and their phenotypic responses to *Fos* isolates is lacking. Insight into these associations will foster our understanding of the variable susceptibility patterns of *S. hermonthica* to various *Fos* isolates, which will then guide the selection of a particular *Fos* isolate to combat a specific *S. hermonthica* population efficiently. This study hypothesizes that the inconsistent effectiveness of a *Fos* isolate against differing *S. hermonthica* populations is explained by population genetic structure in *S. hermonthica*, rather than by the *S. hermonthica* sampling zone (i.e., East Africa and West Africa). In other words, *S. hermonthica* populations susceptible to Foxy-2 and FK3 are associated with certain molecular marker(s), which will not be the case for *S. hermonthica* populations susceptible to either Foxy-2 or FK3. Hence, the research objectives were to determine: (i) population genetic structure in *S. hermonthica* sampled from Eastern and Western Africa, based on sampling zones and phenotypic responses to *Fos* isolates Foxy-2 and FK3; and (ii) SSR locus/loci associated with the susceptibility trait of *S. hermonthica* to Foxy-2 and FK3.

3.3 Materials and methods

3.3.1 Preparatory work

3.3.1.1 Plant materials and microbial isolates

Sorghum (*Sorghum bicolor* L. Moench) cultivar PI563294 from Niger was used as host (model) crop for the sampled *Striga hermonthica* (Delile) Benth. Seeds of 10 *S. hermonthica* populations were sampled from Eastern and Western Africa (Fig. 3.1). Glycerol (20%) stocks of *Fos* isolates Foxy-2 and FK3 were obtained from a −80°C microbial cell bank located at the Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Stuttgart, Germany.

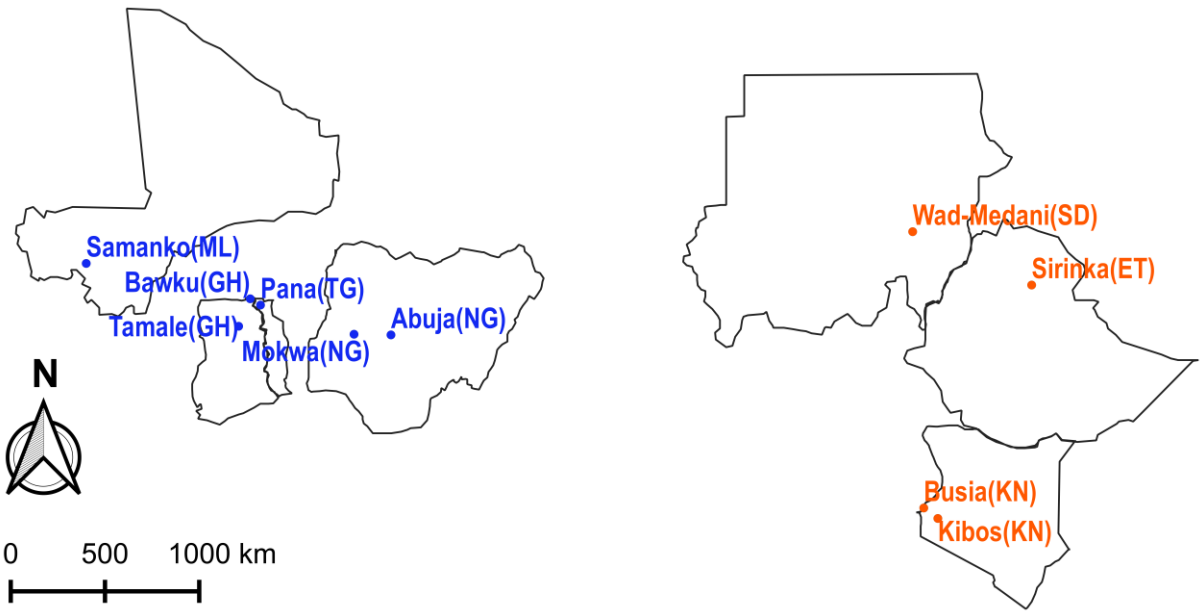

Fig. 3.1: Origins of the 10 *Striga hermonthica* populations in the West and East African sampling zones. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

3.3.1.2 Seed sterilization

Striga hermonthica seeds were surface sterilized by immersion in a blend of 5% (v/v) sodium hypochlorite (containing 12% chlorine) (Carl Roth GmbH, https://www.carlroth.com) and 0.02% (v/v) Tween 20® (Carl Roth GmbH). Then the seeds were cleaned by sonication for 3 min (Bandelin Sonopuls GmbH, https://bandelin.com), followed by rinsing five times with double-distilled water (ddH_2O). The surface sterilization of the sorghum seeds involved soaking the seeds in 70% ethanol for 2 min then rinsing three times with ddH_2O. Then the seeds were immersed in a solution of 1% sodium hypochlorite and 0.02% (v/v) Tween 20® for 3 min, and finally rinsed five times with ddH_2O. Thereafter, the surface-sterilized seeds were separately air dried on glass-fibre filter papers (Macherey-Nagel GmbH, https://www.mn-net.com) under a laminar flow hood for 48 h. The glass-fibre filter papers were initially oven sterilized at 65°C for 48 h followed by exposure to UV light for 2 h.

3.3.1.3 Microbial culturing and inocula concentration (CFU mL^{-1})

Separate mycelia suspensions of Foxy-2 and FK3 inocula were prepared by flooding the surface of 14-day-old actively growing potato dextrose agar (PDA) (Carl Roth GmbH) cultures in 90-mm Petri dishes with 10 ml of 25% potato dextrose broth (PDB) (Carl Roth GmbH). After standing for 30 min, the mycelia masses were gently scraped into sterile flasks containing 2 L of 25% PDB. The flasks were loosely covered with their lids and kept in a dark incubating

shaker for 15 days at 65 rpm with gentle shaking and 28°C for colony growth. Inocula concentration in colony-forming units per mL (CFU ml^{-1}) was determined from six replicates of each fungal isolate. Both Foxy-2 and FK3 had a concentration of 10^4–10^5 CFU ml^{-1}. The prepared fungal inocula served as treatments for subsequent planting trials.

3.3.2 Phenotypic analysis of S. hermonthica populations

3.3.2.1 Planting trials

Rhizoboxes made from polyvinyl chloride (PVC; 20 cm × 5 cm × 2 cm) were filled with 65 g of sterilized modular seed substrate (Klasmann-Deilmann GmbH, https://klasmann-deilmann.com). The front side of each rhizobox was covered with plexiglass. About 65 mg of surface-sterilized *S. hermonthica* seeds was drawn from each seed lot of the 10 sampled populations and sown 5 cm below the substrate surface. This procedure was performed in three rhizoboxes (replications), with the mixing of the population seed lot between successive draws to ensure homogeneity. Then, 65 ml of ddH_2O or the prepared fungal inocula (Foxy-2 or FK3) was added to each rhizobox containing an *S. hermonthica* population. The rhizoboxes were wrapped in a black polythene sheet and incubated at 30°C for 10 days in a dark climate chamber (Percival Intellus Environmental Controller, EA-75HIL; Percival Scientific, https://www.percival-scientific.com), as an essential preconditioning step to facilitate the germination of *S. hermonthica* seeds. Afterwards, a sorghum seed was sown in each rhizobox, and climate chambers were then maintained at 31°C and 27°C for day and night temperatures, respectively. Light was provided at 46 000 lx (mean illuminance) for 12 h for day and deactivated for night. From 2 weeks after sowing the sorghum, irrigation was performed twice weekly with nutrient solution, made up of 0.2% (v/v) Wuxal® universal liquid fertilizer (Aglukon Spezialdünger GmbH, https://www.aglukon.com), blended with 40% Yoshida nutrient solution (Yoshida et al. 1976). The treatments for the climate chamber planting trial included: (i) sorghum without *S. hermonthica* but with ddH_2O (Sorghum − *S. hermonthica* + ddH_2O); (ii) sorghum with *S. hermonthica* and Foxy-2 (Sorghum + *S. hermonthica* + Foxy-2); (iii) sorghum with *S. hermonthica* and FK3 (Sorghum + *S. hermonthica* + FK3); and (iv) sorghum with *S. hermonthica* and ddH_2O (Sorghum + *S. hermonthica* + ddH_2O). Treatments 1 and 4 represented positive and negative controls, respectively. Each of the four treatments were in three replications and the experiment was set up in a randomized complete block design. The growing period was set to 6 weeks. The climate chamber planting trials were repeated twice.

3.3.2.2 Phenotyping (quantitative biostatistics)

The aboveground sorghum biomass was harvested and oven-dried at 65°C for 10 days, then the dry mass was measured. To determine the susceptibility or non-susceptibility responses of the *S. hermonthica* populations to Foxy-2 and FK3, ANOVA and *post-hoc* analysis by

Tukey's range test were performed using sas 9.4 (SAS Institute, 2013, https://www.sas.com). *Striga hermonthica* susceptibility to a specific *Fos* isolate was indicated when the aboveground dry biomass of sorghum from a rhizobox containing a particular *S. hermonthica* population, and treated with a *Fos* isolate, was significantly higher ($\alpha = 0.05$) than the aboveground dry biomass of sorghum from a rhizobox containing the same *S. hermonthica* population but treated with ddH_2O, i.e., comparted with its respective negative control. In contrast, when the aboveground dry biomass of sorghum from a rhizobox containing a particular *S. hermonthica* population, and treated with a *Fos* isolate, was not significantly higher ($\alpha = 0.05$) than its respective negative control, this indicated *S. hermonthica* non-susceptibility to that *Fos* isolate. The *post-hoc* assisted classification was chosen to eliminate personal bias in assigning phenotypic responses to the sampled *S. hermonthica* populations.

Also, the indirect estimation of *S. hermonthica* susceptibility and non-susceptibility from the sorghum aboveground dry biomass was established from our repeated preliminary studies, which presented a significant negative correlation between *S. hermonthica* incidence and the aboveground dry biomass of the adopted sorghum cultivar ($r = -0.98$, $P < 0.0001$) (see appendix 1). Moreover, it was a reliable estimator because newly attached *S. hermonthica* plantlets belowground are very small and could easily be missed during counting, especially for the large-scale experimental observations in this study.

3.3.3 Genomic analysis of S. hermonthica populations

3.3.3.1 DNA extraction and SSR genotyping

Surface-sterilized *S. hermonthica* seeds (65 mg) were drawn from a seed lot of each of the 10 sampled populations. This was performed in three replications, with the mixing of the population seed lot between successive draws to ensure homogeneity. The triplicate draws were then combined (approximately 200 mg seeds). This was performed in three separate repetitions. Total genomic DNA was extracted separately from the combined *S. hermonthica* seeds of the 10 populations, using the Fast DNA® spin kit (MP Biomedicals, https://www.mpbio.com). The extracted DNA was amplified by PCR using peqSTAR 96X Universal Gradient thermocycler (VWR International GmbH, https://vwr.com), with 12 genomic SSR markers: E1005, E1008, E1009, E1012, E1014, E1016, E1029, E1030, E1032, E1038, E1041 and E1042 (originally designated as SH1005, SH1008, SH1009, SH1012, SH1014, SH1016, SH1029, SH1030, SH1032, SH1038, SH1041 and SH1042; Estep et al. 2010). The DNA was also amplified with 10 EST-SSR markers: Y17, Y26, Y33, Y43, Y50, Y53, Y57, Y58, Y59 and Y63 (originally defined as SSR17, SSR26, SSR33, SSR43, SSR50, SSR53, SSR57, SSR58, SSR59 and SSR63; Yoshida et al. 2010). All forward primers were labelled with the fluorescent dye Fam-6. A 50-µl PCR reaction system was used, which included 1 µl of

template DNA, 10× PCR buffer, 50 mm $MgCl_2$, 10 mm dNTPs, 5 µm of each primer and 2.5 U of Accuzyme™ DNA Polymerase (Bioline, https://www.bioline.com). For the 12 genomic SSR markers, the PCR conditions consisted of an initial denaturation at 94°C for 5 min, followed by 10 cycles at 94°C for 45 sec, 68°C for 5 min and 72°C for 1 min, then five cycles at 94°C for 45 sec, 58°C for 2 min and 72°C for 1 min, then 25 cycles at 94°C for 45 sec, 50°C for 2 min and 72°C for 1 min. The reaction was completed with a final elongation at 72°C for 30 min (Estep et al. 2010). PCR conditions for the 10 EST-SSR markers consisted of an initial denaturation at 95°C for 1 min, followed by 40 cycles at 94°C for 15 sec, 60°C for 30 sec and 72°C for 30 sec. A final elongation at 72°C for 5 min completed the reaction (Yoshida et al. 2010). Amplified DNA fragments were verified by 1.5% agarose gel electrophoresis to ascertain that their band sizes were in line with the expected range for each primer. To obtain the microsatellite genotype data set (codominant data) for downstream population genetic analyses, the lengths of the amplified DNA fragments were determined by capillary electrophoresis using the ABI 3130 XL genetic analyser (ThermoFisher Scientific, https://www.thermofisher.com), with the ABI-D filter set and ROX 500 size standard at Eurofins Genomics (https://www.eurofinsgenomics.eu).

3.3.3.2 Population genetics

The sampled *S. hermonthica* were differentiated primarily by sampling zones (i.e., East Africa and West Africa) and by phenotypic response to *Fos* isolates (i.e., susceptible or non-susceptible to Foxy-2 and FK3). Using the complete panel of 22 SSR markers in this study, the *S. hermonthica* were also differentiated by genetic distance, through the simple matching dissimilarity index and weighted neighbour-joining (WNJ) tree diagram in darwin 6.0.021 (Perrier and Jacquemoud-Collet 2006). Population genetic structure was determined by analysis of molecular variance (AMOVA) (Excoffier et al. 1992). Genetic differentiation between structured *S. hermonthica* groups was calculated by the fixative index or F_{ST} (Wright 1965). F_{ST} values for the structured groups were as follows: 0, complete panmixis (absolute interbreeding); <0.05, small/low genetic differentiation; 0.05–0.15, intermediate/moderate genetic differentiation; 0.15–0.25, large/high genetic differentiation; >0.25, very large/very high genetic differentiation; and 1, complete population structure (i.e., the populations share absolutely no genetic diversity) (Wright 1978; Hartl and Clark 1997). Gene flow was measured by the effective number of migrants (*Nm*) exchanged between the groups per generation, where $Nm = [(1/F_{ST}) - 1]/4$ (Wright 1969). Gene flow estimates of $Nm < 1$ were taken as low gene flow, whereas *Nm* values of $1 \leq 5$ indicated moderate gene flow and *Nm* values of >5 indicated high gene flow (Wolf and Soltis 1992; Waples and Gaggiotti 2006; McManus et al. 2015). AMOVA, F_{ST} and *Nm* were calculated using genalex 6.5 (Peakall and Smouse 2006; 2012), with 999 permutations. Departures from Hardy–Weinberg equilibrium (HWE) proportion

by exact test method were used for revealing SSR marker(s) significantly associated with the structured *S. hermonthica* groups. The exact test of HWE is valid for any sample size and minor (rare) allele frequency (Wang and Shete 2012). Phase-unknown LD between all loci pairs (i.e., phase-unknown pairwise LD) was used for determining SSR loci pairs in significant LD. Marker–trait association and LD analyses were carried out in arlequin 3.5 (Excoffier and Lischer 2010). 1 000 000 Markov chain steps with 100 000 dememorization steps was used for the exact test of HWE. Phase-unknown pairwise LD was set at 10 000 permutations, with five random initial conditions from which the expectation maximization (EM) algorithm was started to repeatedly estimate the sample likelihood, with a significance level of 0.05, for phase-unknown pairwise LD. The matrix of pairwise SSR loci with significant LD was illustrated with Morpheus (Morpheus Software 2018). Evidence of a recent population bottleneck in the structured groups, i.e., groups with a significant number of loci with excess heterozygosity (Piry et al. 1999), was analysed using bottleneck 1.2.02 (Cornuet and Luikart 1996) by assuming a two-phase mutation model (TPM), 95% single-step mutation (SSM) in TPM, a variance of 12, with 10 000 simulation iterations, and Wilcoxon's signed-rank test.

3.3.3.3 Bioinformatics

DNA fragments of the sampled *S. hermonthica* were amplified with the associated SSR marker(s) identified, then sequenced by Sanger method to obtain nucleotide sequences (Eurofins Genomics GmbH). To identify variations in the nucleotide sequences and in the translated protein sequences across the sampled *S. hermonthica* populations, the nucleotide sequences were aligned by the multiple sequence comparison by log-expectation (muscle) algorithm (Edgar 2004). Based on similarity in the aligned nucleotide sequences of the associated SSR locus/loci, computational phylogenetics was inferred by the neighbour-Joining method, with evolutionary distance calculated by p-distance using MEGA X (Kumar et al. 2018). MEGA X was also used for determining the translated protein sequences of the aligned nucleotide sequences.

3.4 Results

3.4.1 Population genetic structure in the sampled S. hermonthica

Analysis of variance (ANOVA) showed the significantly varying susceptibility of the sampled *S. hermonthica* populations to Foxy-2 and FK3, as expressed by their phenotypic responses (indirectly estimated from the aboveground dry biomass of sorghum) to the *Fos* isolates ($P < 0.0001$) (Fig. 3.2).

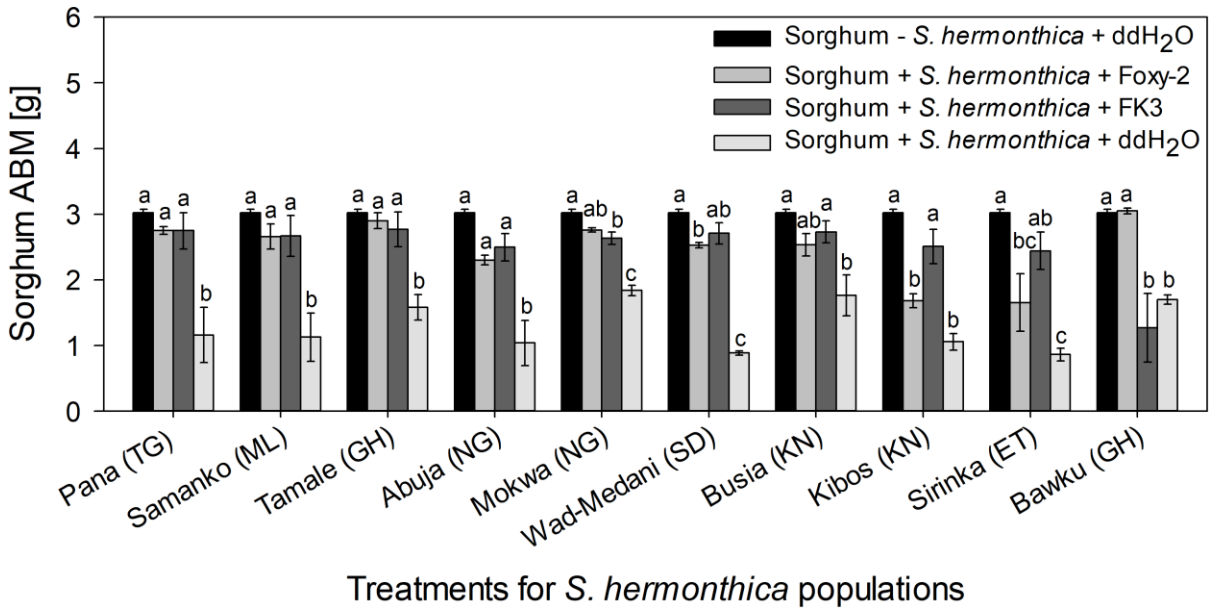

Fig. 3.2: Tukey's range test for *Sorghum bicolor* aboveground dry biomass (sorghum ABM) in response to the treatment of *Striga hermonthica* populations. Positive control: sorghum – *S. hermonthica* + ddH_2O. Negative control: sorghum + *S. hermonthica* + ddH_2O. Bars with at least one letter in common represent treatments that do not significantly differ ($\alpha = 0.05$). Error bars indicate standard deviations. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

The phenotypic responses to *Fos* isolates in the sampled *S. hermonthica* plants classified the *S. hermonthica* populations into four groups: A, B, C and D. The effects of Foxy-2 and FK3 were higher than the negative control for *S. hermonthica* populations from Pana (Togo), Samanko (Mali), Tamale (Ghana), Abuja (Nigeria), Mokwa (Nigeria) and Wad-Medani (Sudan). This indicated that these *S. hermonthica* populations were susceptible to both Foxy-2 and FK3, and thus they were classified as group A. The effect of FK3 was higher than the negative control for the *S. hermonthica* population from Busia (Kenya), but the effect of Foxy-2 neither differed from the negative control nor from the positive control. This implied susceptibility to FK3, but only intermediate (or partial) susceptibility to Foxy-2. Therefore, this

population was classified as group B. For the *S. hermonthica* populations from Kibos (Kenya) and Sirinka (Ethiopia), the effect of FK3 was higher than that of the negative control, whereas the effect of Foxy-2 did not differ from the negative control (but was lower than the positive control). This indicated that these *S. hermonthica* populations were only susceptible to FK3, and hence they were classified as group C. The *S. hermonthica* population from Bawku (Ghana) was only susceptible to Foxy-2, as evidenced by Foxy-2 inducing a higher effect than the negative control, and thus it was classified as group D.

Using the complete set of 22 SSR markers, differentiation of the *S. hermonthica* by genetic distance separated the East African *S. hermonthica* into two clusters, and the West African *S. hermonthica* into three clusters: cluster 1, Kibos (Kenya) and Busia (Kenya); cluster 2, Sirinka (Ethiopia) and Wad-Medani (Sudan); cluster 3, Mokwa (Nigeria) and Abuja (Nigeria); cluster 4, Bawku (Ghana), Pana (Togo) and Samanko (Mali); and cluster 5, Tamale (Ghana) (Fig. 3.3).

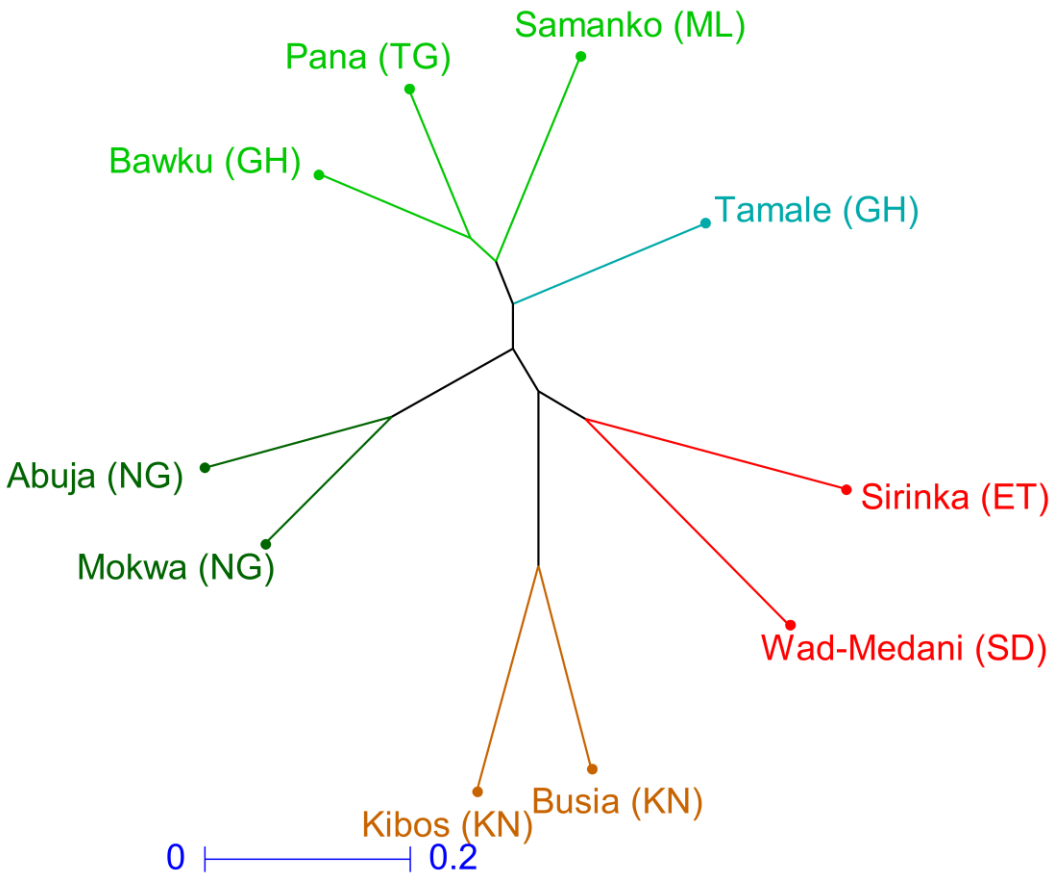

Fig. 3.3: Weighted neighbour-joining (WNJ) tree for five clusters of the *Striga hermonthica* populations based on genetic distance differentiation. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

Population genetic structure demonstrated low genetic differentiation between the East African and West African *S. hermonthica* populations (F_{ST} = 0.012, P < 0.05). An intermediate genetic differentiation was revealed for *S. hermonthica* when differentiated based on phenotypic response to *Fos* isolates (F_{ST} = 0.143, P < 0.01) and genetic distance (F_{ST} = 0.097, P < 0.01) (Table 3.1). Accordingly, *Nm* (number of migrants successfully entering a population) values of 21.42 (high), 1.493 (moderate) and 2.34 (moderate) were calculated for the *S. hermonthica* groups when differentiated based on sampling zones, phenotypic response to *Fos* isolates and genetic distance, respectively. The Mantel isolation-by-distance test indicated an intermediate positive correlation (r = 0.56, P < 0.01) between geographic distance and genetic distance (Fig. 3.4).

Table 3.1: Analysis of molecular variance (AMOVA) and fixative index (F_{ST}) for the sampled *Striga hermonthica* populations differentiated based on sampling zones, phenotypic response to *Fusarium oxysporum* f. sp. *strigae* (*Fos*) isolate and genetic distance.

Source	**East and West Africa sampling zones (two groups)**		**Phenotypic response to *Fos* isolate-based differentiation pattern (four groups)**		**Genetic distance-based differentiation pattern (five groups)**	
	Estimated variance	F_{ST}	Estimated variance	F_{ST}	Estimated variance	F_{ST}
Between groups	1%	0.012 *	13%	0.143 **	9%	0.097 **
Within groups	99%		87%		91%	
Total	100%		100%		100%	

* - P < 0.05. ** - P < 0.01.

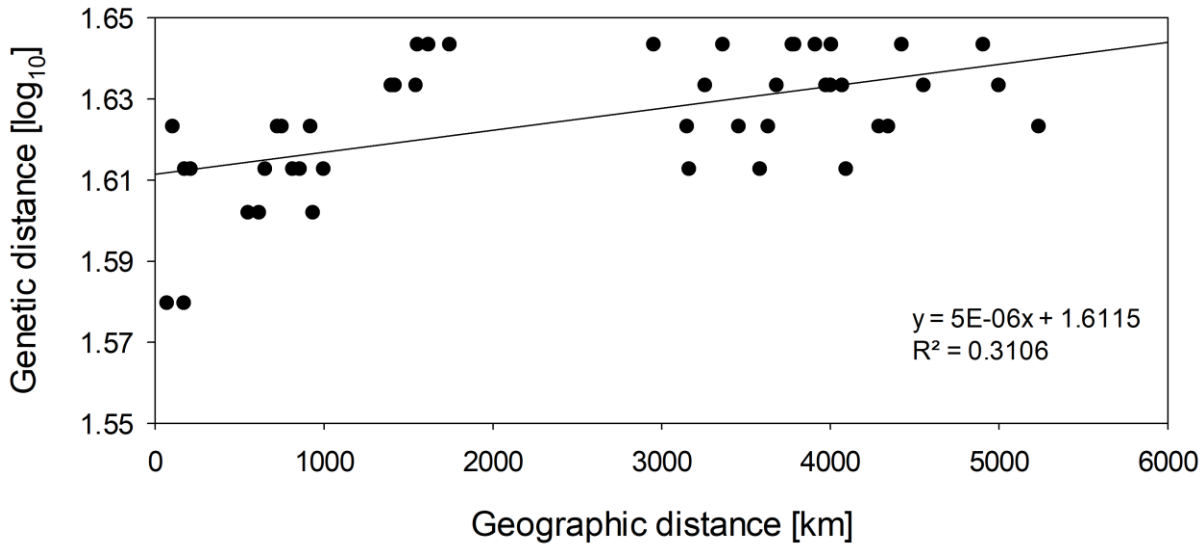

Fig. 3.4: Mantel isolation-by-distance test to show the correlation between geographic distance and genetic distance in the 10 *Striga hermonthica* populations.

3.4.2 SSR markers associated with S. hermonthica susceptibility to Foxy-2 and FK3

An EST-SSR marker (Y53) was associated with the West African *S. hermonthica* populations ($P < 0.05$), but no marker was associated with the East African *S. hermonthica* populations. The *S. hermonthica* class manifesting susceptibility to both Foxy-2 and FK3 (group A) was also associated with the same EST-SSR marker (Y53) ($P < 0.01$) in addition to a genomic SSR marker (E1009) ($P < 0.05$). No marker–trait association was shown for the non-group-A *S. hermonthica* class, i.e., a class containing groups with intermediate susceptibility to Foxy-2 (group B) and susceptibility to either FK3 (group C) or Foxy-2 (group D). Based on the classification of the differentiated *S. hermonthica* groups, the West African *S. hermonthica*, the class containing groups A and B, and group A only, had higher numbers of SSR loci with significant linkage disequilibrium (LD), i.e., 16.9, 16.9 and 14.0%, respectively (Table 3.2). This was followed by lower percentages of SSR loci pairs in significant LD for the *S. hermonthica* class comprising groups B–D (2.9%), the East African *S. hermonthica* (0.8%), and the class containing groups C and D (0%) (Table 3.2). The number of populations in a *S. hermonthica* class was not a determinant of the extent of SSR loci in significant LD, especially as shown by group A (six populations) and the non-group-A class, containing groups B–D (four populations) (Fig. 3.5).

Table 3.2: Marker-trait associations and percentage of simple sequence repeat (SSR) loci pairs in significant linkage disequilibrium (LD) (α = 0.05) in the sampled *Striga hermonthica* populations differentiated based on sampling zones and phenotypic response to *Fusarium oxysporum* f. sp. *strigae* (*Fos*) isolate.

Differentiation pattern	Class	Number of populations	Manifested trait	Associated SSR marker	Percentage of SSR loci pairs in significant LD
Sampling zone	West Africa	6	†	Y53 *	16.9
	East Africa	4	†	Nil	0.8
Phenotypic response to Fos isolate	Group A	6	Susceptibility to both Foxy-2 and FK3	E1009 * Y53 **	14.0
	Groups A and B	7	Susceptibility/intermediate susceptibility to Foxy-2 and FK3	E1009 * E1012 * Y53 *	16.9
	Groups B, C and D	4	Intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3	Nil	2.9
	Groups C and D	3	Susceptibility to either Foxy-2 or FK3	Nil	0

† - Not applicable, * - $P < 0.05$, ** - $P < 0.01$.

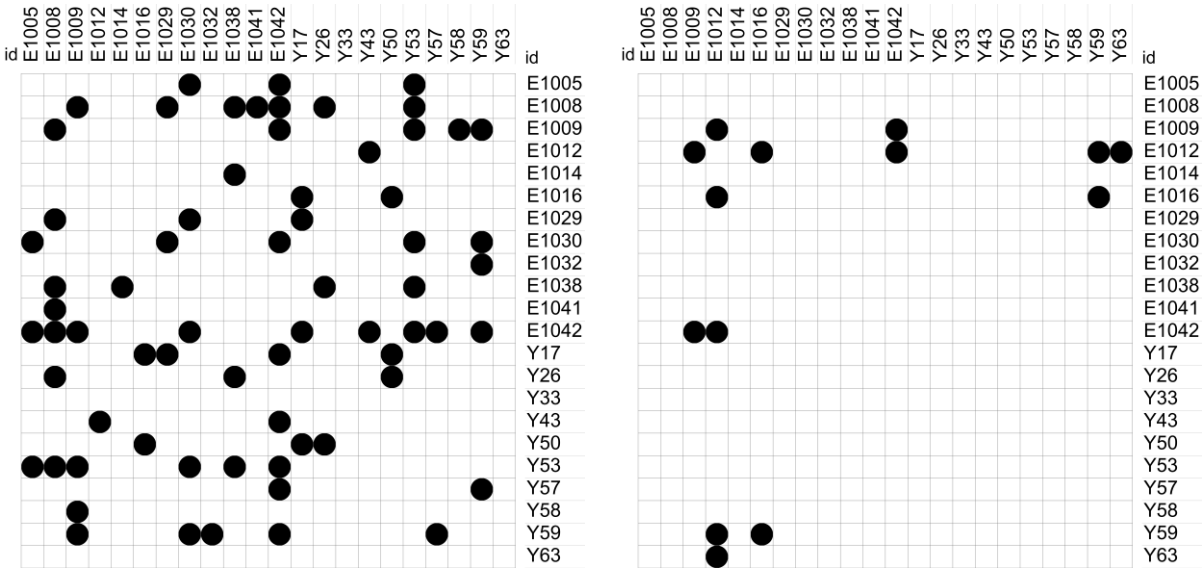

Fig. 3.5: Pairwise simple sequence repeat (SSR) loci in significant linkage disequilibrium (LD) (α = 0.05) for the 10 *Striga hermonthica* populations. Left panel: class with susceptibility to both Foxy-2 and FK3 (group A). Right panel: class containing groups with intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3 (groups B, C and D).

Owing to the rather short DNA fragment lengths of the EST-SSR locus Y53 (mean 86 bp) in *S. hermonthica* populations, it was not possible to perform nucleotide sequencing and alignment. Hence, SSR locus E1009 with relatively longer DNA fragment lengths (>200 bp), suitable for sequencing, was the focus for downstream bioinformatic analysis in this study. muscle-aligned nucleotide sequences of the SSR locus E1009 in the 10 *S. hermonthica* populations revealed genomic variations in the form of point substitutions and insertion–deletion mutations across the four groups. This presented a completely identical nucleotide sequence among *S. hermonthica* in group A that was partially dissimilar to that of group B, but was totally distinct from groups C and D (Fig. 3.6). This resulted in a uniquely translated protein sequence at the E1009 locus in group A, which was partly different to group B but was completely distinct from groups C and D (Fig. 3.7) (for the definition of the letter codes used in Figs. 3.6 and 3.7, see appendix 2).

>Pana (TG) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Samanko (ML) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Tamale (GH) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Abuja (NG) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Mokwa (NG) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Wad-Medani (SD) Group-A
ATGGA-GTATATAATTATTGTTTTAGGTTTTAGTTCCTGTGATCAATTTAAAAAATGCTTGATTATTGCTTTATAGATAAAATGA----------AGTCTCACTCACTCAACACAACCC---
>Busia (KN) Group-B
ATGGA-GTATATAATTATTGTTTTTTGTTTTACTGCCTGTGATCTATTAAAAAAATGCTTGATTATTGCTTTATAAATAAAATGA----------ACTCTCACTCACTCCACCCAAACC---
>Kibos (KN) Group-C
ATGTATATATATATTTATTGATGTTTTTTTTCCTGCCTGCGATTTATAAAAAAAATGATTAATTATTTATTTATAAAATGAATG----------CTCTCTCACTCACCCCAACCCAAC
>Sirinka (ET) Group-C
ATGGT----AAAAATCATGACTTTCGCCTTCCAAC-------TCAACCCAAAG-------GAATATGATCTTCAAAGTCAAGTCATGAACCAACCTCCCCATCTCTCTCCGTCTGAACAAAGCCTC
>Bawku (GH) Group-D
ATGGT----AAGAATGATGATTTTCTTCATTCGATC------TCAGCCCAAAG-------GAATACCAGGTTCAAAGTCAAGTGGGCGTGAAAATCTCTATCTCCACTTTGACTGAGAAATGATT-

- = insertion/deletion

Fig. 3.6: Aligned nucleotide sequences of the simple sequence repeat (SSR) locus E1009 for the 10 *Striga hermonthica* populations. Sequences are in the 5′ → 3′ direction. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

>Pana (TG) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Samanko (ML) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Tamale (GH) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Abuja (NG) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Mokwa (NG) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Wad-Medani (SD) Group-A
M?VYNYCFRF*FL*SI*KMLDYCFIDKM?----SLTHSTQP-
>Busia (KN) Group-B
M?VYNYCFLFYCL*SIKKMLDYCFINKM?----TLTHSTQT-
>Kibos (KN) Group-C
MYIYIY*CFFSCLRFIKKMINYLFIK*M------LSHSPQPN
>Sirinka (ET) Group-C
M?-KNHDFRLP?--STQ?--EYDLQSQVMNQPPHLSPSEQSL
>Bawku (GH) Group-D
M?-KNDDFLHSI--SAQ?--EYQVQSQVGVKISISTLTEK*?

? = missing, * = termination or stop, - = insertion/deletion

Fig. 3.7: Translated protein sequences at the E1009 locus for the 10 *Striga hermonthica* populations. Sequences are in the 5′ → 3′ direction. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

Phylogenetic computation of the genomic variations at the E1009 locus presented an evolutionary relationship network of the *S. hermonthica* groups, whereby group-A *S. hermonthica* were identically clustered. The group-B branch was calculated to sit next to the group-A cluster, and this was followed by group C and then group D (Fig. 3.8).

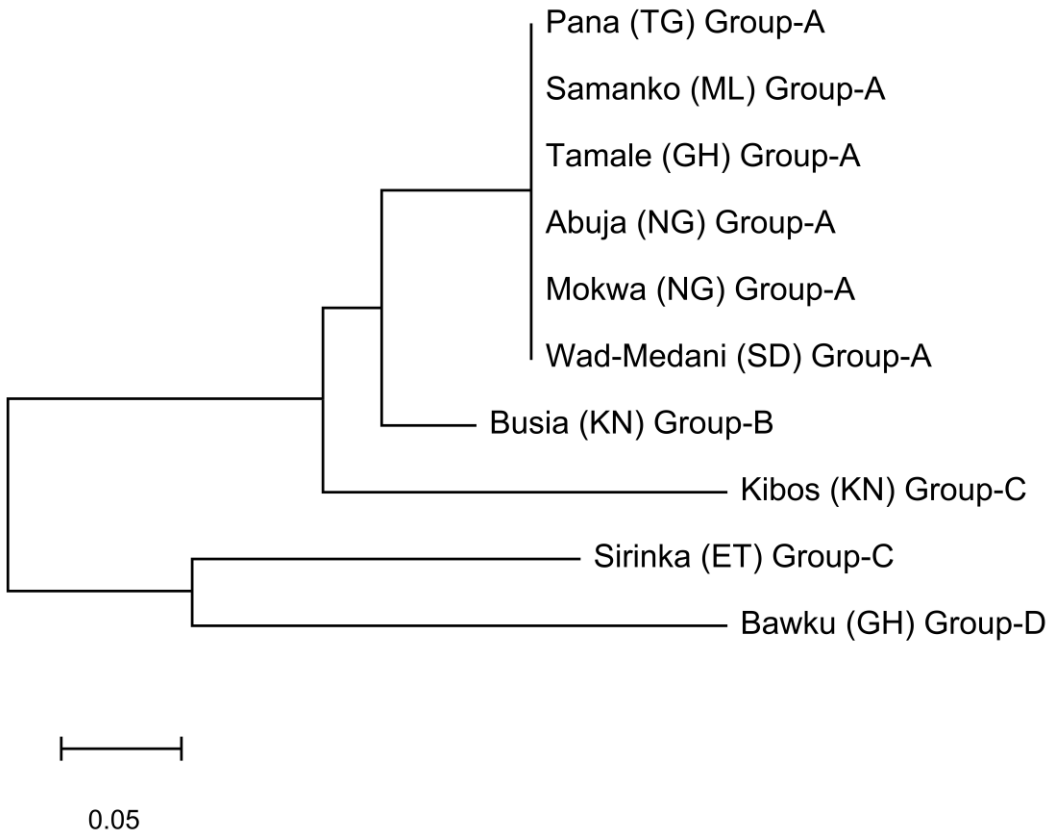

Fig. 3.8: Phylogenetic tree of the 10 *Striga hermonthica* populations based on aligned DNA sequence similarity at the simple sequence repeat (SSR) locus E1009 associated with *S. hermonthica* showing susceptibility to Foxy-2 and FK3. Country codes: ET, Ethiopia; GH, Ghana; KN, Kenya; ML, Mali; NG, Nigeria; SD, Sudan; TG, Togo.

3.5 Discussion

3.5.1 Population genetic structure in S. hermonthica and the role of geography

The differentiation patterns for the sampled *S. hermonthica* (i.e., by sampling zones, phenotypic response to *Fos* isolates and genetic distance) demonstrated population genetic structure, with higher variation within groups than between groups. This was substantiated by their significant F_{ST} values. This finding was in line with earlier reports by Estep et al. (2011), Bozkurt et al. (2015) and Joel et al. (2018). Similar population genetic structure has been described in other outcrossing species with or without a parasitic lifestyle, e.g., *Orobanche*

crenata (Román et al. 2002), *Rheum tanguticum* (Hu et al. 2014) and *Ruellia nudiflora* (Vargas-Mendoza et al. 2015).

As *S. hermonthica* is an obligate outcrossing species, it is not surprising to detect low genetic differentiation between populations, largely caused by heterozygosity resulting from high interbreeding. It is, however, interesting that characterization by phenotypic response to *Fos* isolates genetically differentiated the *S. hermonthica* populations to reveal a stronger population structure (intermediate genetic differentiation) than when characterized by sampling origins (low genetic differentiation). This means that although the East African and West African *S. hermonthica* populations are from geographically widespread locations, only low genetic differentiation occurs between them. Hence, other differentiation patterns used in this study provided a stronger genetic structure of more genetically diverse *S. hermonthica* groups.

The high values of *Nm* measured between the East and West African *S. hermonthica* populations indicated high gene flow between these zones. The overall arbitrary trans-Saharan trade network (Keyser 2014; Chimee 2018) may explain the high gene flow between the East and West African *S. hermonthica* groups. This is because in subsistence agroecosystems, as mainly practiced in sub-Saharan Africa (Sibhatu and Qaim 2017), the dispersal of *S. hermonthica*-contaminated crop seeds is mainly facilitated through trading activities, but dispersal by animal, wind or water is of lesser significance because of limitations in the spatial distances that they cover (Berner et al. 1994; van Delft et al. 1997). The moderate gene flow (moderate *Nm*) between *S. hermonthica* groups differentiated by phenotypic response to *Fos* isolate suggests that in spite of the high transfer of *S. hermonthica* genetic material between Eastern and Western Africa, the genetic factors regulating the susceptibility pattern of a *S. hermonthica* population to Foxy-2 and FK3 are only moderately affected. Therefore, this affirmed the stability (to an extent) of a characteristic response by a *S. hermonthica* population towards an *Fos* isolate.

On a different note, indigenous agroecological factors may influence *S. hermonthica* incidence. For instance, in field studies performed by Ekeleme et al. (2014) in Bauchi (North-Eastern Nigeria) and Kano (North-Western Nigeria), where *S. hermonthica* incidence in Bauchi (3.1 plants per m^2) was over two times higher than that in Kano (1.4 plants per m^2), *S. hermonthica* was positively related to latitude and soil pH, but was negatively related to soil clay content, as well as to soil-exchangeable potassium (K) and calcium (Ca) contents in Bauchi. In Kano, however, *S. hermonthica* was positively related to soil sand and silt contents, but was negatively related to latitude, total soil nitrogen (N) content and soil-exchangeable K content. Additionally, at five *S. hermonthica* endemic areas in Eastern, Central and Western

Sudan, Dafaallah et al. (2017) reported *S. hermonthica* incidence as positively related to total soil carbon (C) content, but negatively related to soil pH, exchangeable K, and available phosphorus (P) and N contents. Whether agroecological factors native to a *S. hermonthica* sampling zone can determine population genetic structure in *S. hermonthica*, or explain the variable susceptibility pattern of *S. hermonthica* to *Fos* isolates, remains elusive, however.

The significant intermediate positive correlation between geographic distance and genetic distance demonstrated the effect of geographic distance on gene flow limitations. This correlation also corresponded to the moderate gene flow (moderate *Nm*) between the *S. hermonthica* clusters differentiated by genetic distance. Welsh and Mohamed (2011) detailed geographic barriers as major factors underlying population genetic structure in Ethiopian *S. hermonthica*, in which the Ahmar mountains and the Rift Valley were relevant obstacles to *S. hermonthica* seed dispersal, including a significant correlation between geographic distance and genetic distance. Estep et al. (2011) and Bozkurt et al. (2015) similarly documented geographic effects on *S. hermonthica* population genetic structure. The susceptibility pattern of the *S. hermonthica* populations to Foxy-2 and FK3 did not correspond with sampling zones in a strict sense, because *S. hermonthica* from Wad-Medani, Sudan (East Africa), was susceptible to both Foxy-2 and FK3, like almost all West African *S. hermonthica* populations. On the other hand, *S. hermonthica* from Bawku, Ghana (West Africa), was susceptible to Foxy-2 but not to FK3, unlike all other West African *S. hermonthica* populations. This confirmed that the source of genetic variation, which was responsible for the variable susceptibility of *S. hermonthica* to Foxy-2 and FK3, was much higher in the East African than West African populations. Nonetheless, the variable susceptibility of *S. hermonthica* to Foxy-2 and FK3 was better explained by genomic variation in *S. hermonthica*, rather than by *S. hermonthica* sampling zones.

3.5.2 Linkage disequilibrium decay in S. hermonthica with variable susceptibility to Foxy-2 or FK3

It is worth noting that statistical bias arising from a small sample population size could be difficult to eliminate when determining LD (as is the case in this study i.e., $n = 10$), as a result of genetic drift and a population bottleneck (Flint-Garcia et al. 2003). The population genetic structure presented in the *S. hermonthica* populations sampled was the result of gene flow and not genetic drift, however, as the *Nm* values calculated were >1. A migration rate of >1 every other generation (i.e., $Nm > 0.5$) is adequate to overcome population differentiation resulting from genetic drift. Hence, population genetic structure is controlled by gene flow when $Nm > 0.5$, but is controlled by genetic drift when $Nm < 0.5$ (Wright 1931; Wolf and Soltis

1992). Also, a non-significant Wilcoxon's signed-rank test suggested no evidence of a recent population bottleneck.

The disparities in the extent of LD for SSR loci in the *S. hermonthica* classes based on sampling zones and phenotypic response to *Fos* isolates cannot be substantiated by the differences between population members in the respective differentiation patterns. Rather, it is an indication of LD decay arising from various uncertain evolutionary forces besides genetic drift and population bottleneck, such as mutation, selection, mating system, genetic recombination rate, population genetic structure and hybridization (Gaut and Long 2003; Slatkin 2008; Fox et al. 2019). The aligned protein-coding nucleotide sequence and phylogeny of the *S. hermonthica* populations, with regards to the associated marker E1009, support the explanation for uncertain evolutionary forces that underlie the variable susceptibility of *S. hermonthica* to Foxy-2 and FK3. Generally, small population sizes give rise to relatively high LD values, whereas large population sizes give rise to low LD values (Sved et al. 2013). But in this case, the reverse was shown. Also, *S. hermonthica* class with six and four populations each, appear twice, but with different LD values.

3.5.3 Genomic variations in S. hermonthica with variable susceptibility to Foxy-2 and FK3

Strigolactone perception, haustorial development/attachment and host vascular recognition/connection are indispensable criteria for *S. hermonthica* incidence (Saucet and Shirasu 2016). In this regard, Ejeta (2005) described low germination stimulant production, low haustorial factor, hypersensitive response and incompatible response induction upon infection as systems of sorghum (host) resistance mechanisms to *S. hermonthica*. Ejeta (2005) further listed the SSR marker TXp358 to be associated with low haustorial factor locus, and SSR markers TXP96 with SBKAFGK1 to be associated with the hypersensitive response. In our study, the *S. hermonthica* class containing groups with intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3 (groups B–D) was not associated with any of the SSR markers used. The *S. hermonthica* class with susceptibility to both Foxy-2 and FK3 (group A) showed associations with the two SSR markers Y53 and E1009, however.

For now, we cannot attribute a functional role or biological activity of the associated loci in *S. hermonthica* with respect to *S. hermonthica* susceptibility to both *Fos* isolates, Foxy-2 and FK3, as this was not examined in this study. We can speculate that the intactness of the DNA sequence of E1009 facilitates *Fos* pathogenicity in *S. hermonthica*, however. So, mutations affecting the integrity of the unique nucleotide sequence in these loci may result in differently translated proteins, which in turn may lead to variable susceptibility of *S. hermonthica* to the

Fos isolates. Further research is required to reveal the biological implications of the associated loci for the susceptibility of *S. hermonthica* to both Foxy-2 and FK3.

Among the non-group-A *S. hermonthica* class, the *S. hermonthica* population from Busia was the closest to group A, as it displayed a susceptibility response to FK3 and a partially susceptible response to Foxy-2. This meant that Foxy-2 appeared to suppress the Busia *S. hermonthica* to an extent; however, Tukey's *post-hoc* analysis did not support the classification of this *S. hermonthica* population to be part of group A. Additionally, the moderate phenotypic resemblance of the Busia *S. hermonthica* to group-A *S. hermonthica* corresponded with fairly similar nucleotide sequences and translated protein sequence between Busia *S. hermonthica* and group-A *S. hermonthica*, with reference to the associated SSR locus E1009. In parallel, computational phylogenetics presented the Busia *S. hermonthica* (among the non-group-A *S. hermonthica*) to have the closest distance to group-A *S. hermonthica*. The non-susceptibility of the Kenyan *S. hermonthica* populations to Foxy-2 in this study was in line with the findings reported by Avedi et al. (2014), where post-entry quarantine field studies at five stations in Western Kenya (that included Busia and Kibos) showed Foxy-2 as not being effective against *S. hermonthica* incidence. Instead, FK3 was proven effective against *S. hermonthica* in Kenya (Beed et al. 2013; Kangethe et al. 2016). A specific *F. oxysporum* isolate has been reported to be effective against *S. hermonthica* in Ethiopia (Rebeka et al. 2013), but we have only limited knowledge about the inconsistent effectiveness of differing *Fos* isolates on Ethiopian *S. hermonthica*. In our study, the *S. hermonthica* population sampled from Sirinka (Ethiopia) was deemed susceptible to FK3, but non-susceptible to Foxy-2. Further research must corroborate the susceptibility pattern of *S. hermonthica* from this less well understood region to varying *Fos* isolates.

3.6 Conclusion

Genomic variation in *S. hermonthica* plays a key role in the inconsistent effectiveness of *Fos* isolates (i.e., Foxy-2 and FK3) against *S. hermonthica* from different locations of SSA. The varying susceptibility of *S. hermonthica* populations to Foxy-2 and FK3 is connected to mutations at certain SSR loci, which possibly arose through evolutionary forces, such as genetic recombination rate, non-recent common ancestry or hybridization events between *S. hermonthica* and other less popular *Striga* spp., e.g., *Striga aspera* (Aigbokhan et al. 1998; 2000). This mutational occurrence engenders population genetic structure that guides the differing phenotypic response of *S. hermonthica* to *Fos* isolates. The SSR loci (Y53 and E1009) associated with the *S. hermonthica* group demonstrating susceptibility to both Foxy-2 and FK3 represent promising candidates for developing stable molecular genetic markers for

identifying *S. hermonthica* with susceptibility to both Foxy-2 and FK3. The relatively high LD uncovered between SSR loci pairs in this *S. hermonthica* class with susceptibility to both Foxy-2 and FK3 (compared with the other class demonstrating intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3) paves the way for further LD mapping to gain deeper insights into the genes underlying this trait (Weir 2008; de Souza et al. 2018).

Although this study employed forward genetics methods to study the variable susceptibility of genetically diverse *S. hermonthica* populations to *Fos* isolates Foxy-2 and FK3, it is also important to investigate further the biological significance or functional role of the identified (associated) SSR loci. For this aim, reverse genetics methods such as site-directed mutagenesis and gene silencing could be employed to create knockout or knockdown mutant *S. hermonthica* lines for the identified (associated) SSR loci. Thorough validation with larger *S. hermonthica* populations across SSA is required to verify the linkage strength and stability for the marker–trait associations, with a careful focus on the associated EST-SSR marker Y53, which showed a stronger statistical association but was not deeply investigated in this study owing to the fragment size limitations. As a result of the highly conserved and less polymorphic nature of ESTs, Y53 is expected to show slower LD decay than would be expected for outcrossing species like *S. hermonthica*.

Chapter 4. Role and *in vivo* localization of *Fusarium oxysporum* f. sp. *strigae* and *Bacillus subtilis* in an integrated *Striga hermonthica* biocontrol system

Williams O. Anteyi[1] and Frank Rasche[1]

[1]Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), University of Hohenheim, 70593 Stuttgart, Germany.

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4.1 Abstract

To improve the efficiency of *Striga hermonthica* biological control in the context of an integrated biocontrol system, the role/impact of coinoculating the mycoherbicide *Fusarium oxysporum* f. sp. *strigae* (*Fos*) with a plant growth promoting rhizobacterium, *Bacillus subtilis* isolate GB03, on *S. hermonthica* attachment and sorghum biomass was investigated. *Fos* isolates (Foxy-2, FK3) and GB03, including *Trichoderma viride* (IMB12098 strain) as reference, were applied as single and combined treatments to *S. hermonthica*-infested rhizoboxes with sorghum as the host crop. In vivo localization/interaction of *Fos* and GB03 in *S. hermonthica* was monitored by fluorescent gene expression of transformed *Fos* and transformed GB03. Combined treatments of FK3 + GB03 and Foxy-2 + GB03 increased sorghum aboveground dry biomass ($P < 0.05$), but not IMB12098 + GB03. None of the combined treatments suppressed *S. hermonthica* attachment. Single treatments of FK3 and GB03 increased sorghum aboveground dry biomass ($P < 0.05$), but Foxy-2 and IMB12098 did not. Only FK3, of all single treatments, suppressed *S. hermonthica* attachment ($P < 0.05$). GB03 promoted sorghum yield when applied alone or combined with *Fos* ($P < 0.05$). *Fos* penetration of *S. hermonthica* through trichome entry was revealed. Although *Fos*, either as sporal (conidia) or vegetative form (mycelium), co-occupied common ecological niches with GB03 (they colocalize) in diseased *S. hermonthica* shoots, mainly in flavonoid-rich regions. Nevertheless, GB03 thwarted *Fos* suppressive activity against *S. hermonthica* attachment. In the given setup, coinoculation of *Fos* with GB03 presented no added advantage in suppressing the incidence of the sampled *S. hermonthica*.

Keywords: *Striga hermonthica, Fusarium oxysporum* f. sp. *strigae*, Foxy-2, FK3, *Bacillus subtilis* GB03, ecological niche, plant/microbe-microbe interactions.

4.2 Introduction

The noxious, hemiparasitic witchweed *Striga hermonthica* (Delile) Benth. constitutes one of the most serious threats to cereal production, especially in tropical agroecosystems (Atera et al. 2011; Dawud 2017; Mounde et al. 2020; Teka 2014). Generally, *S. hermonthica* incidence is managed through four main approaches, which include traditional/cultural practices such as hand pulling, crop rotation, intercropping, trap/catch crop planting, and soil fertility improvement (nitrogen fertilization). The other *S. hermonthica* management strategies rely on chemical (weedicide, e.g., imazapyr), genetic (*S. hermonthica*-resistant crop varieties), and biological (biological control agents [BCAs], e.g., fungi, bacteria, and certain insects) approaches. Each of these approaches alone is not completely efficient in combating *S.*

hermonthica; thus, an integrated *S. hermonthica* management strategy is often recommended (Baiyegunhi et al. 2018; Berner et al. 1996; Mounde et al. 2020; Sibhatu 2016).

The mycoherbicidal effectiveness of the most renowned fungal BCA against *S*. *hermonthica*, which is *Fusarium oxysporum* f. sp. *strigae* (*Fos*), under both controlled and field conditions, has been well documented (Ciotola et al. 1995; Nzioki et al. 2016; Schaub et al. 2006). *Fos* has proven to inhibit *S*. *hermonthica* germination when applied pre-*Striga* seed conditioning period (Kroschel et al. 1996; Yonli et al. 2010) and/or to suppress *S*. *hermonthica* when applied at post-*Striga* attachment period (Abbasher et al. 1998; Marley et al. 1999). *Fos* attacks *S. hermonthica* plants by its hyphal colonization of xylem vessels at inception, followed by total vessel obstruction in mature (emerged) plants or complete tissue digestion of younger plantlets belowground, thereby killing *S. hermonthica* from wilting and tissue degradation (Ndambi et al. 2011). The *Fos* isolate Foxy-2 from Ghana has been widely reported for successful *Striga* spp. biocontrol management in West Africa (Abbasher et al. 1998; Elzein and Kroschel 2004a). Similarly, FK3 is a Kenyan *Fos* isolate with proven antagonism against *S. hermonthica* in the East African region (Beed et al. 2013; Kangethe et al. 2016). A major drawback of *Fos* is the inconsistent effectiveness of its isolates, especially (but not limited to) M12-4, Foxy-2, and FK3, against diverse *S. hermonthica* populations of various origins (Avedi et al. 2014; Mounde et al. 2020; Venne et al. 2009). With respect to *S. hermonthica*, it has recently been shown that genomic variation in *S. hermonthica* is a superior determinant of the inconsistent effectiveness of *Fos* isolates (Foxy-2, FK3) against *S. hermonthica*, rather than the geographic origin of *S. hermonthica* populations (Anteyi and Rasche 2020). The inconsistency of *Fos* isolates in effectively controlling genetically diverse *S. hermonthica* populations lowers *Fos* reliability as an efficient mycoherbicide against *S. hermonthica* in various agro-ecological zones. Consequently, it hinders widespread acceptability of *Fos* as a BCA against *S. hermonthica* (Massart et al. 2015; Velivelli et al. 2014).

Comparably, plant growth promoting rhizobacteria (PGPR), particularly of the genera *Pseudomonas* sp., *Azospirillum* sp., and *Bacillus* sp., have been reported to ameliorate the menace of *S. hermonthica*, by reducing the seed germination, halting haustorial development/attachment, and delaying aboveground emergence (Ahonsi et al. 2002a; Hassan et al. 2009b; Mounde et al. 2015). PGPR are a heterogeneous group of rhizosphere-colonizing beneficial bacteria that improve plant health and/or promote plant growth directly (biofertilization, phytostimulation, rhizoremediation) or indirectly (phytopathogen elimination, e.g., antibiosis, systemic resistance induction, competitive colonization for nutrients, and niche) (Backer et al. 2018; Lugtenberg and Kamilova 2009; Mariutto and Ongena 2015). In assessing the activity of four common PGPR strains in a *S. hermonthica*–sorghum pathosystem, Mounde et al. (2015) verified *Bacillus subtilis* isolate GB03 as the most

promising, compared with *B. subtilis* isolate Bsn5, *B. amyloliquefaciens* isolate FZB42, and *Burkholderia phytofirmans* isolate PsJN, in terms of inhibiting *S. hermonthica* germination with haustorial attachment and promoting the biomass of *S. hermonthica*-free sorghum plants.

Notwithstanding the advancements in *S. hermonthica* biocontrol research, related to the ecology and biocontrol potential of *Fos* and PGPR against *Striga* spp., it is still unknown if the combined application of these *S. hermonthica*-attacking agents will optimize *S. hermonthica* suppression. The goal is that technically a synergistic action of *Fos* attacking *S. hermonthica* and PGPR improving the host cereal growth can be achieved. It is also uncertain if the coinoculation of *Fos* and PGPR would be equally effective when applied either as pre-*Striga* seed conditioning or post-*Striga* attachment treatment. Furthermore, the localization/interaction of *Fos* and PGPR, in affected *S. hermonthica*, is unclear. Hence, the main research questions of this study were as follows: Will the coinoculation of *Fos* and PGPR improve the efficiency of combating *S. hermonthica*, while simultaneously promoting sorghum yield in a *S. hermonthica*–sorghum pathosystem? Does the response pattern of *S. hermonthica* and sorghum differ, suggesting a preference for an application period (pre-*Striga* seed conditioning or post-*Striga* attachment) of the coinoculating *Fos*-PGPR treatments? How do *Fos* and PGPR localize/interact in *S. hermonthica*, when coinoculated in an integrated *S. hermonthica* biocontrol system? Answering these questions will foster the understanding of the functional role of the fungal–bacterial coinoculation in a *S. hermonthica*–sorghum pathosystem, the impact of the microbial treatments at different *Striga* stages/lifecycles (i.e., germination inhibition or attachment/emergence suppression), and the physiology of the host (i.e., *S. hermonthica*)–microbe (i.e., *Fos*, PGPR) interaction in an integrated *S. hermonthica* biocontrol system. This study hypothesized that the coinoculation of *Fos* and *B. subtilis* will jointly infect and co-occupy (colocalize) the diseased *S. hermonthica*, thereby optimizing *S. hermonthica* suppression by circumventing the limitations from both the inconsistent effectiveness of *Fos* isolates against *S. hermonthica* (Anteyi and Rasche 2020) and the instability of *S. hermonthica* germination inhibitor(s) produced by PGPR (Miché et al. 2000). It was expected that this would result to an improved growth promotion (biomass) of *S. hermonthica*-infected sorghum, because PGPR alone, as exemplified by *B. subtilis*, *B. amyloliquefaciens*, and *B. phytofirmans*, were shown not to improve the biomass of *S. hermonthica*-infected sorghum (Mounde et al. 2015). Our expectation for a synergistic interaction between *Fos* and PGPR was based on the compatibility of *Fos* with indigenous maize rhizosphere microbiota (Musyoki et al. 2016; Zimmermann et al. 2016b). Thus, in the framework of a *S. hermonthica*–sorghum pathosystem, the study aimed at examining: (i) the role/impact of *Fos* isolates (Foxy-2, FK3) with *B. subtilis* isolate GB03 coinoculation on *S. hermonthica* attachment and sorghum biomass, when applied as *Striga* preconditioning and

Striga post-attachment treatments, and (ii) the localization/interaction of the *Fos*-GB03 coinoculants in affected *S. hermonthica* plants.

4.3 Materials and methods

4.3.1 Preparatory work

4.3.1.1 Plant materials, microbial isolates/strains, and plasmids

S. hermonthica seeds, sampled from Sirinka, Ethiopia, were used as parasitic (model) plant in this study. *S. hermonthica*-susceptible sorghum (*Sorghum bicolor* L. Moench) cultivar PI563294 from Niger was used as host (model) crop. From our previous study (Anteyi and Rasche 2020), the sampled *S. hermonthica* population displayed selective susceptibility to FK3 but not to Foxy-2. Also, the utilized sorghum cultivar exhibited susceptibility to diverse *S. hermonthica* populations from East and West Africa. *B. subtilis* isolate GB03, designated as 3A37 strain, was obtained from the *Bacillus* Genetic Stock Centre, Ohio State University, Columbus, Ohio. It is noteworthy to state that the GB03 isolate was first identified as *B. subtilis* and later as *B. amyloliquefaciens*; however, due to its genome sequence, the strain 3A37 has been reclassified as *B. velezensis* (D. R. Zeigler, *personal communication*). *B. subtilis* isolate GB03 was selected as a representative PGPR for this study, following its outstanding ability to inhibit *S. hermonthica* germination with haustorial attachment and promote the biomass of *S. hermonthica*-free sorghum plants in a *S. hermonthica*–sorghum pathosystem, compared with other PGPR (i.e., *B. subtilis* isolate Bsn5, *B. amyloliquefaciens* isolate FZB42, and *B. phytofirmans* isolate PsJN) (Mounde et al. 2015). *Trichoderma viride* strain IMB12098, DSM number 63065, was procured from the Leibniz-Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) GmbH, Braunschweig, Germany. *Fos* isolates Foxy-2 and FK3 were obtained as 20% glycerol stocks from a –80°C microbial cell bank at the Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Germany.

pFPL-Ch (61647) and pFPL-Rh (61649) plasmids were procured from Addgene (2019) repository. Development of these plasmids were described by Gong et al. (2015). Both plasmids contain antibiotic resistance genes for chloramphenicol, kanamycin, and hygromycin (as selectable markers). pFPL-Ch contained a 720-bp gene insert, enhanced cyan fluorescent protein, for cyan fluorescence expression. A 678-bp gene insert, monomeric red fluorescent protein in pFPL-Rh, is responsible for red fluorescence expression (Fig. 4.1).

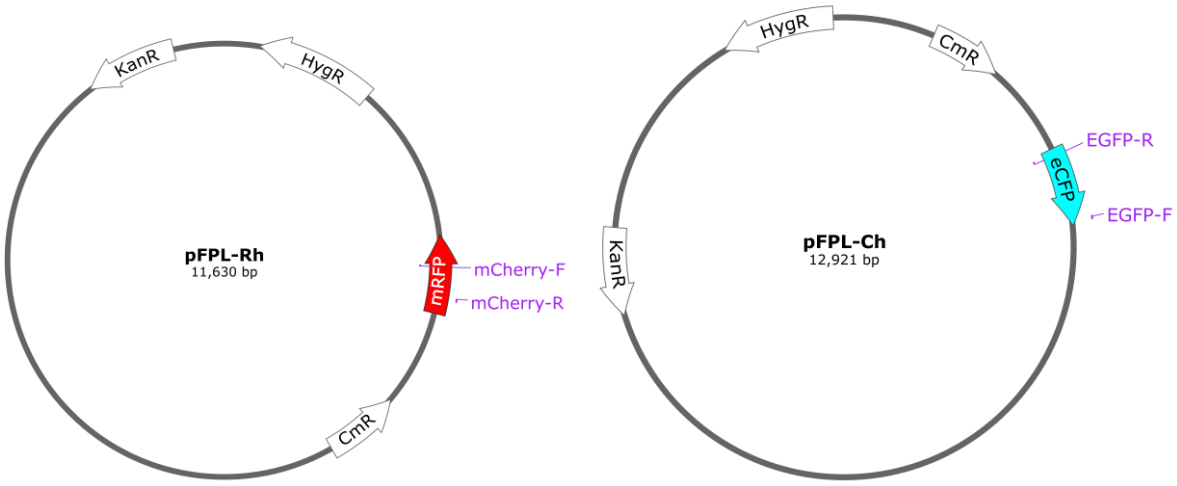

Fig. 4.1: Schematic plasmid maps of pFPL-Rh and pFPL-Ch with fluorescent proteins, specific primers used, and antibiotic resistance. CmR = chloramphenicol; KanR = kanamycin; HygR = hygromycin; eCFP = enhanced cyan fluorescent protein; and mRFP = monomeric red fluorescent protein. Plasmid maps were created with SnapGene Viewer 5.0.7.

4.3.1.2 Seed sterilization

Surface sterilization of *S. hermonthica* and sorghum seeds was performed according to the method of Anteyi and Rasche (2020). For *S. hermonthica*, the seeds were first submerged in a mixture of 5% (v/v) sodium hypochlorite (containing 12% chlorine) (Carl Roth GmbH, Karlsruhe, Germany) and 0.02% (v/v) Tween 20 (Carl Roth GmbH). Then, sonication for 3 min (Bandelin Sonopuls GmbH, Berlin, Germany) was followed by five to six rinses with double-distilled water (ddH_2O). Surface sterilization of sorghum seeds was by immersing in 70% ethanol for 2 min, followed by rinsing thrice with ddH_2O. Then, seeds were submerged in a blend of 1% sodium hypochlorite and 0.02% (v/v) Tween 20 for 3 min; finally, they were rinsed five to six times with ddH_2O. Glass fibre filter papers (90 mm, Macherey-Nagel GmbH, Düren, Germany) were oven sterilized for 48 h at 65°C and then placed under a laminar flow hood with UV for 2 h. Surface-sterilized *S. hermonthica* and sorghum seeds were separately air dried on the sterilized glass fibre filter papers under a laminar flow hood without UV for 48 h.

4.3.1.3 Germination percentage of surface-sterilized S. hermonthica seeds

Approximately 200 surface-sterilized *S. hermonthica* seeds were spread on an 8-mm punched disc of sterilized glass fibre filter papers. Six discs (replicates) carrying the *S. hermonthica* seeds were placed on two layers of 90-mm sterilized glass fibre filter papers, within a 90-mm Petri dish. Six millilitres of ddH_2O were added to the Petri dish. Then, the Petri dish was covered, sealed with Parafilm, and incubated in a dark chamber at 30°C for 14 days, for

preconditioning. Thereafter, the discs (with preconditioned seeds) were transferred onto a fresh glass fibre filter paper, within a Petri dish, and 3 ml of a 0.1-ppm synthetic strigolactone analog, rac-GR24 (Chiralix B.V., Nijmegen, Netherlands) was applied to the Petri dish. The setup was re-incubated at 30°C for 24 h for germination. Afterward, germinated seeds were counted under a binocular microscope (Zeiss Stemi 2000-C Stereomicroscope, Carl Zeiss Microscopy GmbH, Jena, Germany), coupled with the Zeiss AxioCam HRc (Carl Zeiss Light Microscopy, Göttingen, Germany). This germination assay was done in two experimental repetitions.

4.3.2 Fos-GB03 coinoculation study

4.3.2.1 Pre-Striga seed conditioning treatment application

Microbial culturing and inocula concentrations: Mycelial suspensions of Foxy-2 and FK3, including IMB12098, were separately prepared by flooding the surface of their 2-week-old actively growing potato dextrose agar (PDA) (Carl Roth GmbH) cultures in 90-mm Petri dishes, each with 10 ml of 25% potato dextrose broth (PDB) (Carl Roth GmbH). Then, they were left to stand for 30 min, before gently scraping the mycelial mass into sterile flasks containing 2 litres of 25% PDB. For colony growth, the flasks were loosely covered with their lids and placed in a dark incubating shaker for 15 days at 65 rpm and 28°C. A colony of GB03 was separately transferred from overnight Luria-Bertani (LB) agar cultures into sterile flasks containing 2 litres of 15% LB broth. The loosely covered flasks were placed in a dark incubating shaker for 4 days at 80 rpm and 35°C. Inocula concentrations in colony forming units per millilitre (CFU/ml) were determined from six replicates of each microbial inoculum: Foxy-2 and FK3, 10^4 to 10^5; GB03, 10^6 to 10^7; and IMB12098, 10^7. The prepared fungal and bacterial inocula were subsequently used as pre-*Striga* seed conditioning planting treatments.

Planting trials: Sixty grams of sterilized modular seed substrate (Klasmann-Deilmann GmbH, Geeste, Germany) was filled into polyvinyl chloride rhizoboxes of dimensions 20 × 5 × 2 cm. Then, 60 mg of surface-sterilized *S. hermonthica* seeds were sown 5 cm below the substrate surface, and the front side of each rhizobox was covered with Plexiglas. Sixty millilitres of ddH_2O or the prepared microbial inocula (as single or combined fungi + bacteria coinoculation) were applied to each rhizobox as treatments. For rhizoboxes containing combined fungi + bacteria treatments, 30 ml of each microbial inoculum was added. Also, *T. viride* (IMB12098 strain) was introduced among the planting treatments to serve as a check BCA treatment, because *T. viride* was reported to completely inhibit *S. hermonthica* seed germination at 10^6 spores/ml inoculum concentration (Hassan et al. 2013). Hence, treatments included (i) ddH_2O without *S. hermonthica* (negative control); (ii) *S. hermonthica* with ddH_2O (positive control); (iii) *S. hermonthica* with FK3 and GB03 (*Striga* + FK3 + GB03); (iv) *S. hermonthica* with Foxy-

2 and GB03 (*Striga* + Foxy-2 + GB03); (v) *S. hermonthica* with IMB12098 and GB03 (*Striga* + IMB12098 + GB03); (vi) *S. hermonthica* with GB03 (*Striga* + GB03); (vii) *S. hermonthica* with FK3 (*Striga* + FK3); (viii) *S. hermonthica* with Foxy-2 (*Striga* + Foxy-2); (ix) *S. hermonthica* with IMB12098 (*Striga* + IMB12098). The setup was incubated at 30°C for 10 days in a dark climate chamber (Percival Intellus Environmental Controller, EA-75HIL, Perry, IA) for *S. hermonthica* seed preconditioning. The experiment was set up in a randomized complete block design, with three replications for each treatment. Thereafter, a single sorghum seed was sown in every rhizobox, and climate chambers were set at 31 and 27°C, for day and night temperatures, respectively. Light of mean illuminance 46,000 lx was available for 12 h (as day), but without light as night. Plants were irrigated twice per week, first with ddH_2O and then with nutrient solution comprising a blend of 0.2% (v/v) Wuxal universal liquid fertilizer (Aglukon Spezialdünger GmbH, Düsseldorf, Germany) and Yoshida nutrient solution (Yoshida et al. 1976) in a ratio of three parts Wuxal to two parts Yoshida. The planting trials were done in two experimental repetitions, with a 5-week growing period for each trial.

4.3.2.2 Post-Striga attachment treatment application and localization study

<u>*Antimycotic and antibiotic sensitivity determination*</u>: Freshly prepared, molten PDA was supplemented with hygromycin B (Carl Roth GmbH) at 0, 25, 50, 75, 100, 125, 150, 175, and 200 µg/ml. An 8-mm agar plug from actively growing Foxy-2 and FK3 mycelia was each transferred to 90-mm Petri dishes containing the solidified media. The experiment was set up in three replications. The cultures were incubated in the dark for 7 days at 28°C, followed by determination of mycelial radial growth. Hygromycin B concentration of 125 and 150 µg/ml completely inhibited mycelial growth of wild-type Foxy-2 and FK3, respectively (Fig. 4.2). Sensitivity assay revealed that 200 µg/ml of hygromycin B completely inhibited wild-type GB03. For kanamycin sensitivity, 50 µg/ml was used (http://www.addgene.org/mol-bio-reference/). Hence, growth medium supplemented with 200 µg/ml of hygromycin B and 50 µg/ml of kanamycin sulphate (Carl Roth GmbH), hereafter abbreviated as Hyg-B/Kan, was adopted for selectively screening transformed *Fos* or transformed GB03.

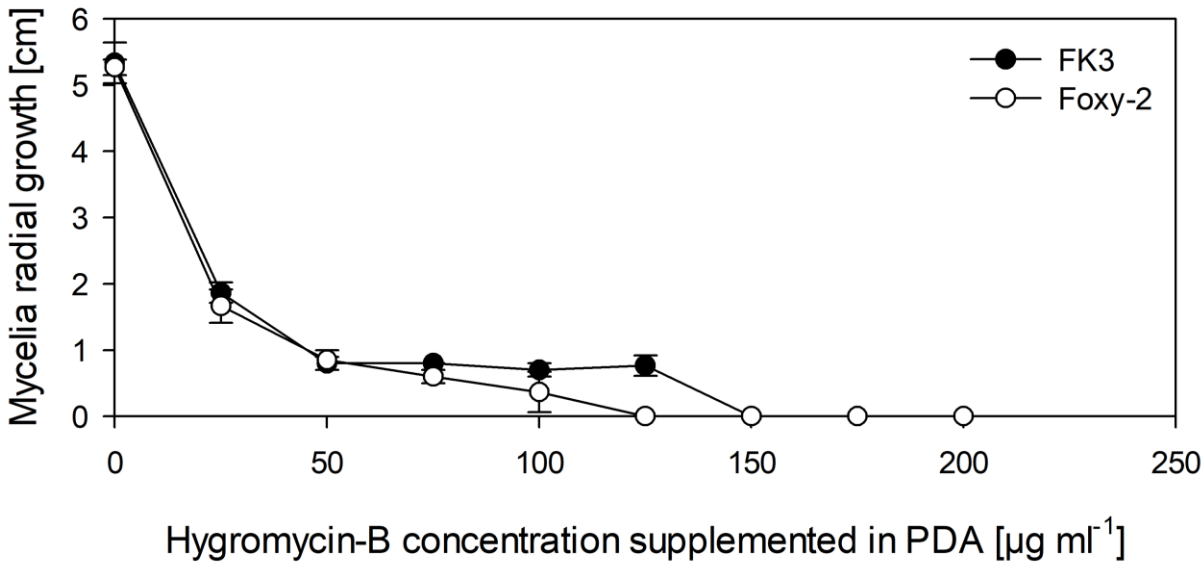

Fig. 4.2: Hygromycin B sensitivity test to determine mycelial growth inhibition in wild-type FK3 and wild-type Foxy-2. Error bars indicate standard deviation. PDA = potato dextrose agar.

Plasmid DNA isolation: Using the Invisorb Spin Plasmid Mini Two (Stratec Molecular GmbH, Berlin, Germany), plasmid DNA (pDNA) was isolated from the bacteria containing the plasmids (i.e., pFPL-Ch and pFPL-Rh) after culturing a single colony of the bacteria in 15-ml tubes containing 2 ml of 15% LB broth (Carl Roth GmbH) with Hyg-B/Kan for 15 h at 100 rpm and 37°C in a dark shaker. Diagnostic restriction digestion with *Hind*III, *Fsp*I, *Kpn*I, and *Afl*II (New England Biolabs GmbH, Frankfurt am Main, Germany) was performed to verify the plasmids. Digested DNA products confirmed the plasmids, as shown by 1.5% agarose gel electrophoresis (see appendix 3).

Fos transformation mediated by polyethylene glycol (PEG): The procedure for PEG-mediated transformation of *Fos* was modified from the protocol for PEG-mediated transformation of *F. oxysporum* f. sp. *conglutinans* (Zhang et al. 2014). The detailed methodology is given in appendix 4.

GB03 transformation ("mixed salts" mediated): The protocol for GB03 transformation was adapted from the International Genetically Engineered Machine (iGEM 2014) (Ludwig Maximilian University of Munich, Germany), but with minor adjustments. The detailed methodology is provided in appendix 5.

Screening and verification of Fos and GB03 transformants: *Fos* and GB03 transformants were screened by their ability to grow on antibiotic-supplemented selective medium, in which wild-type *Fos* and GB03 were unable to grow (Fig. 4.3). The mycelia of transformed *Fos* and a

colony of transformed GB03 were also screened for fluorescence expression using the Zeiss Axioskop-2 Plus Upright Fluorescence Microscope (Carl Zeiss Microscopy GmbH), and AxioVision SE64 Rel. 4.8 software (Jülich GmbH, Bonn, Germany). Red fluorescence expression in transformed *Fos* was detected at 546 nm (excitation) and 580 nm (emission), whereas cyan fluorescence expression in transformed GB03 was detected at 475 nm (excitation) and 500 nm (emission) (Fig. 4.4).

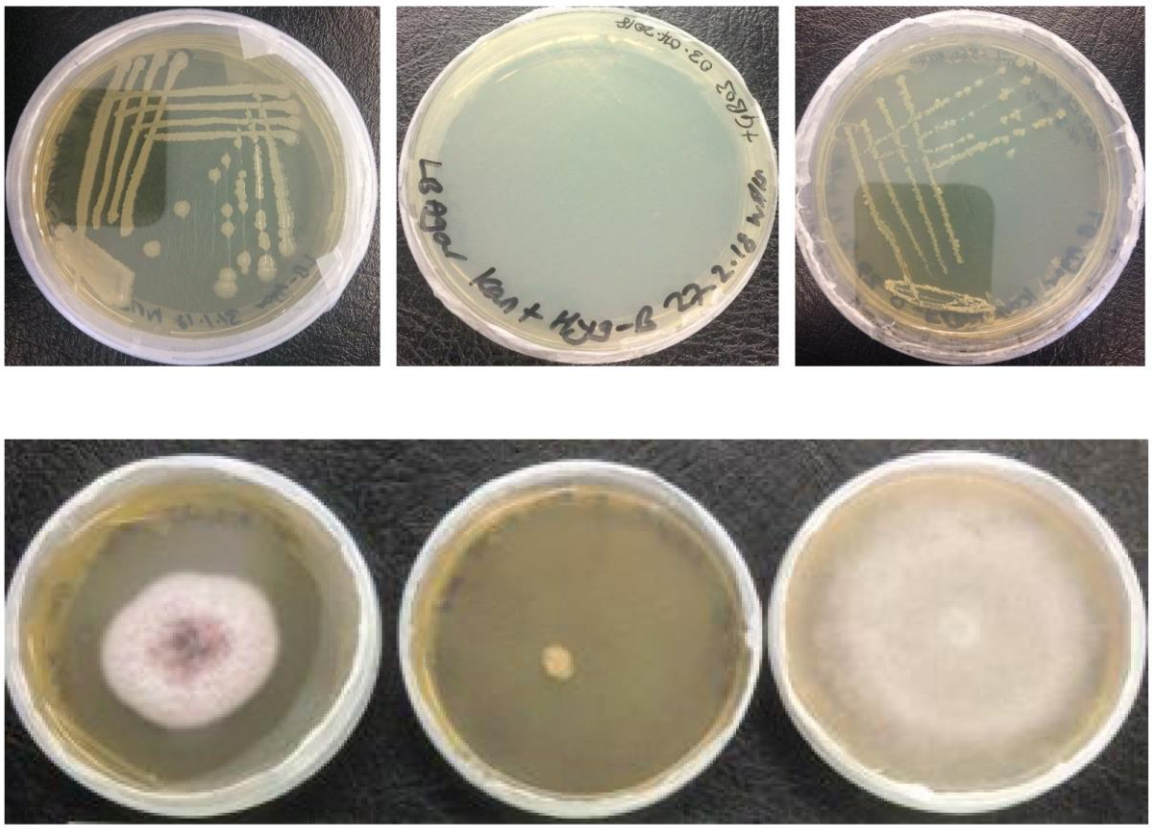

Fig. 4.3: Wild-type GB03 growing on Luria-Bertani (LB) agar (top left). Wild-type GB03 unable to grow on LB agar supplemented with 200 µg/ml of hygromycin B and 50 µg/ml of kanamycin sulfate (Hyg-B/Kan) (top center). Transformed GB03 growing on LB agar supplemented with Hyg-B/Kan (top right). Wild-type FK3 growing on potato dextrose agar (PDA) (bottom left). Wild-type FK3 unable to grow on PDA supplemented with Hyg-B/Kan (bottom center). Transformed FK3 growing on PDA supplemented with Hyg-B/Kan (bottom right).

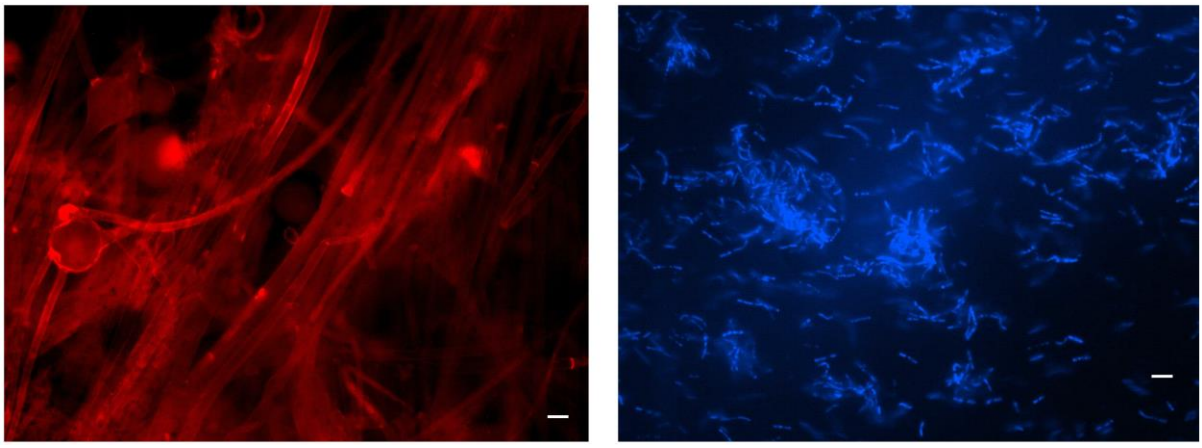

Fig. 4.4: Mycelia of transformed FK3 from potato dextrose agar supplemented with 200 µg/ml of hygromycin B and 50 µg/ml of kanamycin sulfate (Hyg-B/Kan) expressing red fluorescence (left). Short chains of transformed GB03 from Luria-Bertani agar supplemented with Hyg-B/Kan expressing cyan fluorescence (right). Scale bars = 50 µm.

To verify successful fluorescence gene integration in *Fos* and GB03 transformants, total genomic DNA (gDNA) was extracted from a 100-mg biomass of *Fos* hyphae and GB03 cellular pellets transformants (Fast DNA spin kit, MP Biomedicals, LLC). Primer sequences in the fluorescent gene inserts of the plasmids were identified with SnapGene Viewer 5.0.7 (GSL Biotech, https://www.snapgene.com/): mCherry primers for pFPL-Rh (mCherry-F, 5′-CCCCGTAATGCAGAAGAAGA-3′; mCherry-R, 3′-TTGGTCACCTTCAGCTTGG-5′) and EGFP primers for pFPL-Ch (EGFP-F, 5′-CATGGTCCTGCTGGAGTTCGTG-3′; EGFP-R, 3′-CGTCGCCGTCCAGCTCGACCAG-5′). Polymerase chain reaction (PCR) was done by a 50-µl reaction system, consisting of template DNA (pDNA, 5 ng; gDNA, 200 ng), 10× PCR buffer, 50 mM $MgCl_2$, 10 mM dNTPs, 20 µM forward and reverse primers for both mCherry and EGFP primers (biomers.net GmbH, Ulm, Germany), and 2.5 U of Accuzyme DNA Polymerase (Bioline GmbH, Luckenwalde, Germany). PCR conditions with mCherry primers included initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s. Reaction was completed with a final extension at 72°C for 4 min. With EGFP primers, PCR conditions were initial denaturation at 95°C for 3 min, then 30 cycles at 95°C for 15 s, 60°C for 15 s, 72°C for 45 s, and a final elongation at 72°C for 4 min.

Microbial transformants culturing and inocula concentrations: Preparation of transformed *Fos* and GB03 microbial cultures and determination of their inocula concentrations in CFU/ml were similar to the procedures described above for their corresponding wild-type microbial treatment cultures. Inocula concentrations of the transformed microbial cultures in CFU/ml were 10^5 to 10^6 for transformed Foxy-2 and transformed FK3 and 10^6 for transformed GB03.

The prepared transformed microbial inocula, together with their wild types, were used as post-*Striga* attachment planting treatments.

Planting trials and localization study: The planting trials made use of wild-type microbial treatments to study the impact of the post-*Striga* attachment treatment application on the sorghum–*S. hermonthica* pathosystem. It utilized the transformed microbial treatments to examine the localization/interaction of *Fos* and GB03 in affected *S. hermonthica*, to give further insight into the post-*Striga* attachment treatment application of *Fos*-GB03. Planting for the post-*Striga* attachment treatment application study followed similar technical steps, experimental conditions, setup, and iterations as described for the pre-*Striga* seed conditioning treatment application study (see above). However, here, treatments were applied following *S. hermonthica* attachment to sorghum root, precisely 3 weeks after sowing sorghum. This timing was also ascertained from the average period for visible *S. hermonthica* attachment. Sixty millilitres of the treatments, that is, ddH_2O or the prepared microbial inocula (as single or combined fungi + bacteria coinoculation, in separate wild-type and transformed setups) was directly applied on the substrate surface and/or on emerged (aboveground) *S. hermonthica* in each rhizobox. Similarly, in rhizoboxes for combined fungi + bacteria treatments, 30 ml of each microbial inoculum was added. Thus, treatments were (i) ddH_2O without *S. hermonthica* (negative control); (ii) ddH_2O with *S. hermonthica* (positive control); (iii) *S. hermonthica* with wild-type FK3 and wild-type GB03 (*Striga* + FK3 + GB03); (iv) *S. hermonthica* with wild-type Foxy-2 and wild-type GB03 (*Striga* + Foxy-2 + GB03); (v) *S. hermonthica* with wild-type GB03 (*Striga* + GB03); (vi) *S. hermonthica* with wild-type FK3 (*Striga* + FK3); (vii) *S. hermonthica* with wild-type Foxy-2 (*Striga* + Foxy-2); (viii) *S. hermonthica* with transformed FK3 and transformed GB03 (*Striga* + FK3[T] + GB03[T]); (ix) *S. hermonthica* with transformed Foxy-2 and transformed GB03 (*Striga* + Foxy-2[T] + GB03[T]); (x) *S. hermonthica* with transformed GB03 (*Striga* + GB03[T]); (xi) *S. hermonthica* with transformed FK3 (*Striga* + FK3[T]); and (xii) *S. hermonthica* with transformed Foxy-2 (*Striga* + Foxy-2[T]). Planting period was for 7 weeks to allow for adequate microbial colonization of sorghum for microscopic analysis.

In vivo localization analysis (microscopy and PCR): Localization of *Fos* and GB03 in infected and diseased *S. hermonthica* was studied through fluorescence expression by transformed *Fos* and transformed GB03 in infected *S. hermonthica* plants. Histological sections of *S. hermonthica* belowground and aboveground shoots and leaves were analysed by digital imaging, using Zen software (version 2010) with a Zeiss laser scanning microscope (Carl Zeiss Microscopy GmbH). The presence of *Fos* and GB03 transformants in *S. hermonthica* was further confirmed by PCR, using primers specific to the fluorescence gene regions in the plasmids (see above).

4.3.3 Plant data collection and biostatistical analysis

The total counts of attached *S. hermonthica*, belowground and aboveground, in each rhizobox were recorded. Sorghum aboveground biomass was harvested and oven dried at 65°C for 10 days, and dry mass was determined. Quantitative data of the attached *S. hermonthica* and the sorghum aboveground dry biomass were tested by analysis of variance (ANOVA) and post hoc analysis by Tukey's range test. For purposes of clarity and easy comparison between the *S. hermonthica* attachment and sorghum aboveground dry biomass, graphical presentation of Tukey's range test for the mean data values was standardized (as percentage), relative to the positive control (without altering the output of the analyses).

Calculation by Pearson correlation coefficient was used to corroborate the negative correlation between the sampled *S. hermonthica* attachment and sorghum aboveground dry biomass (of the adopted sorghum cultivar) in the presence of microbial treatments. This was because from repeated preliminary studies, in the absence of microbial treatments, a strong negative correlation (r = –0.98, P < 0.0001) was calculated between the sampled *S. hermonthica* attachment and the aboveground dry biomass of the same sorghum cultivar (see appendix 1). Evaluation of the impact of *Fos* and GB03 on *S. hermonthica*, when the microbial treatments were applied at post-*Striga* attachment, was indirectly determined from the sorghum aboveground dry biomass. This was a reliable estimator for the microbial treatments' effects on the already attached *S. hermonthica* plants. This indirect estimation was also supported by the stable and negative correlation between the *S. hermonthica* attachment and the sorghum aboveground dry biomass. All statistical analyses were carried out using SAS 9.4 (SAS Institute, Cary, NC).

4.4 Results

4.4.1 Role/impact of Fos-GB03 coinoculation on S. hermonthica attachment and sorghum biomass

4.4.1.1 Pre-Striga seed conditioning treatment application

The surface-sterilized *S. hermonthica* seeds had a mean percentage germination of 55%. ANOVA revealed that pre-*Striga* seed conditioning treatment application had different effects on *S. hermonthica* attachment (P < 0.01) and sorghum aboveground dry biomass (P < 0.01). Combined treatments of FK3 + GB03 and Foxy-2 + GB03 increased sorghum aboveground dry biomass (P < 0.05), but IMB12098 + GB03 did not. Neither FK3 + GB03, Foxy-2 + GB03, nor IMB12098 + GB03 reduced *S. hermonthica* attachment (P > 0.05). FK3 and GB03, applied as single treatments, increased sorghum aboveground dry biomass (P < 0.05). Single

treatments of Foxy-2 and IMB12098 did not increase sorghum aboveground dry biomass ($P > 0.05$). FK3 suppressed *S. hermonthica* attachment ($P < 0.05$), an effect not revealed for GB03, Foxy-2, and IMB12098 (Fig. 4.5). GB03 applied as a single treatment was ineffective in suppressing the attachment of the sampled *S. hermonthica*, and it thwarted *Fos* suppressive activity against *S. hermonthica* attachment. Nevertheless, GB03 promoted sorghum yield, either when applied alone or in combination with *Fos* ($P < 0.05$). This indicated that *Fos* (FK3) was primarily responsible for causing disease and death to *S. hermonthica*, whereas *B. subtilis* (GB03) was responsible for relieving the menace of *S. hermonthica* on sorghum productivity by promoting sorghum growth. In the presence of the microbial treatments, sorghum aboveground dry biomass showed a strong negative correlation to *S. hermonthica* attachment ($P < 0.05$) (Fig. 4.6).

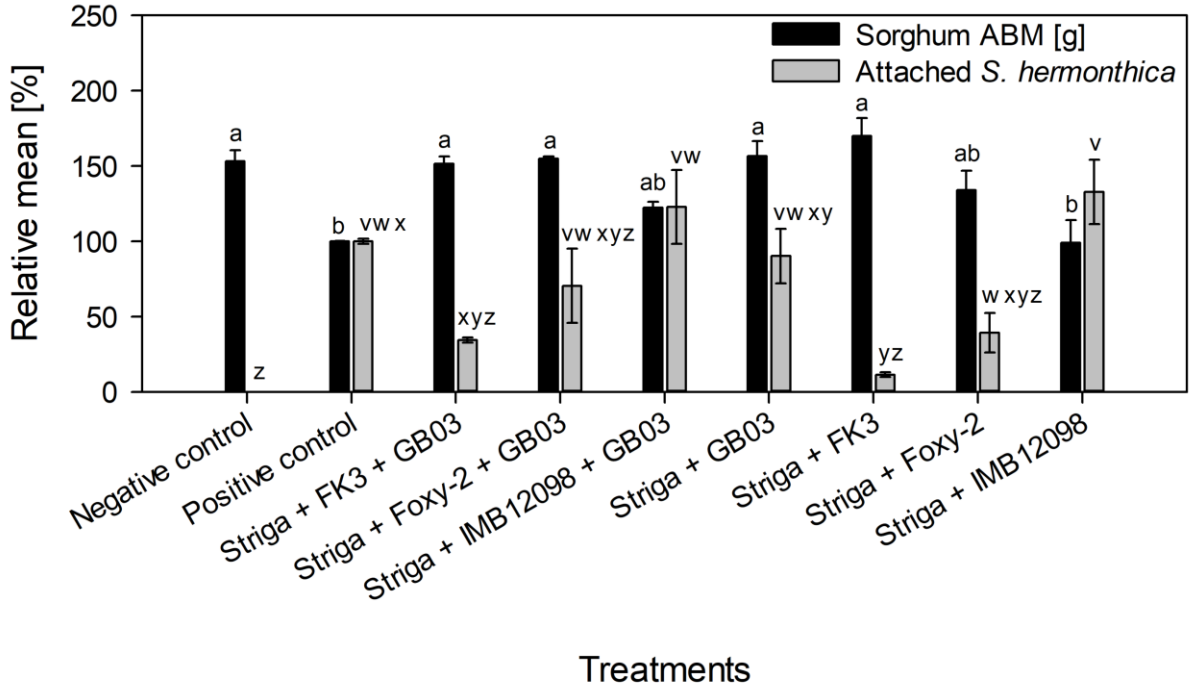

Fig. 4.5: Tukey's range test for relative mean values of sorghum aboveground dry biomass (sorghum ABM) and *Striga hermonthica* attachment responses to pre-*Striga* seed conditioning treatment application. Negative control = ddH_2O without *S. hermonthica*. Positive control = *S. hermonthica* with ddH_2O. Bars having at least a letter in common represent treatments means that are not significantly different ($\alpha = 0.05$). Error bars indicate standard error.

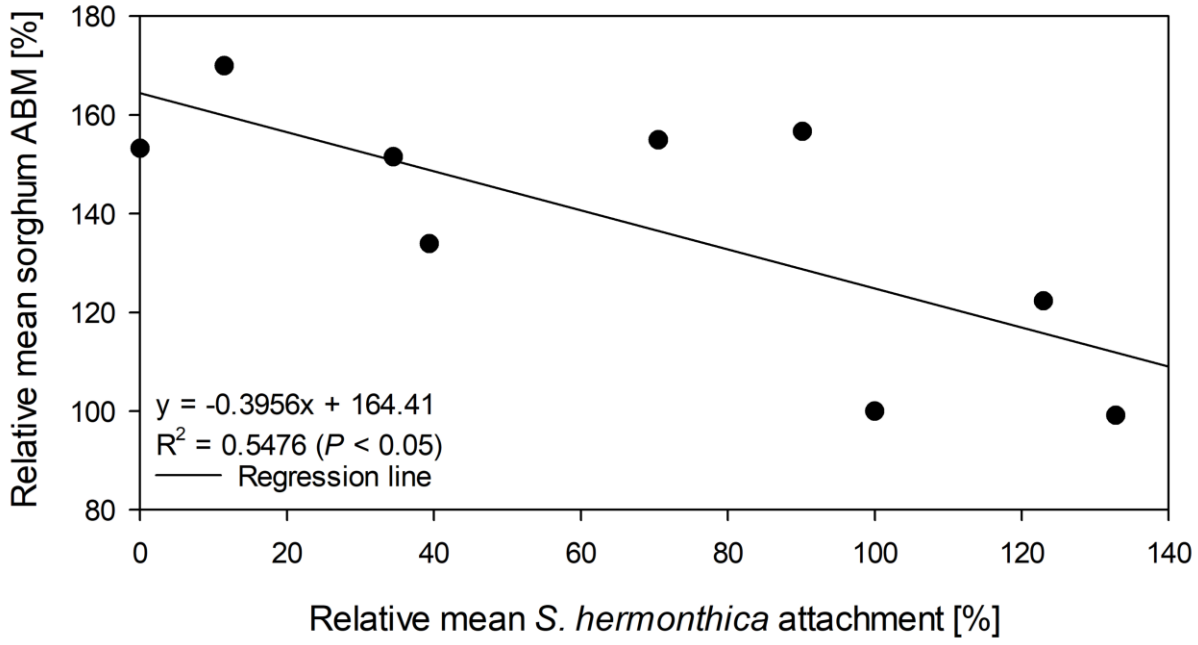

Fig. 4.6: Correlation and regression between relative mean values of sorghum aboveground dry biomass (sorghum ABM) and *Striga hermonthica* attachment in presence of microbial treatments.

4.4.1.2 Post-Striga attachment treatment application

Similarly, post-*Striga* attachment microbial treatment application had a different effect on sorghum aboveground dry biomass ($P < 0.0001$). In both wild-type or transformed microbial treatments, all *Fos*-GB03 combined treatments and single treatments of FK3 and GB03 improved sorghum aboveground dry biomass ($P < 0.05$). However, with single treatments of Foxy-2, the sorghum aboveground dry biomass was not higher than the positive control ($P > 0.05$) (Fig. 4.7).

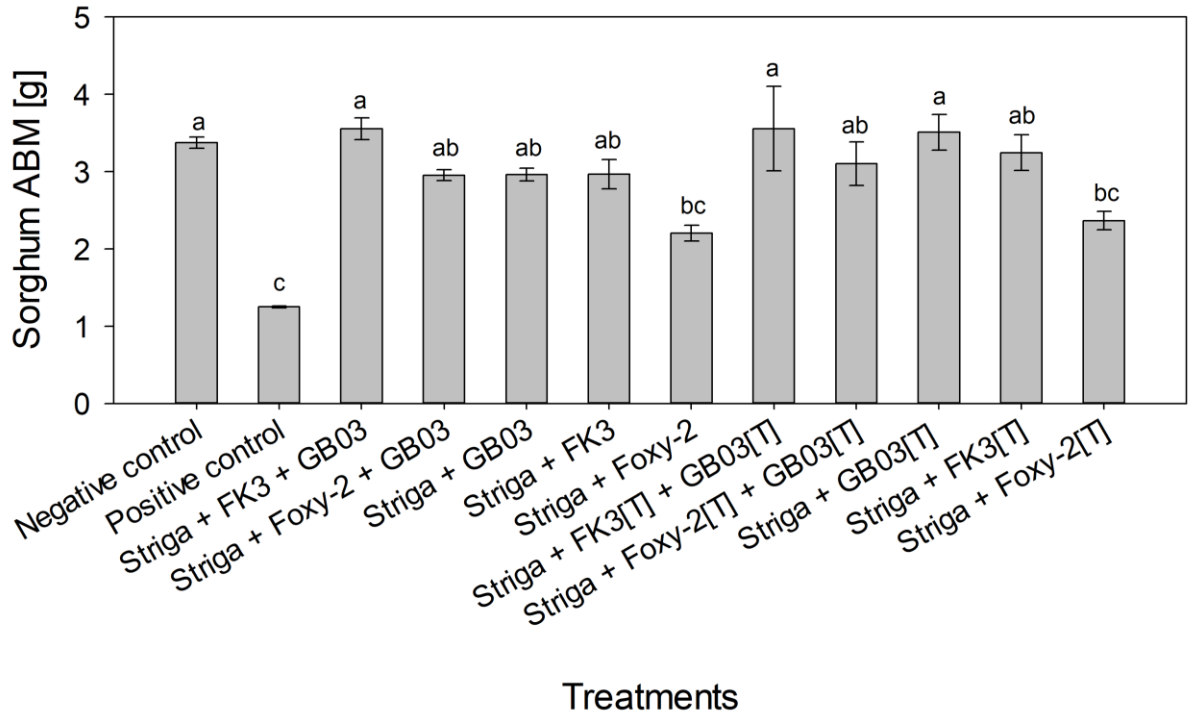

Fig. 4.7: Tukey's range test for sorghum aboveground dry biomass (sorghum ABM [g]) response to post-*Striga* attachment application of wild-type and transformed (indicated with [T]) microbial treatments. Negative control = sorghum + ddH_2O without *Striga hermonthica*. Positive control = sorghum + ddH_2O with *S. hermonthica*. Bars having at least a letter in common represent treatments means that are not significantly different ($\alpha = 0.05$). Error bars indicate standard error.

4.4.2 Localization/interaction of Fos and B. subtilis in emerged S. hermonthica

Morphologically, *S. hermonthica* from the positive control (i.e., treated with ddH_2O) showed no infection signs (Fig. 4.8A). Also, microscopic examination of the positive control showed healthy *S. hermonthica* leaf, phloem, and xylem tissues (Fig. 4.8B to D). For *S. hermonthica* treated with *Fos* (particularly FK3), infection of *S. hermonthica* through trichome entry was revealed (Fig. 4.8E). *Fos* infection and pathological damage of emerged (aboveground) *S. hermonthica* originated (and was more intense) on *S. hermonthica* shoots, before spreading throughout the entire plant structure (Fig. 4.8F and G). Following *Fos* (FK3) colonization of *S. hermonthica*, *Fos* degraded *S. hermonthica* tissues until plant death (Fig. 4.8H). Then, it thrived on the dead and decaying *S. hermonthica* matter (this was also substantiated by the persistent fluorescence expression) (Fig. 4.8I). This necrotrophic attribute of FK3 was not displayed by GB03. Cyan fluorescence detection at 475 to 500 nm indicated high GB03 localization in flavonoid-rich spots of *S. hermonthica* shoot tissue (Fig. 4.8J). *Fos*, either as sporal (conidia) or vegetative form (mycelium), infected and co-occupied common ecological niches with GB03 in diseased *S. hermonthica* shoots (i.e., both *Fos* and GB03 colocalized degrading shoot tissue of infected *S. hermonthica*) (Fig. 4.8K and L). The interaction of *Fos* and GB03 (colocalization) was not distinctly observed in *S. hermonthica* leaves. Colocalization of *Fos* and GB03 in the infected *S. hermonthica* was further confirmed by PCR of fluorescent genes carried by the transformed microbial inoculants, from planting trials of the post-*Striga* attachment treatment application (see appendix 6).

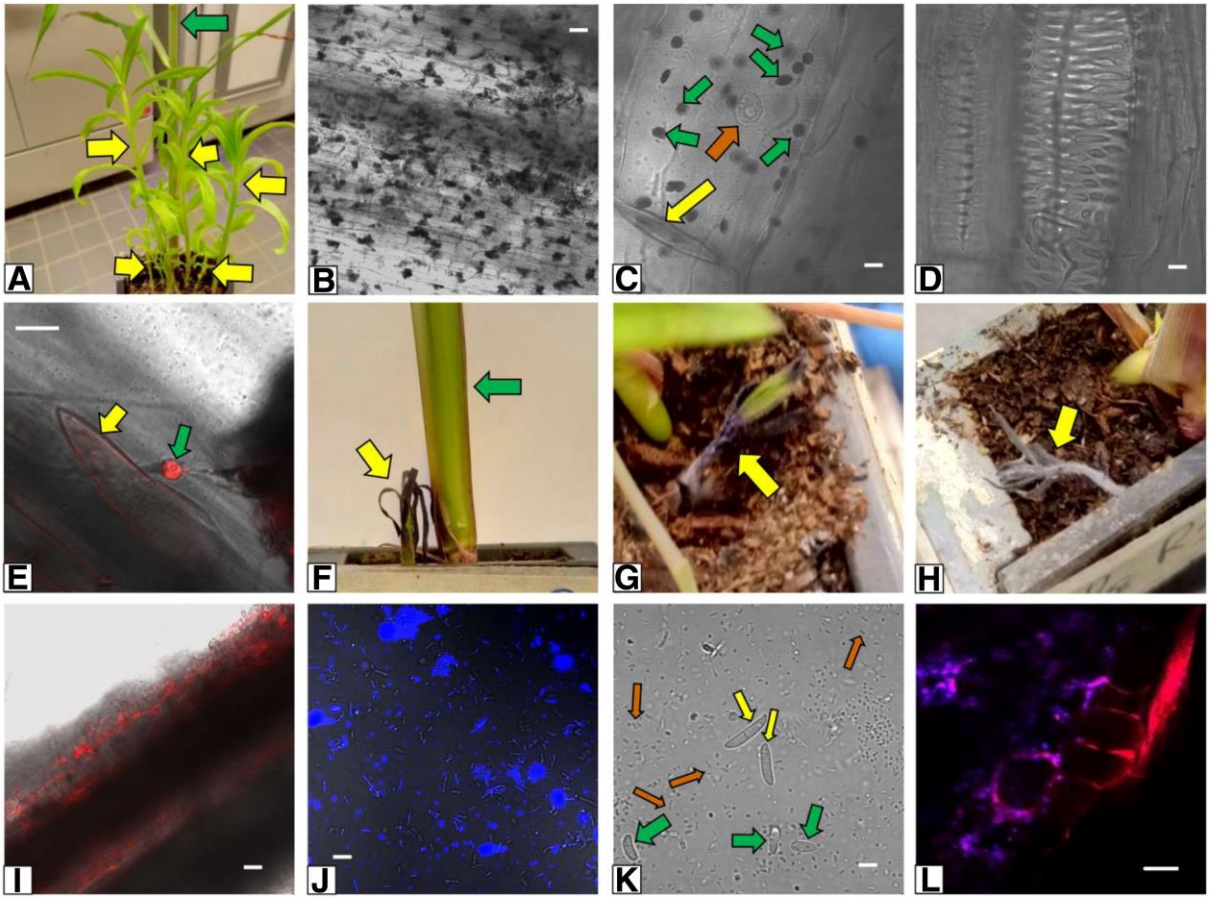

Fig. 4.8: (A) Healthy *Striga hermonthica* plants (yellow arrows) parasitizing sorghum (green arrow) in the positive control rhizobox. **(B)** Healthy *S. hermonthica* leaf from the positive control showing chloroplasts (numerous dark spots). **(C)** Sieve plate of sieve tube element (yellow arrow), chloroplasts (green arrows), and a companion cell nucleus (brown arrow) of healthy *S. hermonthica* phloem from the positive control. **(D)** Reticulate vessel of noninfected *S. hermonthica* xylem from the positive control. **(E)** A germinating FK3 chlamydospore (green arrow), with its germ tube penetrating the *S. hermonthica* trichome (yellow arrow). **(F)** FK3-infected *S. hermonthica* (yellow arrow), parasitizing sorghum (green arrow). **(G)** FK3 colonizing diseased *S. hermonthica* shoot (yellow arrow). **(H)** Dead and degrading *S. hermonthica* plant (yellow arrow) completely colonized by FK3. **(I)** Transformed FK3 expressing red fluorescence in diseased, necrotic, and decaying *S. hermonthica* tissue. **(J)** Transformed GB03 expressing cyan fluorescence within shoot tissue of diseased *S. hermonthica* (ubiquitous autofluorescence is due to flavonoids). **(K)** Consortia of FK3 macroconidia (yellow arrows), FK3 microconidia (green arrows), and short chains of GB03 (brown arrows) colocalizing in a diseased *S. hermonthica* shoot. **(L)** Transformed FK3 hyphae and transformed GB03 occupying a common ecological niche of degrading *S. hermonthica* shoot tissue. Scale bars are 20 µm.

4.5 Discussion

4.5.1 FK3 controlled S. hermonthica attachment, and it infected S. hermonthica through trichome entry when applied post-Striga attachment

Among the microbial treatments, only FK3 effectively controlled the attachment of the sampled *S. hermonthica*. In assessing the variable susceptibility pattern of genetically diverse *S. hermonthica* populations from East and West Africa to *Fos* isolates, similarly sampled *S. hermonthica* from Sirinka, Ethiopia, displayed significant susceptibility to FK3 but not to Foxy-2 (Anteyi and Rasche 2020). This implied that the susceptibility pattern of a *S. hermonthica* population to *Fos* isolates, thus far, is a stable feature that is primarily explained by the innate attributes (genetic) of *S. hermonthica*. It therefore explains a finding from this study, as to why the effectiveness of a *Fos* isolate against *S. hermonthica* was not altered by the fungal treatment application period during planting. This was shown by the significant susceptibility of the sampled *S. hermonthica* to FK3 but not to Foxy-2, either when the microbial treatments were applied pre-*Striga* seed conditioning or post-*Striga* attachment. Hence, this corroborated the narratives that *Fos* mycoherbicidal action against *S. hermonthica* may occur through chemical (i.e., release of certain metabolites that inhibit *S. hermonthica* germination and/or attack young *S. hermonthica* plantlets belowground, before haustorial attachment) as well as mechanical (i.e., epidermal penetration of emerged or mature *S. hermonthica* shoot, especially through natural opening or wounds) modes (Abbasher and Sauerborn 1992; Yonli et al. 2010). For instance, *Fusarium* have been reported to produce chemical substances (e.g., fusaric acid, 9,10-dehydrofusaric acid, trichothecenes, and β-lactones) that inhibit *S. hermonthica* seed germination (Sugimoto et al. 2002; Xiang et al. 2017; Zonno et al. 1996). Likewise, the successful post-*Striga* attachment foliar application of *Fos* isolate PSM-197 against *S. hermonthica* has been earlier noted (Marley et al. 1999).

Fluorescence microscopic analysis revealed that when the microbial treatments were applied post-*Striga* attachment, trichome entry was among the mechanical means by which *Fos* (FK3) penetrated *S. hermonthica*. This finding contrasted the acknowledged indirect mode by which *Fos* infects *S. hermonthica*, whereby *Fos* hyphae penetrate *S. hermonthica* through the “sorghum central cylinder-*S. hermonthica* haustorial” connecting tissues within sorghum root, without causing any known pathological damage to sorghum (Elzein et al. 2010; Ndambi et al. 2011). *Fos* infection of *S. hermonthica* by trichome entry is possible, because *S. hermonthica* shoots and leaves are largely covered with unique trichomes (CABI 2019). Apart from the primary roles of trichomes, which include protection, water/moisture absorption, storage or secretion of specialized metabolic compounds, and elimination of excess toxic substances (Huchelmann et al. 2017; Werker 2000), they are also host sites for plant relationships with

endophytic fungi. Injured trichomes give rise to open bases or stalks, which can serve as favorable entry points for phytopathogens, thereby encouraging fungal infection (penetration and colonization). Thus, trichomes serve as an uncommon fungal niche (Andargie and Li 2016; Calo et al. 2006; Kim 2019).

FK3 colonized, degraded, killed, and thrived on the dead tissue of *S. hermonthica*. This is a typical necrotrophic lifestyle of *F. oxysporum* (Moore et al. 2018). The necrotrophic feature of *Fos*, together with its chlamydospore-forming ability, facilitates its existence as a soil-borne phytopathogen (Elzein et al. 2008b; 2010). This supports its ability to persist and proliferate in substrate or soil for long periods, even beyond the completion of a particular *S. hermonthica* biocontrol season (Ciotola et al. 1995; Sauerborn et al. 1996), although without underrating the importance of site-specific factors (i.e., soil physicochemical properties and climatic conditions) (Zimmermann et al. 2016a).

4.5.2 B. subtilis counteracted the fungal activity against S. hermonthica attachment

B. subtilis (GB03) did not prevent the attachment of the sampled *S. hermonthica*. This outcome was not in agreement with the observations of Mounde et al. (2015), in which they reported GB03 suppressive activity toward *S. hermonthica* attachment, through reduction of *Striga* germination and haustorial attachment, including tubercle death. It is uncertain if this disaccord was also due to a possible inconsistency of GB03 effectiveness against differing *S. hermonthica* populations (as existent with *Fos* isolates), especially going by GB03 effectiveness against a Malian (West African) *S. hermonthica* population used by Mounde et al. (2015), whereas our study was based on an Ethiopian (East African) *S. hermonthica* population.

Furthermore, integration of GB03 with fungal treatments (FK3, Foxy-2, IMB12098) counteracted their activity against *S. hermonthica* attachment. This resulted in an undesirable promotion of *S. hermonthica* attachment, in the order of the suppressive tendency of the applied fungal treatments toward *S. hermonthica*: FK3 < Foxy-2 < IMB12098. This fungal counteractivity by GB03 could be explained by potential fungal antagonizing substances released by *B. subtilis*. Generally, PGPR secrete extracellular metabolites in the form of lytic enzymes, lipopeptides, and hydrogen cyanide, which can antagonize a variety of fungi and other phytopathogens (Beneduzi et al. 2012; Goswami et al. 2016). For instance, *B. subtilis* produces a bioactive metabolite, bacillaene, a polyene polyketide that confers antifungal activity against the genera *Fusarium* sp. and *Trichoderma* sp. (Um et al. 2013). Other *B. subtilis* metabolites, such as bacillomycin D, macrolactins, iturin, and fengycin, are also important antagonists to *F. oxysporum* (Caulier et al. 2019; Cawoy et al. 2015; Yuan et al. 2012). This, however, does not imply that in field conditions or natural ecologies *Fos* will be

easily subdued (and restrained from controlling *S. hermonthica*) by *B. subtilis* or other comparable members of the soil microbiota. It was recently shown that apart from *Fos* being a naturally occurring, soil-borne phytopathogen with a successful biocontrol record against *S. hermonthica* in various agroecosystems, it is also compatible with indigenous maize rhizosphere microbiota, which include prokaryotic and fungal communities (Musyoki et al. 2016; Zimmermann et al. 2016b).

Cyan fluorescence detection revealed high GB03 localization in flavonoid-abundant spots of *S. hermonthica* shoot tissue. Typically, *Striga* spp. are rich in a variety of flavones (Hiremath et al. 1997; Kim et al. 2010; Nakanishi et al. 1985), and at 470 to 525 nm, flavonoids emit in the blue or cyan region of the visible spectrum (Müller et al. 2013; Roshchina 2012). Flavonoids are important signaling compounds for initiating plant-microbe interactions (Straney et al. 2002). For instance, it stimulates *Fusarium solani* macroconidia germination (Ruan et al. 1995), it is a carbon source for some bacteria (Hassan and Mathesius 2012), and it acts as a chemo-attractant for symbiotic rhizobacterial interaction with plants (Steinkellner and Mammerler 2007). Microbial recognition of *S. hermonthica* characteristic flavonoids (and/or other specific metabolites) may explain the attraction/infection of FK3 and GB03, in colocalizing *S. hermonthica* shoot tissue.

4.5.3 T. viride was not effective against the sampled S. hermonthica

T. viride (IMB12098) was initially included among the microbial treatments of the pre-*Striga* seed conditioning treatment application study, to serve as a reference to the investigated treatments. The decision was taken based on reports of the *S. hermonthica* seed germination inhibition activity of *Trichoderma* spp. For instance, Hassan et al. (2013) had reported *T. viride* of 10^6 spore/ml concentration to completely inhibit *S. hermonthica* germination (0%), but it enhanced *S. hermonthica* germination (90%) compared with their control (85%) at 10^1 spore/ml. In our study, a slightly higher *T. viride* concentration of 10^7 CFU/ml was used; nonetheless, it was not effective in controlling *S. hermonthica* germination and attachment. It could be speculated that this nonconformity of *T. viride* suppressive activity against *S. hermonthica*, as revealed by Hassan et al. (2013), was due to a different strain of *T. viride* used in their study. Alternatively, it may be ascribable to the different *S. hermonthica* population from Sinnar State (Sudan) used by Hassan et al. (2013), which was susceptible to *T. viride*, whereas our *S. hermonthica* from Sirinka (Ethiopia) was resistant to *T. viride*. The reason for the nonconformity remained unclear.

4.6 Conclusions

Despite the colocalization of *Fos* and GB03 in infected *S. hermonthica* shoots, the combined BCA treatment did not result in a stronger *S. hermonthica* control compared with the single FK3 treatment. The sampled *S. hermonthica* maintained its selective susceptibility to FK3 but not to Foxy-2. This variable susceptibility of *S. hermonthica* was perhaps also responsible for the inconsistent effectiveness of other acclaimed *S. hermonthica* BCAs (e.g., *B. subtilis* and *T. viride*) against our differing *S. hermonthica* population. Hence, prior to any *S. hermonthica* biocontrol program, a BCA strain/isolate confirmed for its effectiveness against a particular *S. hermonthica* population should be adhered to. This is clearly preferable, rather than applying a combination of contrasting BCAs, for which knowledge of their interaction or awareness on the susceptibility pattern of the *S. hermonthica* population toward the BCA is unknown or insufficient.

The effectiveness of a specific *Fos* isolate (i.e., FK3) against the sampled *S. hermonthica* was shown to be unaffected with changing *Fos* treatment application period during planting (i.e., pre-*Striga* seed conditioning or post-*Striga* attachment). Nevertheless, it is suggested to apply the BCA pre-*Striga* seed conditioning, because this would prevent *S. hermonthica* from infecting the host crop in the first place. Most times, the physiological damage initiated to the host crop by *S. hermonthica* could be irreversible (Beed et al. 2007; Rumsey 2012). Thus, exometabolites that specifically inhibit germination of *S. hermonthica* seeds, and/or haustoria development with attachment, shall be identified and validated from potent BCAs of *S. hermonthica* through tracer-assisted metabolomic techniques such as stable isotopy, positron emission tomography, nuclear magnetic resonance, and liquid/gas chromatography–mass spectrometry (Jang et al. 2018; Lin and Chung 2014). This could lead to the discovery of ecologically safe, specific metabolites, with stronger and broader efficacy range against diverse *S. hermonthica* populations.

Lastly, the discovery from this study, involving a phyllosphere-transmitting, direct *S. hermonthica* infection by *Fos* through trichome entry, offers an alternative entry point. This is an addition to the recognized, rhizosphere-transmitting, indirect infection mechanism through the “sorghum central cylinder-*S. hermonthica* haustorial” connecting tissues within sorghum root (Elzein et al. 2010; Ndambi et al. 2011). This revelation paves the way for further research possibilities that centre on exploiting this newly identified anatomical pathway for better *S. hermonthica* biocontrol. For instance, the induction of *Fos* sporal germination by particular *S. hermonthica* secondary metabolites can be further investigated through molecular identification and characterization of transcription factors with regulatory constituents by which a potent, *S. hermonthica*-attacking microbial strain specifically recognizes its host.

Chapter 5. Diacetoxyscirpenol, a *Fusarium* exometabolite, prevents efficiently the incidence of the parasitic weed *Striga hermonthica*

Williams O. Anteyi[1], Iris Klaiber[2], Frank Rasche[1]

[1]Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg-Institute), University of Hohenheim, 70593 Stuttgart, Germany.

[2]Core Facility Hohenheim, University of Hohenheim, 70593 Stuttgart, Germany.

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5.1 Abstract

Background: Certain *Fusarium* exometabolites have been reported to inhibit seed germination of the cereal-parasitizing witchweed, *Striga hermonthica*, *in vitro*. However, it is unknown if these exometabolites will consistently prevent *S. hermonthica* incidence *in planta*. The study screened a selection of known, highly phytotoxic *Fusarium* exometabolites, in identifying the most potent/efficient candidate (i.e., having the greatest effect at minimal concentration) to completely hinder *S. hermonthica* seed germination *in vitro* and incidence *in planta*, without affecting the host crop development and yield.

Results: *In vitro* germination assays of the tested *Fusarium* exometabolites (i.e., 1,4-naphthoquinone, equisetin, fusaric acid, hymeglusin, neosolaniol (Neo), T-2 toxin (T-2) and diacetoxyscirpenol (DAS)) as pre-*Striga* seed conditioning treatments at 1, 5, 10, 20, 50 and 100 µM, revealed that only DAS, out of all tested exometabolites, completely inhibited *S. hermonthica* seed germination at each concentration. It was followed by T-2 and Neo, as from 10 to 20 µM respectively. The remaining exometabolites reduced *S. hermonthica* seed germination as from 20 µM ($P < 0.0001$). *In planta* assessment (in a *S. hermonthica*-sorghum parasitic system) of the exometabolites at 20 µM showed that, although, none of the tested exometabolites affected sorghum aboveground dry biomass ($P > 0.05$), only DAS completely prevented *S. hermonthica* incidence. Following a 14-d incubation of DAS in the planting soil substrate, bacterial 16S ribosomal RNA (rRNA) and fungal 18S rRNA gene copy numbers of the soil microbial community were enhanced; which coincided with complete degradation of DAS in the substrate. Metabolic footprinting revealed that the *S. hermonthica* mycoherbicidal agent, *Fusarium oxysporum* f. sp. *strigae* (isolates Foxy-2, FK3), did not produce DAS; a discovery that corresponded with underexpression of key genes (Tri5, Tri4) necessary for *Fusarium* trichothecene biosynthesis ($P < 0.0001$).

Conclusion: Among the tested *Fusarium* exometabolites, DAS exhibited the most promising herbicidal potential against *S. hermonthica*. Thus, it could serve as a new biocontrol agent for efficient *S. hermonthica* management. Further examination of DAS specific mode of action against the target weed *S. hermonthica* at low concentrations (≤ 20 µM), as opposed to non-target soil organisms, is required.

Keywords: *Striga hermonthica*, *Fusarium oxysporum* f. sp. *strigae*, *Fusarium* exometabolites, Diacetoxyscirpenol, Biopesticides, Targeted metabolomics, Metabolic footprinting, Trichothecene gene expression.

5.2 Background

Cereal production in sub-Saharan Africa (SSA), like many crops in tropical/subtropical agroecosystems, is challenged by various biotic, abiotic, socio-cultural and socio-economic factors (Reynolds et al. 2015; Amelework et al. 2016). In terms of biotic constraints, the obligate hemiparasitic witchweed, *Striga* spp., especially *Striga hermonthica* (Delile) Benth., is a major factor (Emechebe et al. 2004; Ouedraogo et al. 2017). According to Gressel et al. (2004), out of the 26.23 million ha of all crop fields that were infested by *Striga* spp. in SSA, sorghum fields alone accounted for roughly 20 million ha, thereby causing an estimated yield loss of 6.5 to 6.9 million t per annum. Infestation by *S. hermonthica* begins after the release of organic stimulants (i.e., strigolactones) by the host cereal plants, inducing germination of preconditioned *S. hermonthica* seeds. Then, the elongating radicle of the *S. hermonthica* seedling develops into a haustorium, which is used to attach and penetrate the host cereal root (Saucet and Shirasu 2016). Successful parasitism of the host plant by *S. hermonthica* is ensured by vascular continuity between the parasite and the host. Through this channel, *S. hermonthica* accesses the host resources to support its life cycle, while the host plant health deteriorates (until death in serious cases), owing mainly to water and nutrient deficiency (Spallek et al. 2013).

Fusarium oxysporum f. sp. *strigae* (*Fos*) is a well-known bioherbicide (mycoherbicide), that has proven effective for controlling parasitic *Striga* spp., under both natural and artificial environments (Rebeka 2013; Nzioki et al. 2016; Shayanowako et al. 2018). Although, various *Fos* isolates with significant pathogenicity towards *S. hermonthica* have been reported (Zimmermann et al. 2015), in some cases, the bioherbicidal effectiveness of *Fos* isolates against *S. hermonthica* has shown to be inconsistent (Venne et al. 2009; Avedi et al. 2014; Anteyi and Rasche 2021). The inconsistent effectiveness of *Fos* isolates against differing *S. hermonthica* populations is, amongst others, a major challenge that deters the widespread application of any bioherbicide against its target weed (El-Sayed 2005; Harding and Raizada 2015). Like every functioning ecosystem (natural or artificial), the *Fos*–*S. hermonthica*–cereal crop pathosystem is made up of complex networks of bipartite, tripartite or multipartite interactions that occur both within and between the biotic (cereal crop, *S. hermonthica*, microorganisms) and abiotic (climate, water, soil physico-chemistry) components (Olff et al. 2009; Mounde et al. 2020). These ecologically complex interactions may eventually reduce or in some cases enhance the efficacy of mycoherbicides against their target weeds (Knudsen and Dandurand 2014). However, with respect to the bipartite interaction between *Fos* and *S. hermonthica*, the role of genetic diversity as an underlying factor for this inconsistent effectiveness has been well studied. For instance, the classification of genetically contrasting *Fos* isolates (e.g., Foxy-2 and FK3) into separate vegetative

compatibility groups (VCG) and mating types (de Klerk 2017), and population genomic structure in *S. hermonthica* populations with varying susceptibility response to Foxy-2 and FK3 (Anteyi and Rasche 2020). As a result, variation in the metabolome between genetically structured *S. hermonthica* groups were revealed through the sequenced genomic regions of the associated loci to show the differences in the protein-coding nucleotide sequences (Anteyi and Rasche 2020). Meanwhile, in *Fos*, metabolomic studies have been mainly limited to the detection of particular secondary metabolites that are produced by the fungi and released into culture media e.g., fusaric acid and dehydrofusaric acid (Savard et al. 1997; Amalfitano et al. 2002), and/or into the infected *S. hermonthica* shoot to cause phytotoxicity, e.g., beauvericin (Ndambi 2011). Screening of the differential exometabolome of genetically contrasting *Fos* isolates to identify specific secondary metabolites with proven biopesticidal efficacy against *S. hermonthica* incidence *in planta* is lacking. This knowledge would bring awareness on specific bioactive metabolites that constitute the *Fos* exometabolomic arsenal for preventing *S. hermonthica* incidence.

The ability of several fungal secondary metabolites, especially *Fusarium* toxins, to hamper early growth stages (germination, germ tube development/attachment), and later stages (post-attachment/aboveground development) of the life cycle of parasitic weeds have been mentioned in numerous studies. With this, they have a considerable potential as biological tools for the parasitic weed management (Vurro et al. 2009; Cimmino et al. 2015; Triolet et al. 2020). In the context of *S. hermonthica*, some specific *Fusarium* extracellular metabolites (exometabolites) have shown complete inhibition of the seed germination *in vitro*, when used as post-*Striga* seed conditioning treatments at very low concentrations (≤ 1 mM). For example, T-2 toxin at 10 µM (Zonno and Vurro 1999), neosolaniol at 240 µM (Sugimoto et al. 2002), and fusaric acid at 1 mM (Idris et al. 2003). However, it is unknown if these highly bioactive *Fusarium* exometabolites will maintain their potency against *S. hermonthica* incidence *in planta*. Also, in non-parasitic plants, some *Fusarium* exometabolites have similarly shown to cause complete germination inhibition at very low concentrations, for instance; 1,4-naphthoquinone at 63 µM against *Pinus thunbergii* Parl. pollen germination (Kimura et al. 1988), and hymeglusin at 50 µM against *Brassica juncea* (L.) Czern. seed germination (Liao et al. 2014). For other *Fusarium* exometabolites, such as equisetin, the concentration causing complete inhibition of germination in plants has not been determined (Wheeler et al. 1999). Unfortunately, the potential of these latter *Fusarium* exometabolites to inhibit *S. hermonthica* seed germination or incidence is not known. Thus, the identification of highly bioactive *Fusarium* exometabolites with broadscale efficacy against diverse *S. hermonthica* populations has been suggested as a promising direction for overcoming the limitations of the inconsistent effectiveness of *Fos* isolates against *S. hermonthica* (Anteyi and

Rasche 2021). In fact, fungal exometabolites, especially mycotoxins, play important roles in shaping the soil microbial community dynamics. This could impact the microbial abundance positively e.g., by serving as carbon source to support soil microbial proliferation; or adversely (antibiosis) e.g., by acting as antimicrobials to suppress soil microbial growth (McCormick 2013; Venkatesh and Keller 2019). Hence, in the latter case, microbial elimination (degradation) of the mycotoxin from the environment becomes a crucial ecological concern, as soil microorganisms are an integral part of any balanced ecosystem.

In spite of the progress made in identifying some particular *Fusarium* exometabolites that are effective at very low concentrations against *S. hermonthica* seed germination, there are still major knowledge gaps, especially regarding the exometabolite consistency in preventing *S. hermonthica* incidence *in planta*. Therefore, the main research questions of this study were: Among a set of known highly phytotoxic, germination-inhibiting *Fusarium* exometabolites (Kimura et al. 1988; Vurro et al. 2009; Liao et al. 2014), which is the most potent/efficient candidate (i.e., having the greatest effect at minimal concentration) against *S. hermonthica* seed germination? Is the potency of this candidate exometabolite consistent against *S. hermonthica* incidence *in planta*, whilst unaffecting the host crop development/yield? These questions serve as fundamental determiner for drawing greater attention into this strategy and encourage the use of fungal exometabolites as biopesticides to better combat the *S. hermonthica* menace. The study hypothesized that among the tested *Fusarium* exometabolites, the most potent/efficient candidate exometabolite against *S. hermonthica*, is part of the *Fos* exometabolome composition which is used for attacking *S. hermonthica*. Thus, the main objectives of the study were to: (i) examine the performance of a set of highly phytotoxic *Fusarium* exometabolites against *S. hermonthica* seed germination (*in vitro*) and incidence (*in planta*), towards identifying the most potent/efficient candidate exometabolite; and (ii) screen the exometabolome of contrasting *Fos* isolates (Foxy-2 and FK3), in determining if these agents produce the candidate exometabolite.

5.3 Methods

5.3.1 Preparatory activities: seed materials, seed sterilization, microbial samples, and exometabolites

Striga hermonthica (Delile) Benth. seeds were sampled from various locations in SSA (Table 5.1). Sorghum (*Sorghum bicolor* L. Moench) seeds (cultivar PI563294) were sampled from Maradi, Niger. Our previous research studies confirmed the susceptibility of this sorghum cultivar to *S. hermonthica* (Anteyi and Rasche 2020; 2021). The *S. hermonthica* and sorghum

seeds were separately surface-sterilized using sodium hypochlorite, Tween 20® and double distilled water (ddH_2O), then air-dried, according to the procedure of Anteyi and Rasche (2020; 2021). *Fusarium oxysporum* f. sp. *strigae* (*Fos*) isolates Foxy-2 and FK3 were obtained from the University of Hohenheim, Stuttgart, Germany. *Fusarium venenatum* strain O86 (DSM number 23361) was obtained from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures) GmbH, Braunschweig, Germany. The following *Fusarium* exometabolites were purchased as isolated and purified forms from their suppliers; 1,4-naphthoquinone (1,4-Nq) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), diacetoxyscirpenol (DAS) (Romer Labs Deutschland GmbH, Butzbach, Germany), equisetin (Equi) (Sigma-Aldrich Chemie GmbH), fusaric acid (FuA) (Sigma-Aldrich Chemie GmbH), hymeglusin (Hym) (Sigma-Aldrich Chemie GmbH), neosolaniol (Neo) (VWR International GmbH, Bruchsal, Germany) and T-2 toxin (T-2) (BIOMOL GmbH, Hamburg, Germany).

Table 5.1: Sampled *S. hermonthica* origin and phenotypic response to *Fos* isolates Foxy-2 and FK3.

***S. hermonthica* origin**	**Geographic coordinates (in decimal degrees)**		**Source**	**Sampled population phenotypic response to *Fos* isolates Foxy-2 and FK3 †**
	Latitude	**Longitude**		
Abuja (Nigeria)	9.066667	7.483333	IITA-Nigeria	Susceptible to Foxy-2 and FK3
Wad-Medani (Sudan)	14.4	33.533333	UG-Sudan	Susceptible to Foxy-2 and FK3
Kibos (Kenya)	-0.066667	34.816667	CIMMYT-Kenya	Susceptible to FK3; non-susceptible to Foxy-2
Sirinka (Ethiopia)	11.75	39.6	SARC-Ethiopia	Susceptible to FK3; non-susceptible to Foxy-2

IITA-Nigeria – International Institute of Tropical Agriculture, Ibadan, Nigeria.

UG-Sudan – University of Gezira, Wad Medani, Sudan.

CIMMYT-Kenya – International Maize and Wheat Improvement Centre, Kibos research facility, Kenya.

SARC-Ethiopia – Sirinka Agricultural Research Centre, Sirinka, Ethiopia.

† – according to Anteyi and Rasche 2020.

5.3.2 Performance of the Fusarium exometabolites against S. hermonthica

5.3.2.1 In vitro seed germination assay

The germination inhibitory potential of the test *Fusarium* exometabolites (i.e., 1,4-Nq, DAS, Equi, FuA, Hym, Neo and T-2) against *S. hermonthica* seeds were examined at 1, 5, 10, 20, 50 and 100 µM concentrations. The exometabolites were initially diluted with acetone (Carl Roth GmbH) to 1 mM, then by serial dilutions with ddH_2O to the respective study concentrations. 100–250 surface-sterilized *S. hermonthica* seeds (collected from Sirinka, Ethiopia) were laid on a sterilized 0.8 cm glass fibre filter paper (GFFP) punched disc. Four *S. hermonthica* seed-carrying punched discs (i.e., four replicates) were placed on a doubled layer of sterilized 9 cm GFFP, contained in a 9 cm petri dish. Then, 6 mL of the exometabolites (or ddH_2O as control) were added as pre-*Striga* seed conditioning treatments to each petri dish. Thus, the treatments included **(1):** ddH_2O (control); **(2):** Hym; **(3):** 1,4-NQ; **(4):** FuA; **(5):** Equi; **(6):** Neo; **(7):** T-2; **(8):** DAS. For *S. hermonthica* seed preconditioning, the petri dishes were covered, sealed with parafilm and incubated for 14 d in a dark chamber at 30 °C. Afterwards, the GFFP punched discs (carrying the preconditioned *S. hermonthica* seeds) were re-placed on a single layer of new, sterilized 9 cm GFFP, in a petri dish. 3 mL of a synthetic strigolactone analog, rac-GR24 (0.1 ppm) (Chiralix B.V., Nijmegen, Netherlands) was added to every petri dish, and they were re-incubated at 30 °C for 24 h. This was followed by counting the germinated *S. hermonthica* seeds using the Zeiss Stemi 2000-C Stereomicroscope (Carl Zeiss Microscopy GmbH, Jena, Germany), together with the Zeiss AxioCam HRc (Carl Zeiss Light Microscopy, Göttingen, Germany). The experiment was performed in two repetitions. The least study concentration where all the tested exometabolites significantly reduced *S. hermonthica* seed germination was utilized as working concentration for further investigations.

5.3.2.2 Planting experiments

Sixty-five g of planting soil substrate, made up of a blend of 80% modular seed substrate (Klasmann-Deilmann GmbH, Geeste, Germany) and 20% maize rhizosphere (collected from the top layer (0–20 cm) of a maize field in Filderstadt, Germany: Latitude 48.6483, Longitude 9.2475) were filled into 20 × 5 × 2 cm rhizoboxes, made from polyvinyl chloride. Then a plexiglass lid was used to cover each of the rhizoboxes. Sixty-five mg of *S. hermonthica* seeds (from Sirinka, Ethiopia) were sown in depth of 5 cm beneath the soil surface. Thereafter, using a 10 mL pipette, each of the rhizoboxes were treated with 65 mL of the test exometabolites at 20 µM working concentration (derived from the *in vitro* seed germination assay), by application on the soil surface. Thus, the treatments included **(1):** ddH_2O without *Striga* (*Striga*-neg. control); **(2):** ddH_2O + *Striga* (*Striga*-pos. control); **(3):** Hym + *Striga*; **(4):** 1,4-Nq + *Striga*; **(5):**

FuA + *Striga*; **(6):** Equi + *Striga*; **(7):** Neo + *Striga*; **(8):** T-2 + *Striga*; **(9):** DAS + *Striga*. Each treatment was replicated thrice, and the set up was arranged in a randomized complete block design. For *S. hermonthica* seed preconditioning, the rhizoboxes were incubated at 30 °C for 14 d in a climate chamber without light (Percival Intellus Environmental Controller, EA-75HIL, Perry, Iowa, USA). This was followed by sowing a sorghum seed in each of the rhizoboxes, and maintaining the climate chamber at an alternating 12-h period of 31 °C with light (mean illuminance 46,000 lx) and 27 °C without light. The rhizoboxes were irrigated with nutrient solution twice weekly, which consisted of a mixture of 3 parts of 0.2% (v/v) Wuxal® universal liquid fertilizer (Aglukon Spezialdünger GmbH, Düsseldorf, Germany) and 2 parts of Yoshida nutrient solution (Yoshida et al. 1976). The planting experiments lasted for 9 weeks after sowing sorghum, and the trials were performed in 2 experimental repetitions. Following a similar experimental setup, additional planting experiments were performed, but without including *S. hermonthica* in the design. At the end of the planting experiments, *S. hermonthica* incidence in a rhizobox was determined from the total amount of attached *S. hermonthica* belowground and aboveground. The sorghum aboveground biomass was harvested, dried in an oven at 65 °C for 10 d, then weighed to obtain the sorghum aboveground dry biomass (ABM) data.

5.3.3 Additional experiments focusing on DAS (the candidate exometabolite)

5.3.3.1 Consistency against diverse S. hermonthica populations

The germination inhibitory potential of DAS against seeds of *S. hermonthica* populations from Abuja (Nigeria), Wad-Medani (Sudan) and Kibos (Kenya) were also evaluated. The experiment followed similar technical procedures as described for the *in vitro* seed germination assay (see above). The treatments included **(1):** ddH_2O (control); **(2):** DAS 1 µM; **(3):** DAS 20 µM. Each of the treatments were in 4 replications, and the experiment was done in two repetitions.

5.3.3.2 Effectiveness as a post-Striga seed conditioning treatment

The experiment followed similar technical procedures as described for the *in vitro* seed germination assay (see above). However, here, the 14-d *S. hermonthica* (from Sirinka, Ethiopia) seed preconditioning was performed with 6 mL ddH_2O. Thereafter, 1.5 mL DAS (1 µM or 20 µM) was applied as a post-*Striga* seed conditioning treatment, together with 1.5 mL rac-GR24 (0.1 ppm). Hence, the treatments included **(1):** rac-GR24 (control); **(2):** DAS 1 µM + rac-GR24; **(3):** DAS 20 µM + rac-GR24. The treatments were in 3 replications each, and the experiment was done in two repetitions.

5.3.3.3 Impact on soil microbial community abundance

10 g of planting soil substrate were filled into 9 cm petri dishes, and supplied with either 10 mL of ddH_2O, DAS 1 µM or DAS 20 µM. In a parallel setup, 10 mg unsterilized *S. hermonthica* seeds were mixed into the planting soil substrate in each of the petri dishes. Therefore, the treatments consisted of (1): ddH_2O (*Striga*-neg. control); (2): ddH_2O + *Striga* (*Striga*-pos. control); (3): DAS 1 µM; (4): DAS 1 µM + *Striga*; (5): DAS 20 µM; (6): DAS 20 µM + *Striga*. The treatments were in 3 replications each. Then, the petri dishes were covered and incubated in a dark chamber at 28 °C for 14 d. Thereafter, total DNA (deoxyribonucleic acid) was isolated from 500 mg of the incubated treatments using the Fast DNA® spin kit for soil (MP Biomedicals LLC, Solon, Ohio, USA). Effect of DAS on the soil microbial community abundance was evaluated from the major soil microbial groups i.e., bacteria and fungi, through the 16S ribosomal RNA (rRNA) and 18S rRNA gene copy numbers, respectively. This was done by real-time quantitative PCR (qPCR) on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Waltham, Massachusetts, USA). At a standardized DNA concentration of 10 ng μl^{-1} for each treatment, a 20 µL qPCR reaction system, with 3-step cycling, was performed using the SensiFAST™ SYBR® Hi-ROX Kit (Bioline Meridian Bioscience, Paris, France), according to the manufacturer's protocol. The primer information is provided in Table 5.2. qPCR runs for each treatment consisted of 3 biological replicates into 3 technical replicates.

Table 5.2: List of primers sets used for qPCR and RT-PCR analyses.

Primer id	Nucleotide sequence (5′→3′)	Target gene	Target group	Reaction	Reference
Eub338 (F)	ACCTACGGGAGGCAGCAG	16S rRNA	Bacteria	qPCR	Lane 1991
Eub518 (R)	ATTACCGCGGCTGCTGG	16S rRNA	Bacteria	qPCR	Muyzer et al. 1993
FF390 (F)	CGATAACGAACGAGACCT	18S rRNA	Fungi	qPCR	Vainio and Hantula 2000
FR1 (R)	AICCATTCAATCGGTAIT	18S rRNA	Fungi	qPCR	Vainio and Hantula 2000
FsTri5 (F)	TGGAGAACTGGATGGTCTGG	Tri5	TpFs	RT-qPCR	Alexander et al. 2008
FsTri5 (R)	GACATAGCCGTGCATGAAGC	Tri5	TpFs	RT-qPCR	Alexander et al. 2008
FsTri4 (F)	GCCACTGCTGCTACTGTTGA	Tri4	TpFs	RT-qPCR	Alexander et al. 2008
FsTri4 (R)	GGTCGTTGTCCAGATGTTCTTG	Tri4	TpFs	RT-qPCR	Alexander et al. 2008
EF1A (F)	GGCTTTCACCGACTACCCTCCTCT	EF1A	Eukaryotes	RT-qPCR	Kim and Yun 2011
EF1A (R)	ACTTCTCGACGGCCTTGATGACAC	EF1A	Eukaryotes	RT-qPCR	Kim and Yun 2011

F – Forward.

R – Reverse.

TpFs – Trichothecene producing *Fusarium* species (especially characterized in *F. graminearum* and *F. sporotrichioides*).

5.3.3.4 Degradation of DAS in the planting soil substrate (targeted metabolomics)

For sample isolation, the left-overs of the soil microbial cultures (see above) were transferred to sterile 50 mL falcon tubes, and 10 mL ethyl acetate (Merck KGaA, Darmstadt, Germany) were added to each tube. The tubes were incubated in a dark shaker overnight, at 100 rpm and 27 °C. Thereafter, the tubes were centrifuged for 20 min, at 4750 rpm and 4 °C, using the Allegra® X-15R centrifuge (Beckman Coulter GmbH, Krefeld, Germany). The ethyl acetate phase was isolated and utilized for targeted metabolomic analysis by liquid chromatography–mass spectrometry (LC-MS) system. For this, 500 µL of the ethyl acetate phase were completely dried under N_2, and resuspended with 150 µL methanol (VWR, Radnor, Pennsylvania, USA). Five µL of the solution were injected into the Agilent 1290 Infinity LC, coupled with the HPLC column ZORBAX Eclipse Plus C18, 95 Å, 1.8 µm, 2.1 × 50 mm, at 40 °C (Agilent Technologies GmbH, Waldbronn, Germany). Mobile phases included 0.2% formic acid (VWR) (solvent A), and acetonitrile (VWR) with 0.2% (v/v) formic acid (solvent B). At a flow rate of 0.3 mL min^{-1}, the 'time – composition' gradient program was set at 0.00(min) – B = 0.0%, 1.50(min) – B = 0.0%, 7.00(min) – B = 15.0%, 19.00(min) – B = 75.0%, 22.00(min) – B = 100.0%, 22.10(min) – B = 0.0%, and 23.10(min) – B = 0.0%. All solvents used were LC-MS grade. The purified fraction, and standard DAS, were measured by electrospray ionization and fourier transform mass spectrometry, using the Q Exactive™ Plus Orbitrap™ System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). It made use of positive polarity mode, with 4.2 kV spray capillary. A desolvation temperature of 380 °C and stepped collision energy of 20, 60, and 110 V were maintained. The data dependent acquisition method was adopted, with a full MS scan of 70,000 resolution, followed by dd-MS^2 (TOP 5) 140 to 1200 *m/z* of 17,500 resolution. From the obtained $[M + H]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ adducts, the more intense $[M + NH_4]^+$ adduct was selected for further analysis. For the standard DAS, at a retention time of 11.17 min, it was measured at 384.201 *m/z*.

5.3.4 Screening of Fos exometabolome for DAS production

5.3.4.1 Fungal culturing and metabolic footprinting

1.6 cm agar-mycelial plug from actively growing potato dextrose agar (PDA) (Carl Roth GmbH) cultures of *Fos* isolates (Foxy-2 and FK3), including *F. venenatum* (as control), were separately transferred to sterile 50 mL falcon tubes containing 35 mL of 25% potato dextrose broth (PDB) (Carl Roth GmbH). *Fusarium venenatum* was used as control because of its ability to produce DAS (Miller and MacKenzie 2000). In a parallel setup, 35 mg unsterilized *S. hermonthica* seeds were added to each tube. Thus, the treatments included **(1):** Foxy-2; **(2):** Foxy-2 + *Striga*; **(3):** FK3; **(4):** FK3 + *Striga*; **(5):** *F. venenatum*; **(6):** *F. venenatum* + *Striga*. The treatments were in 3 replications each. Then, the tubes were

incubated in a dark shaker for 2 weeks, at 65 rpm and 28 °C. Following a similar experimental design, Foxy-2, FK3 and *F. venenatum* were also cultured using Czapek-Dox broth containing 2% peptone (Carl Roth GmbH), with static incubation for 3 weeks at 22 °C, under 16 h light and 8 h dark conditions (Shams et al. 2011). Thereafter, 10 mL ethyl acetate were added to every tube, and they were re-incubated overnight. Then, the tubes were centrifuged for 20 min, at 4750 rpm and 4 °C. The ethyl acetate phase of the treatments was isolated and used for exometabolomic analysis by LC-MS system as described above.

5.3.4.2 RNA isolation and gene expression

Total RNA (ribonucleic acid) was isolated from 100 mg hyphal biomass of actively growing PDA cultures of Foxy-2, FK3 and *F. venenatum* (ISOLATE II RNA Plant Kit, Bioline GmbH, Luckenwalde, Germany). Trichothecene gene expression by the fungal isolates were determined by quantitative reverse transcription PCR (RT-qPCR), using the StepOnePlus™ Real-Time PCR System. At a standardized RNA concentration of 10 ng μl^{-1} for each fungal sample, a 20 µl RT-qPCR reaction system, with 3-step cycling, was performed using the SensiFAST™ SYBR® Hi-ROX One-Step Kit (Bioline Meridian Bioscience), according to the manufacturer's protocol. Trichothecene genes, Tri5 and Tri4, were the genes of interest. These are the first genes involved in the *Fusarium* trichothecene biosynthetic pathway, from the starting compound farnesyl pyrophosphate. Tri5 encodes trichodiene synthase (which converts farnesyl pyrophosphate to trichodiene), while Tri4 encodes a cytochrome P450 monooxygenase (which converts trichodiene to isotrichotriol). Isotrichotriol is converted to isotrichodermol (i.e., the first trichothecene in the pathway) through a non-enzymatic process. In presence of other relevant Tri-genes (i.e., Tri101, Tri11, Tri3, Tri13 and Tri7, and Tri8), isotrichodermol is eventually converted to more complex trichothecene chemical species e.g., DAS (Kimura et al. 2007; Ismail et al. 2011). Thus, *Fusarium* strains lacking trichodiene synthase do not produce trichothecenes (Miller and MacKenzie 2000). A housekeeping gene, Eukaryotic translation elongation factor 1 alpha (EF1A), was used as the reference gene (Kim and Yun 2011). The primer information is given in Table 5.2. RT-qPCR runs for each fungal sample consisted of 3 biological replicates into 3 technical replicates. Relative gene expression was calculated by the Pfaffl equation (Pfaffl 2001).

5.3.5 Biostatistical analysis

Quantitative data from all the experiments in this study were tested by the analysis of variance (ANOVA), and Tukey's range test (post hoc analysis), using SAS 9.4 (SAS Institute, Cary, North Carolina, USA). In presenting the Tukey's range test of the *S. hermonthica* incidence and sorghum ABM from the planting experiments, the mean data were standardized (as percentage) in relation to the respective control treatments, without making alterations to the

analyses output. Thus, the *S. hermonthica* incidence data were standardized relative to the '*Striga*-pos. Control', and the sorghum ABM data were standardized relative to the '*Striga*-neg. Control'.

5.4 Results

5.4.1 The most potent/efficient candidate exometabolite against S. hermonthica

ANOVA indicated that at the various study concentrations, the treatments caused different effect on *S. hermonthica* seed germination ($P < 0.0001$). Tukey's range test further revealed that at 1–10 µM, some exometabolites, particularly DAS, T-2 and Neo, exhibited stronger *S. hermonthica* seed germination inhibition compared to the others (Table 5.3). This concentration range (1–10 µM) was also characterised by wide variability (standard error (SE) > 1) in most of the treatments effect, including high instability of the response (*S. hermonthica* seed germination inhibition) in relation to increasing treatment concentration. Nevertheless, as from 10 µM, inhibition of *S. hermonthica* seed germination followed a concentration-dependent trend in relation to increasing treatment concentration. At ≥20 µM, all the exometabolites significantly reduced *S. hermonthica* seed germination, and they displayed more accurate/stable treatment effects (SE < 1). Hence, 20 µM was utilized as the exometabolite treatments working concentration in the planting experiments. All exometabolites completely inhibited *S. hermonthica* seed germination at 100 µM. Out of all tested exometabolites, only DAS completely inhibited *S. hermonthica* seed germination at every concentration (1–100 µM). The planting experiments revealed that at 20 µM, none of the exometabolites affected the ABM of *S. hermonthica*-free sorghum ($P > 0.05$). However, only DAS and T-2 supressed *S. hermonthica* incidence ($P < 0.0001$) (Fig. 5.1).

Table 5.3: Tukey's range test for *S. hermonthica* germination percentages (average) at different exometabolite concentrations.

	1 µM	**5 µM**	**10 µM**	**20 µM**	**50 µM**	**100 µM**
ddH_2O	55.000 (1.414) ^{a}	55.000 (1.414) ^{a}	55.000 (1.414) ^{ab}	55.000 (1.414) ^{a}	55.000 (1.414) ^{a}	55.000 (1.414) ^{a}
Hym	37.958 (4.495) ^{ab}	40.798 (1.366) ^{a}	11.362 (1.862) ^{de}	0.551 (0.225) ^{c}	0.689 (0.264) ^{b}	0.000 (0.000) ^{b}
1,4-NQ	32.148 (3.309) ^{bc}	34.859 (6.083) ^{ab}	37.958 (8.161) ^{bc}	4.135 (0.574) ^{b}	0.965 (0.347) ^{b}	0.000 (0.000) ^{b}
FuA	28.662 (2.569) ^{bc}	35.117 (5.164) ^{ab}	16.268 (8.521) ^{de}	1.516 (0.823) ^{bc}	0.000 (0.000) ^{b}	0.000 (0.000) ^{b}
Equi	15.106 (6.939) ^{cd}	54.225 (6.512) ^{a}	26.338 (0.894) ^{cd}	0.551 (0.225) ^{c}	0.000 (0.000) ^{b}	0.000 (0.000) ^{b}
Neo	3.486 (2.480) ^{d}	5.423 (0.775) ^{c}	0.775 (0.447) ^{e}	0.000 (0.000) ^{c}	0.000 (0.000) ^{b}	0.000 (0.000) ^{b}
T-2	4.648 (1.096) ^{d}	0.775 (0.775) ^{c}	0.000 (0.000) ^{e}	0.000 (0.000) ^{c}	0.000 (0.000) ^{b}	0.000 (0.000) ^{b}
DAS	0.000 (0.000) ^{d}	0.000 (0.000) ^{c}	0.000 (0.000) ^{e}	0.000 (0.000) ^{c}	0.000 (0.000) ^{b}	0.000 (0.000) ^{b}

Means having at least a letter in common are not significantly different (α = 0.05). Standard error (SE) is in parenthesis.

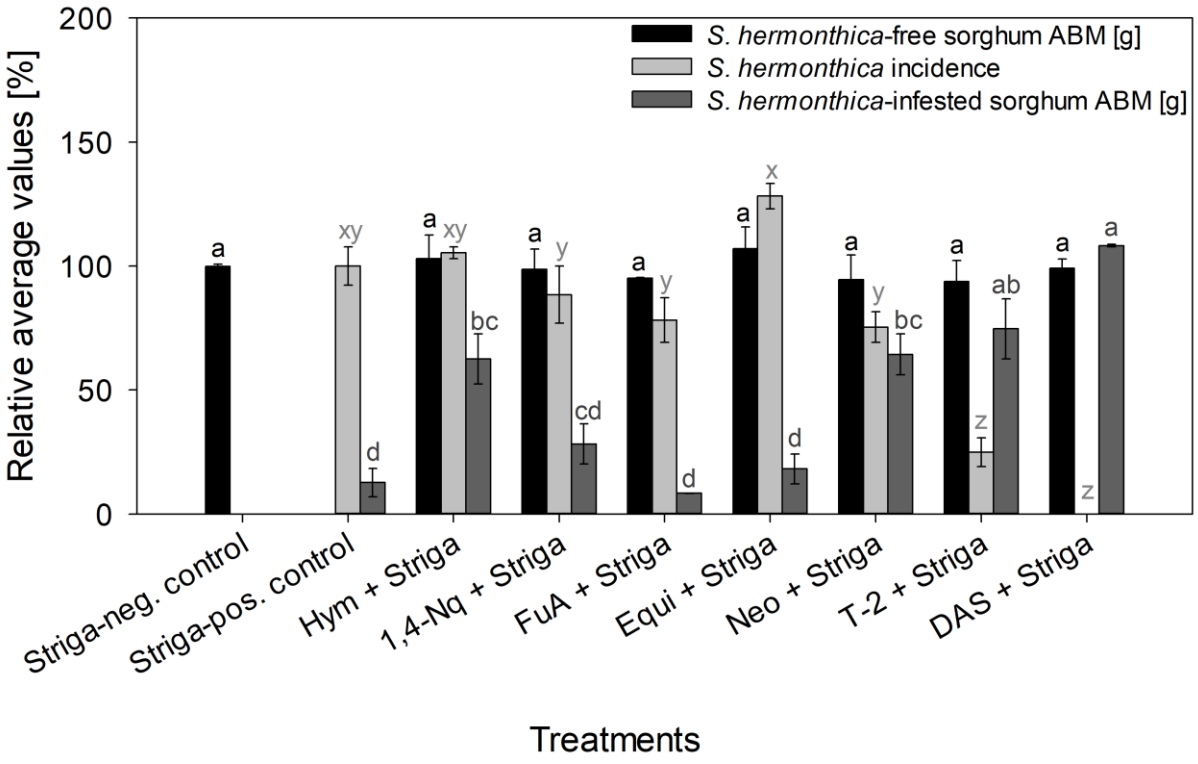

Fig. 5.1: Relative average values of *S. hermonthica*-free sorghum ABM (aboveground dry biomass), *S. hermonthica* incidence and *S. hermonthica*-infested sorghum ABM to the chemical treatments at 20 µM concentration. *Striga*-neg. control = ddH_2O without *S. hermonthica*. *Striga*-pos. control = ddH_2O with *S. hermonthica*. Bars having at least a letter in common represent treatments means that are not significantly different (α = 0.05). Error bars indicate standard error (SE).

Correspondingly, except for DAS and T-2, the ABM of *S. hermonthica*-infested sorghum for the rest exometabolites was lower than the *Striga*-neg. Control ($P < 0.0001$). DAS, nonetheless, was outstanding compared to T-2, because DAS completely hindered *S. hermonthica* incidence. This resulted in a larger ABM of *S. hermonthica*-infested sorghum. Further evaluation of DAS and T-2 showed that their ability to prevent *S. hermonthica* incidence was lower at 1 µM (Fig. 5.2). Nevertheless, DAS still suppressed *S. hermonthica* incidence ($P < 0.05$), while *S. hermonthica* incidence for T-2 was not different from the *Striga*-pos. Control ($P > 0.05$). The ABM of *S. hermonthica*-infested sorghum for both exometabolites were not different to the *Striga*-neg. Control ($P > 0.05$).

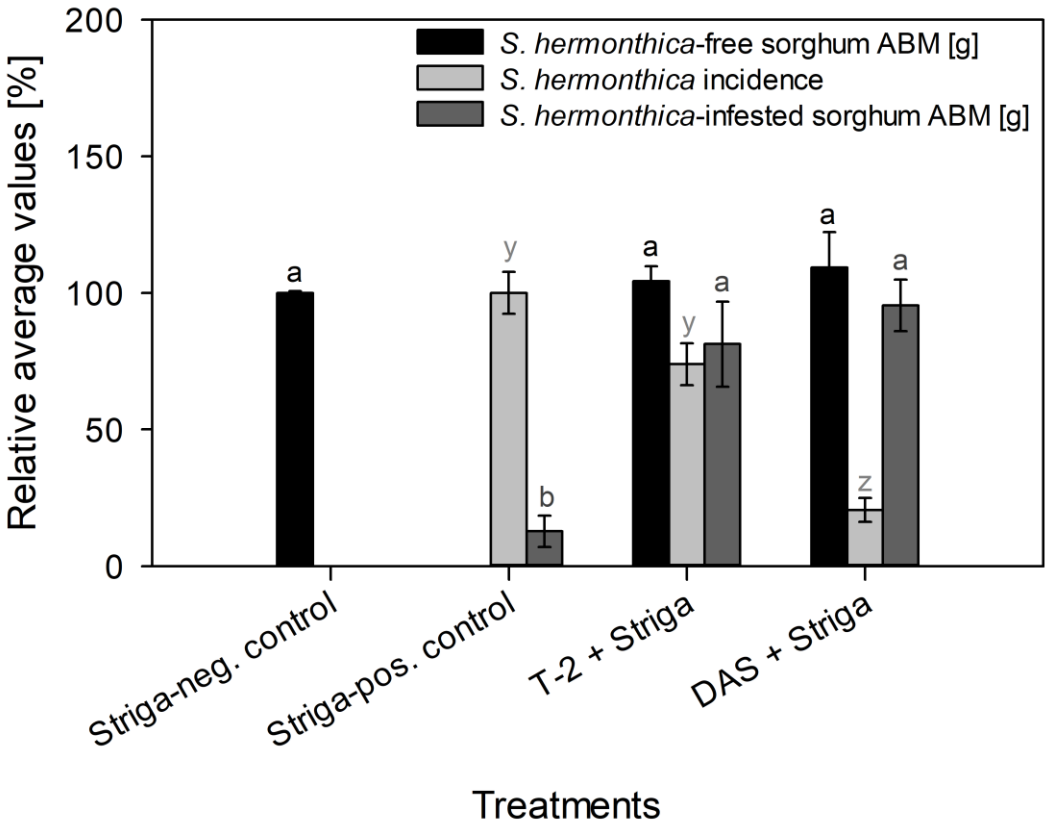

Fig. 5.2: Relative average values of *S. hermonthica*-free sorghum ABM (aboveground dry biomass), *S. hermonthica* incidence and *S. hermonthica*-infested sorghum ABM to T-2 and DAS at 1 µM concentration. *Striga*-neg. control = ddH_2O without *S. hermonthica*. *Striga*-pos. control = ddH_2O with *S. hermonthica*. Bars having at least a letter in common represent treatments means that are not significantly different (α = 0.05). Error bars indicate standard error (SE).

At 20 µM, DAS consistently inhibited the seed germination of diverse *S. hermonthica* populations from Abuja (Nigeria), Wad-Medani (Sudan) and Kibos (Kenya) completely (Table 5.4). Complete germination inhibition of the diverse *S. hermonthica* populations was also achieved at 1 µM DAS, except for the population from Kibos, which was almost completely inhibited ($P < 0.0001$). Similarly, application of DAS as a post-*Striga* seed conditioning treatment resulted in the complete inhibition of *S. hermonthica* seed germination at 20 µM (Fig. 5.3). This potential was reduced at 1 µM DAS, nevertheless, it was effective ($P < 0.0001$).

Table 5.4: Tukey's range test for germination percentages (average) of diverse *S. hermonthica* populations at different DAS concentrations.

	Abuja (Nigeria)	**Wad-Medani (Sudan)**	**Kibos (Kenya)**
ddH_2O (control)	27.375 (2.625) [a]	31.250 (1.750) [a]	34.500 (0.833) [a]
DAS 1 µM	0.000 (0.000) [b]	0.000 (0.000) [b]	0.583 (0.468) [b]
DAS 20 µM	0.000 (0.000) [b]	0.000 (0.000) [b]	0.000 (0.000) [b]

Means having at least a letter in common are not significantly different (α = 0.05). Standard error (SE) is in parenthesis.

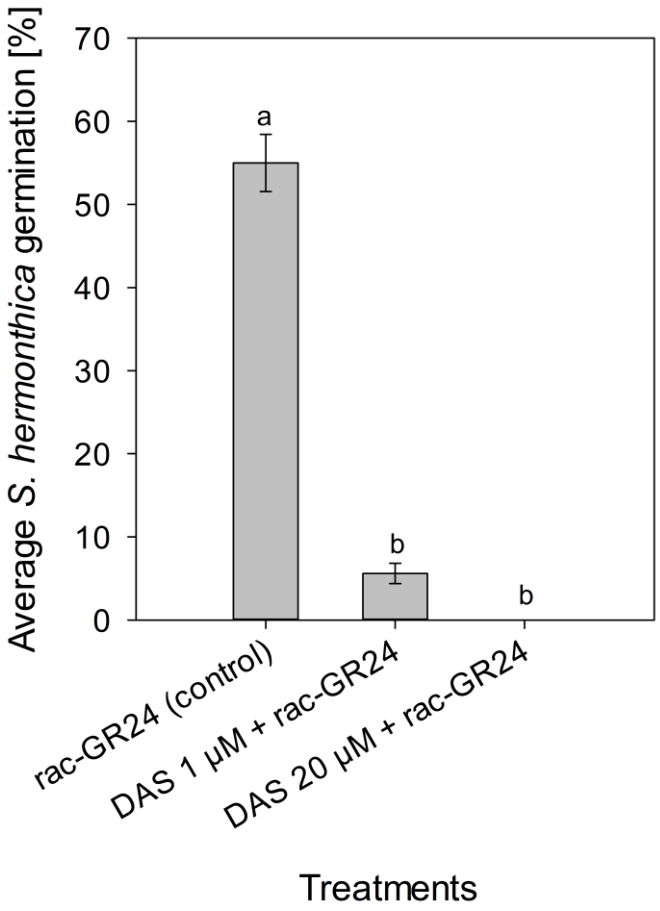

Fig. 5.3: Average *S. hermonthica* germination response to post-*Striga* seed conditioning treatment with DAS. Bars having at least a letter in common represent treatments means that are not significantly different (α = 0.05). Error bars indicate standard error (SE).

Incubation of the planting soil substrate with 20 µM DAS for 14 days, strikingly increased the copy numbers of the 16S rRNA (bacterial community) ($P < 0.0001$) and the 18S rRNA (fungal community) genes ($P < 0.01$) (Fig. 5.4).

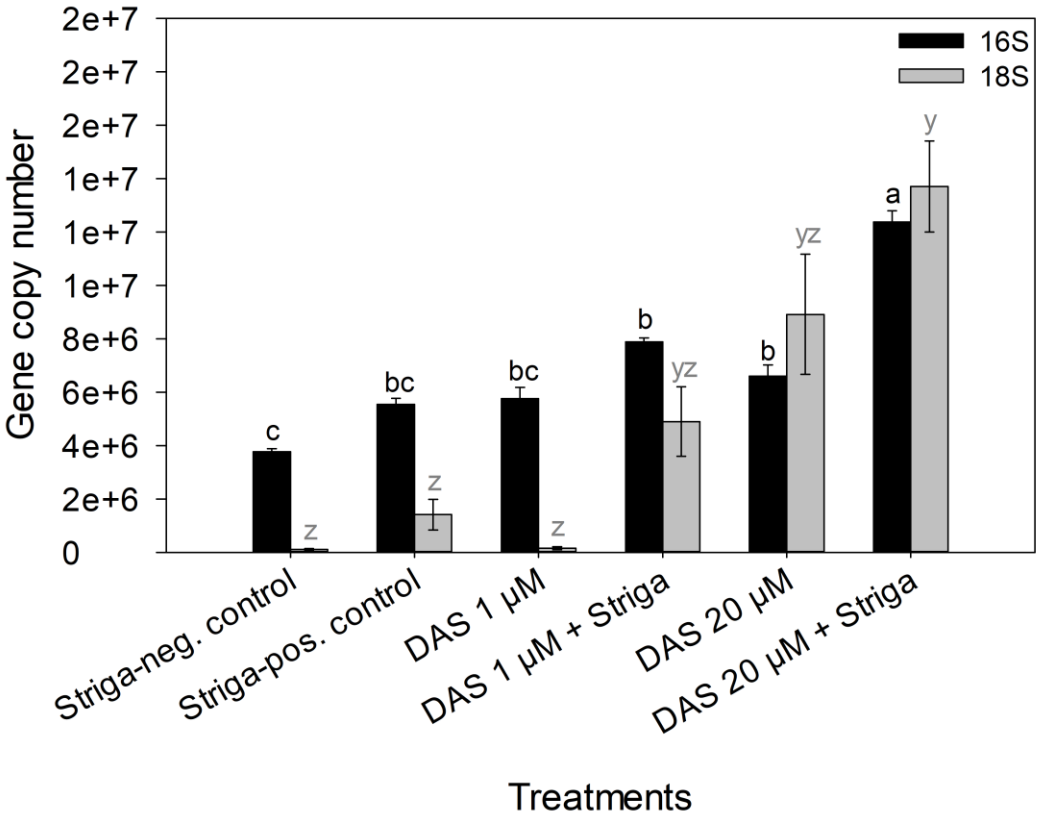

Fig. 5.4: 16S rRNA and 18S rRNA gene copy numbers in planting soil substrate after a 14-d incubation period. *Striga*-neg. control = soil + ddH_2O without *Striga hermonthica* seeds. *Striga*-pos. control = soil + ddH_2O + *S. hermonthica* seeds. Bars having at least a letter in common represent treatments means that are not significantly different (α = 0.05). Error bars indicate standard error (SE).

The difference between the *Striga*-pos. and *Striga*-neg. control treatments indicated that addition of the *S. hermonthica* seeds moderately promoted the microbial abundance in the setup. This could have occurred either by contamination from the unsterilized *S. hermonthica* seeds, or the *S. hermonthica* seeds serving as nutrient substrate for microbial growth. However, comparing the DAS-treated samples to the control treatments revealed that DAS application stimulated proliferation of the bacterial and fungal communities in a concentration-dependent manner (i.e., 20 µM > 1 µM > control). Whereby, the fungal community multiplied faster than the bacterial community, as DAS concentration increased. LC-MS analysis revealed that DAS was eventually degraded in the planting soil substrate after

the 14-d incubation. This was indicated by the absence of DAS signal in the LC-MS data of the DAS-treated samples (Fig. 5.5).

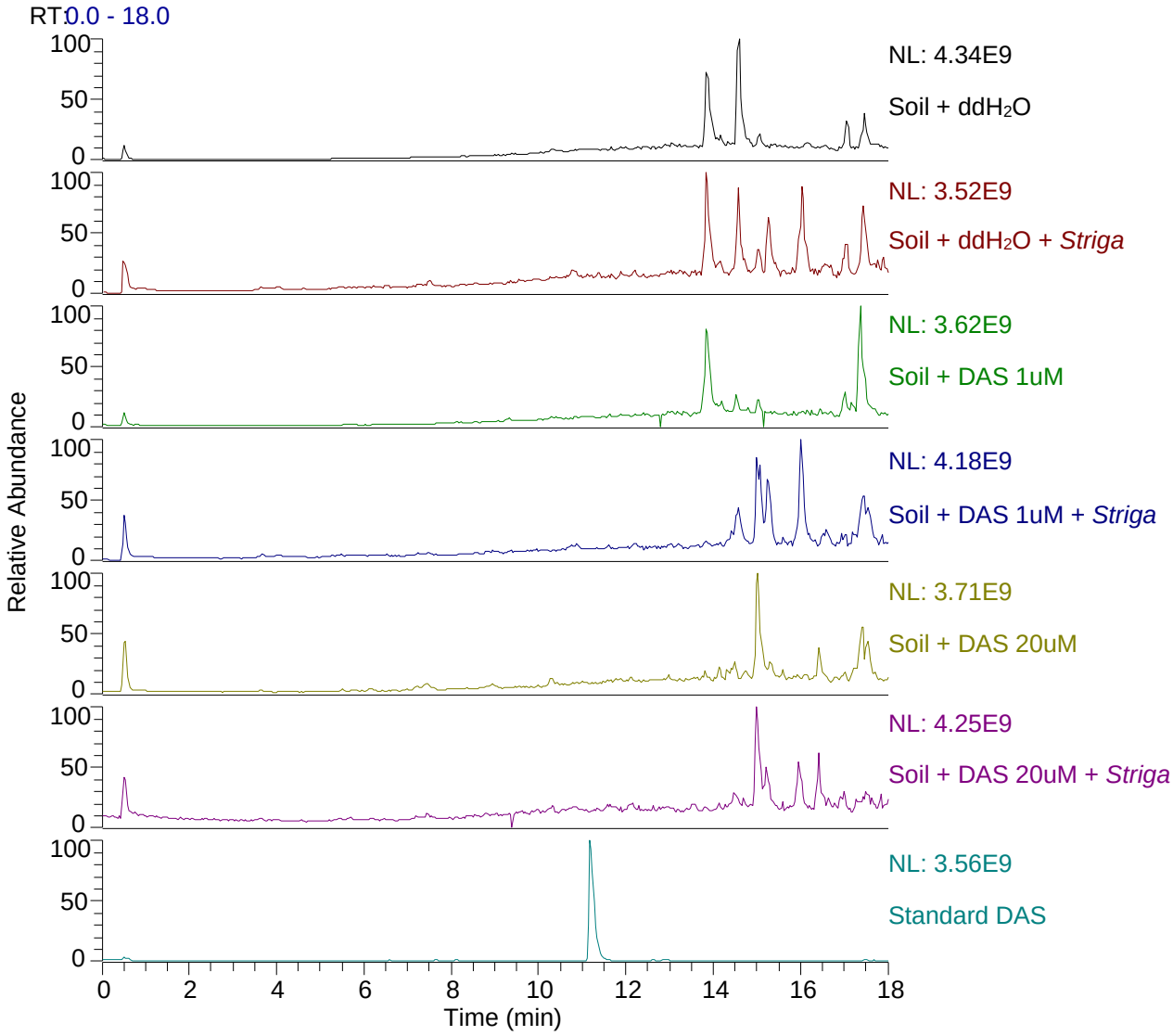

Fig. 5.5: Mass chromatogram of planting soil substrate samples after a 14-d incubation. Standard DAS signal at 11.17 min.

5.4.2 Determination of DAS production by Fos

Exometabolomic investigation revealed that *F. venenatum* produced DAS in both the PDB and Czapek-Dox broth media cultures. This was regardless of the addition or non-addition of *S. hermonthica* seeds to the culturing media. Contrarily, both *Fos* isolates (Foxy-2, FK3) did not produce DAS under similar culturing conditions (Fig. 5.6)

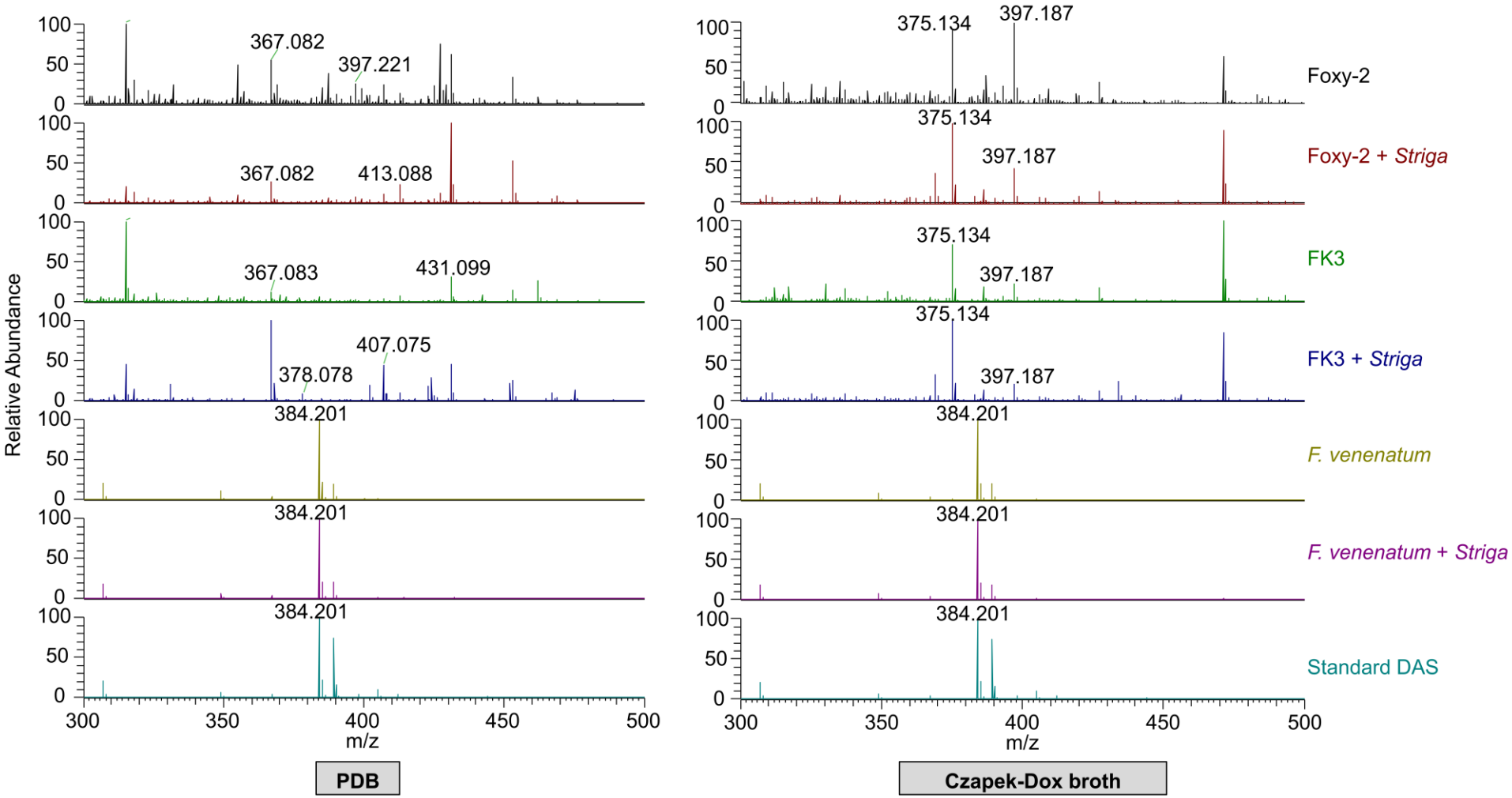

Fig. 5.6: Mass spectrum of fungal samples cultured in PDB and Czapek-Dox broth media.

RT-qPCR analysis further revealed that for both Foxy-2 and FK3, the trichothecene genes Tri5 and Tri4 were underexpressed ($P < 0.0001$) (Fig. 5.7). *Fusarium venenatum* expressed Tri5 gene at 349.8-fold higher than Foxy-2, and 7951.3-fold higher than FK3. Also, *F. venenatum* expressed Tri4 gene at 17.7-fold higher than Foxy-2, and at 23.8-fold higher than FK3.

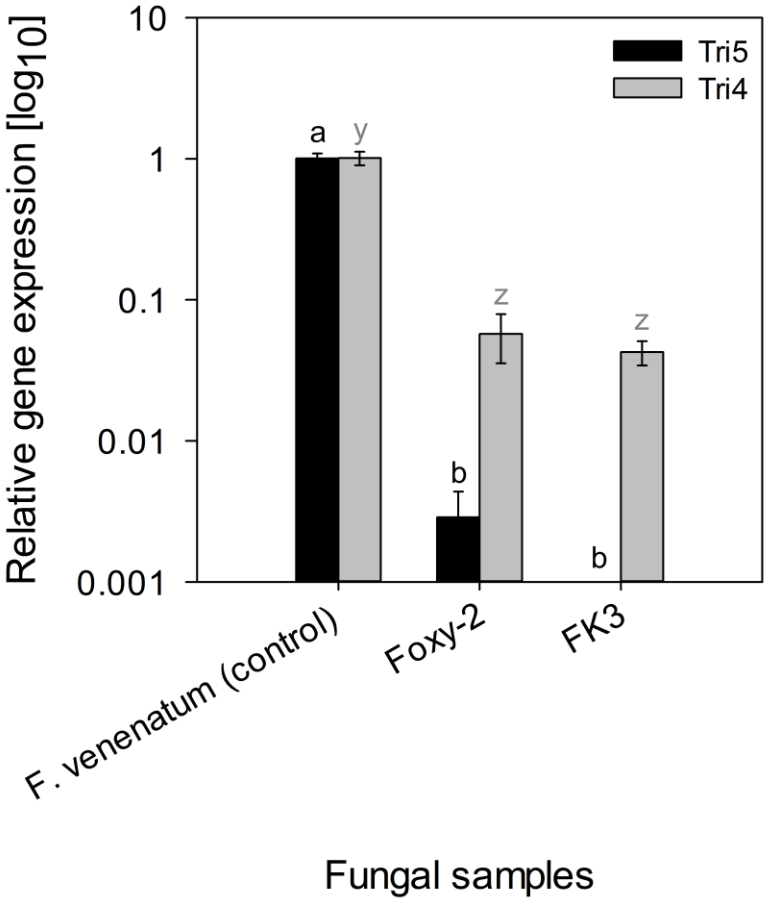

Fig. 5.7: Relative expression of trichothecene genes Tri5 and Tri4 by *F. venenatum* and *Fos* isolates (Foxy-2 and FK3). Bars having at least a letter in common represent treatments means that are not significantly different (α = 0.05). Error bars indicate standard error (SE).

5.5 Discussion

5.5.1 DAS revealed outstanding suppression of S. hermonthica

It was the fundamental finding of this study that out of all tested *Fusarium* exometabolites, DAS exhibited the greatest potential to control *S. hermonthica*. DAS presented a clearly larger advantage than the other tested exometabolites in terms of its ability to completely inhibit *S. hermonthica* seed germination *in vitro*, at all studied concentrations (1–100 µM). Comparably,

in a previous study on *Orobanche ramosa* (also a parasitic weed), DAS was the most promising *Fusarium* toxin tested as well, where it caused complete inhibition of the seed germination at 10 and 100 µM, but at 1 and 0.1 µM, it suppressed the germination to 2 and 7%, respectively (Zonno and Vurro 2002). With non-parasitic plants, on the other hand, 1 µM DAS failed to inhibit *Arabidopsis* seed germination, but at 10 µM, the germination was reduced from about 95 to 40% (Masuda et al. 2007). Furthermore, in *Nicotiana sylvestris*, pollen germination inhibition started from 20 ng mL^{-1} (0.055 µM) DAS at 26.7%, but at 200 ng mL^{-1} (0.55 µM), it was completely inhibited (Siriwardana and Lafont 1978). Because the variable susceptibility of diverse *S. hermonthica* populations to *Fos* isolates has been a major drawback in biocontrol of *S. hermonthica* (Avedi et al. 2014; Anteyi and Rasche 2020), the ability of DAS to consistently inhibit seed germination of diverse *S. hermonthica* populations in our study is a very interesting discovery. After DAS, T-2 was the second best-performing exometabolite tested in our study, followed by Neo. DAS, T-2 and Neo are all type A trichothecenes. Trichothecenes are sesquiterpene mycotoxins that are made up of a core ring structure, which contains an epoxide (tricyclic ether) at the 12,13 carbon positions, including a double bond at the 9, 10 carbon positions (see appendix 7). These functional groups (particularly the former) are mainly responsible for their toxicity (de Oliveira et al. 2014). Also, type A trichothecenes differ from other trichothecene types i.e., type B (e.g., deoxynivalenol), type C (e.g., crotocin) and type D (e.g., satratoxin H), primarily because of the substitution at the carbon 8 of the core ring structure. This could be a hydroxyl group (e.g., Neo), an ester function (e.g., T-2), or no functional group/oxygen substitution (e.g., DAS) (McCormick et al. 2011). Hence, this group sameness may explain the moderately close biopesticidal potential of the type A trichothecenes against *S. hermonthica* in our study, particularly DAS and T-2. Nevertheless, T-2 performance was limited by several factors. For instance, it started causing complete germination inhibition of *S. hermonthica* seeds *in vitro* as from 10 µM (this was in agreement with Zonno and Vurro (1999)), it was unable to completely hinder *S. hermonthica* incidence at 20 µM *in planta*, and it was unable to suppress *S. hermonthica* incidence at 1 µM. Moreover, due to loss of acetyl side groups on carbons 4 and 15, and an isovaleryl group at carbon 8, toxicity of DAS is generally lower (Thompson and Wannemacher 1986; Eudes et al. 2000; Masuda et al. 2007). We assume that the unique chemical structure of DAS may be the basis for its outstanding biopesticidal performance against *S. hermonthica*, compared to all other exometabolites tested in this study.

Preconditioning (i.e., dark stratification over a given period, in presence of moisture and suitably warm-temperature), is an indispensable, preliminary treatment, that is required for the release of parasitic plant seeds (*Orobanche* sp., *Striga* sp.) from primary dormancy, in

preparation for germination (Matusova et al. 2004). During the seed preconditioning stage, essential metabolic pathways in the seed, such as respiration, synthesis of DNA, protein and hormones, are activated (Joel 2000; Ejeta 2005). Previous studies had mainly focused on using fungal exometabolites as post-*Striga* seed conditioning treatment against a single *S. hermonthica* population, mostly sampled from Sudan (Zonno and Vurro 1999; Ahmed et al. 2001; Sugimoto et al. 2002; Idris et al. 2003). However, as shown from our study, pre-*Striga* seed conditioning treatment with DAS consistently inhibited seed germination of diverse *S. hermonthica* populations, and DAS was effective both as a pre- and post-*Striga* seed conditioning treatment. It therefore implied that the mode by which DAS specifically inhibited *S. hermonthica* seed germination is not limited to the general disruption of vital cytological pathways or processes that occurs during *S. hermonthica* seed preconditioning for which trichothecenes are widely known, such as disruption of protein/DNA synthesis, mitochondrial function and mitotic division (Desjardins et al. 1993; Bin-Umer et al. 2011; Gurdaswani and Ghag 2020). Perhaps, it could be connected to the disruption of other post-*Striga* seed conditioning pathways that are essential for *S. hermonthica* seed germination e.g., strigolactone perception. Because the strigolactone signal, which triggers *S. hermonthica* seed germination is perceived by strigolactone receptor, hence disruption of the strigolactone receptor (e.g., by mutation, or physical/chemical inhibitors) could lead to the inability of the receptor to perceive/transduce strigolactone signal (Saucet and Shirasu 2016; Seto et al. 2019). For instance, certain chemical inhibitors of the strigolactone receptors in some flowering plants (i.e., the α/β-hydrolase family proteins e.g., decreased apical dominance *2* in petunia (*DAD2*), and DWARF14 (*D14*) in *Arabidopsis* (*AtD14*) and rice (*OsD14*)), were recently identified. Examples of these inhibitors include, 2MN (Mashita et al. 2016), N-Ph (Hamiaux et al. 2018) and DL1 (Yoshimura et al. 2018). Thus, this major variation in the *S. hermonthica* seed germination pathway may serve as an important point of action for DAS. However, the exact mode of DAS inhibitory action against *S. hermonthica* seed germination remained elusive.

5.5.2 DAS application in planta did not affect sorghum biomass and soil microbial abundance

Treatment of the planting soil substrate with 20 µM DAS did not affect the ABM of *S. hermonthica*-free sorghum. This observation was the same for all tested exometabolites. It was previously reported that shoot morphogenesis was severely inhibited in *Nicotiana tabacum* grown on DAS-treated MS agar as from 5 µM, but was completely blocked at 20 µM DAS (Muhitch et al. 2000). Also, *Arabidopsis* shoot growth was severely inhibited and root elongation completely blocked, when grown on 10 µM DAS-treated MS agar (Masuda et al. 2007). Thus, the extent of toxicity of DAS and other trichothecenes varies with the test plant

(Masuda et al. 2007; Perincherry et al. 2019). This, therefore, corroborates the need to ascertain an exometabolite working concentration on *S. hermonthica* host plants, before the exometabolite is adopted for parasitic weed management.

The soil bacterial and fungal community abundances were increased in relation to increasing DAS concentration applied to the planting soil substrate. Accordingly, this observation coincided with the complete degradation of DAS in the soil substrates after a 14-d incubation of the experimental setup. Numerous microbes, especially bacteria and fungi, possess enzymatic gene products to specifically and efficiently degrade the 12,13-epoxy ring that is important for trichothecene toxicity (Devreese et al. 2013; Vanhoutte et al. 2016). For instance, bovine rumen microorganisms e.g., *Eubacteria* strain BBSH 797, selectively degrade the 12,13-epoxy ring of DAS, T-2 and deoxynivalenol by enzymatic reduction into de-epoxy and deacylated products (Swanson et al. 1987; Fuchs et al. 2002). Also, widely diverse soil and freshwater bacteria species including, but not limited to, *Curtobacterium* sp., *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp. *Agrobacterium* sp., *Brevibacterium* sp., *Blastobacter* sp., *Alcaligenes* sp., *Microbacterium* sp. and *Xanthomonas* sp., have been reported to completely degrade T-2, DAS, Neo and type B trichothecenes (nivalenol and fusarenon-X); whereby, the degraded product is assimilated and utilized as a source of carbon and energy for cellular growth/multiplication (Ueno et al. 1983; Beeton and Bull 1989). On the other hand, fungal degradation of trichothecenes is mainly by acetylation and deacetylation of the trichothecene molecule to produce significantly less-toxic acetoxy products, without necessarily affecting the trichothecene core (Yoshizawa et al. 1980). For example, the degradation of DAS by resting cells of *Mucor mucedo* and growing cells of *F. oxysporum* f. sp. *vasinfectum*, to produce triacetoxyscirpene and 3-acetoxyscirpene-4,15-diol respectively (Claridge and Schmitz 1978; 1979). Thus, the ability of numerous environmental microbes to efficiently degrade trichothecenes, and assimilate the end products as carbon/energy source for cellular multiplication, may explain the correlation we observed between increasing DAS treatment concentration in the planting soil substrate and the soil microbial community abundance, whereby DAS was eventually degraded in the planting soil substrate.

5.5.3 Fos does not produce DAS

Fos, represented by Foxy-2 and FK3 isolates, did not produce DAS according to the exometabolomic (LC-MS) analysis. This observation corresponded to both *Fos* isolates underexpression of key genes (Tri5 and Tri4) that are necessary for initiating the *Fusarium* trichothecene biosynthesis pathway. It was on this note that the expression of Tri101, Tri11, Tri3, Tri13 and Tri7, and Tri8 genes by *Fos* were not further analysed in this study, since Foxy-2 and FK3 showed underexpression of Tri5 and Tri4 genes; an observation

in line with the *Fos* exometabolomic analysis. This discovery gives a new insight that DAS (or essentially, trichothecenes) is not part of *Fos* exometabolome composition. This finding was in accordance with Proctor et al. (2009) and Khatibi et al. (2011), that categorized *F. oxysporum* as a non-producer of trichothecenes. However, it is worthy to note that some authors had reported the isolation of trichothecenes e.g., DAS and T-2, from *F. oxysporum* (Ghosal et al. 1976; Mirocha et al. 1989; Hasan 1999). Although, Ghosal et al. (1976) had clearly specified the *forma specialis* of the *F. oxysporum* they investigated i.e., *F. oxysporum* f. sp. *carthami*, which was different from ours. However, Mirocha et al. (1989) and Hasan (1999 did not further specify the *forma specialis* of *F. oxysporum* used in their experiment. It was revealed that *F. oxysporum* carry both non-functional (pseudo-Tri101) and functional (Tri201) trichothecene 3-*O*-acetyltransferase genes in their genome. The Tri201 gene acts like the Tri101 gene in known *Fusarium* trichothecene producers (e.g., *F. sporotrichioides*), which is responsible for C3 acetylation of 3-hydroxytrichothecenes (e.g., isotrichodermol) into a less-toxic product (e.g., isotrichodermin). This action is both a means of self-defence against toxic trichothecenes, and is also part of the *Fusarium* trichothecene biosynthesis pathway (Kimura et al. 1998; 2003; 2007). Therefore, it might be suggested that the ancestor of *F. oxysporum* was a trichothecene producer, before the divergence of trichothecene producers from non-producers in the evolution of *Fusarium* species (Tokai et al. 2005). Our finding, in contrast to Ghosal et al. (1976), Mirocha et al. (1989), Hasan (1999), perhaps illustrates the proposed divergent evolution of trichothecene producers from non-producers in *F. oxysporum*.

5.6 Conclusion

Among the tested *Fusarium* exometabolites, DAS was unequalled for completely inhibiting *S. hermonthica* seed germination *in vitro*, and preventing *S. hermonthica* incidence *in planta*. Notwithstanding the promising attributes of DAS in this study, there is need to further investigate its specific mode of action against the germination of *S. hermonthica* seeds. This investigation would be a critical step, before performing in situ verification (field trials) of the *S. hermonthica*-biopesticidal efficacy of DAS. Through this, DAS specificity of action against the target weed (*S. hermonthica*) at very low concentrations (≤ 20 µM), as opposed to non-target soil organisms, will be clearly understood.

Also, our study revealed that contrary to *F. venenatum, Fos* is a non-producer of DAS. It therefore raises the question if *F. venenatum* could be a complementary bioherbicide for controlling *S. hermonthica*. In this regard, an additional positive feature of this ubiquitous soil-borne saprophytic fungus is its inability to produce some mycotoxins, including T-2, deoxynivalenol, nivalenol, zearalenone and sambucoin (Miller and MacKenzie 2000).

Furthermore, *F. venenatum* is phylogenetically closely related to the phytopathogenic fungi *F. graminearum*, which is globally notorious for causing Fusarium head blight in cereals and vascular wilt to non-cereal plants, however, the non-phytopathogenic status of *F. venenatum* was confirmed (King et al. 2018). Thus, as part of future research directions, *F. venenatum* could be tested for its *S. hermonthica* incidence prevention *in planta*. This will reveal whether the quantity of DAS produced by *F. venenatum* in the soil will sufficiently prevent *S. hermonthica* germination, whilst unaffecting non-target organisms. Therefore, *F. venenatum* could serve as a sustainable, cheaper (compared to isolated/purified DAS), and proactive biocontrol agent for *S. hermonthica* eradication. Another option would be to test if the co-inoculation of *F. venenatum* and *Fos* isolate (with known pathogenicity towards the given *S. hermonthica* population) will better increase the overall *S. hermonthica* biocontrol efficiency through synergism. This is based on the assumption that DAS from *F. venenatum* will primarily attack *S. hermonthica* germination, while *Fos* will attack the incidence of germinated or attached *S. hermonthica* seedlings that escaped the reach of DAS in the soil.

Chapter 6. General Discussion and Conclusions

6.1 Overview

Witchweeds (i.e., parasitic *Striga* spp.), especially *Striga hermonthica* (Delile) Benth., are a major biotic constraint to cereal production in sub-Saharan Africa (SSA), where they cause enormous crop yield losses estimated at over US$10 billion per annum (Ejeta 2007a, Mandumbu et al. 2017). Sorghum, however, is the most affected cereal of the menace caused by parasitic *Striga* spp. in SSA, whereby it suffers yield loss amounting to 6.5 to 6.9 million t per annum (Gressel et al. 2004). Owing mainly to the excessive costs of developing and registering chemical pesticides, along with potential environmental hazards associated with chemical pesticides, biological control agents (BCA) or bioherbicides on the other hand, have become a highly sought-after, alternative strategy, for cheaper/safer systems of weed control (Boyette et al. 1996; Duke et al. 2015; Jabran and Chauhan 2018). *Fusarium oxysporum* f. sp. *strigae* (*Fos*), is a prominent soil-borne fungal agent for controlling *S*. *hermonthica*. Various *Fos* isolates, for example, Foxy-2 (from Ghana) and FK3 (from Kenya), have proven effective for specifically and significantly tackling *S. hermonthica* under both natural and artificial environments, without negatively affecting the cereal crop (Abbasher et al. 1998; Venne et al. 2009; Kangethe et al. 2016).

Sadly, the inconsistent effectiveness of *Fos* isolates against diverse *S. hermonthica* populations of differing origins, is a primary obstacle that discourages the widespread use of any bioherbicidal agent in general, against its target weed (El-Sayed 2005; Avedi et al. 2014; Harding and Raizada 2015). Therefore, a good understanding of the factors underlying this daunting phenomenon would be a crucial step towards developing workable solutions (e.g., integration of other non-*Fos* inoculum BCA, such as a bioherbicide cocktail of *Fos* and plant growth promoting rhizobacteria (PGPR), or utilizing fungal secondary metabolites) for overcoming this critical problem. Also, despite the *Fos*-induced suppression/death of *S. hermonthica*, the physiological damage *S. hermonthica* initiates to an infested cereal crop is mostly irreversible (Beed et al. 2007; Rumsey 2012). Hence, the integration of other non-*Fos* inoculum BCA (e.g., PGPR combined with *Fos*, or fungal secondary metabolites) for *S. hermonthica* biocontrol is, in a wider sense, expected to also serve as means for circumventing this other challenge (as this would prevent *S. hermonthica* germination and infestation in the first place). It has been reported that besides the renowned activity of PGPR in enhancing crop health and growth, certain PGPR strains (especially *Bacillus subtilis* isolate GB03) have shown promising potentials to prevent *S. hermonthica* seed germination, haustorial development/attachment and tubercle formation (Mounde et al. 2015). Similarly, certain *Fusarium* exometabolites have shown complete inhibition of *S. hermonthica* seed germination

in vitro, at very low concentrations (Zonno and Vurro 1999). However, knowledge of the performance of these suggested strategies for *S. hermonthica* biocontrol under planting conditions was lacking.

Hence, in the framework of this thesis, the tripartite interaction between *Striga* spp., cereal plants and plant root-associated microorganisms was reviewed (chapter 2). This review appraised current knowledge relating to the importance of genetic diversity of *Striga* spp., as well as the biotic interactions that occur in a *Striga*–cereal plant parasitic system, and their roles in determining the effectiveness of a bioherbicide against the parasitic weed. Thus, the molecular genetic basis, underlying the variable susceptibility of differing *S. hermonthica* populations from SSA to contrasting *Fos* isolates (Foxy-2, FK3) was investigated (chapter 3). The study revealed the role and superiority of genomic variation in *S. hermonthica*, over *S. hermonthica* sampling zone/origin, in determining *S. hermonthica* variable susceptibility towards the different *Fos* isolates. Furthermore, the role/interaction and localization of the combined BCA treatment of *Fos* (Foxy-2, FK3) and a PGPR (*B. subtilis* isolate GB03), on *S. hermonthica* incidence and sorghum aboveground biomass in a *S. hermonthica*-sorghum parasitic system was examined (chapter 4). The study showed that despite the colocalization of *Fos* and GB03 in common ecological niches of diseased *S. hermonthica* shoot, the BCA combination was not beneficial for *S. hermonthica* control, because GB03 counteracted *Fos* (FK3) suppressive activity against *S. hermonthica*. Lastly, a set of highly phytotoxic *Fusarium* exometabolites were screened for their bioherbicidal potential against *S. hermonthica*, and if the most potent/efficient candidate exometabolite in this regard, is part of the *Fos* exometabolome composition for attacking *S. hermonthica* (chapter 5). The study showed that diacetoxyscirpenol (DAS) was the most potent among the tested exometabolites, to completely antagonize *S. hermonthica*. However, *F*os was shown to be a non-producer of DAS (i.e., DAS is not part of *Fos* exometabolome composition); a discovery that corresponded to its underexpression of key genes necessary for *Fusarium* trichothecene biosynthesis. In the concluding part of the thesis, findings from the three research studies were discussed in line with the study aims, as well as the study limitations, recommendations, and future research directions (chapter 6).

6.2 Genomic variation in *S. hermonthica* is a superior determinant of the inconsistent effectiveness of *Fos* isolates, rather than the *S. hermonthica* origin

Genomic variation is an important phenomenon in *S. hermonthica*. This is because the obligate outcrossing mating system of *S. hermonthica* ensures a high rate of interbreeding, which thereby result to high genetic diversity of individuals (Musselman and Parker 1983; Safa

et al. 1984; Bozkurt et al. 2015). By definition, genomic variation is the difference in the genetic make-up (or DNA) among individuals of a given species, either within a population or between populations. The primary sources of genomic variation are mutation (the most important), followed by genetic recombination (sexual reproduction), and gene flow (gene migration) (Griffiths et al. 2000; Nevo and Beiles 2011). As geography has been detailed as a major determinant of gene flow (Wolf and Soltis 1992; Welsh and Mohamed 2011), it therefore explained the high rate of gene flow (*Nm* = 21.42) calculated between the West African and East African *S. hermonthica* populations (despite the wide geographic distance separating them), in contrast to the moderate gene flow calculated for the other *S. hermonthica* differentiation patterns i.e., genetic distance (*Nm* = 2.34) and phenotypic response to *Fos* isolates (*Nm* = 1.493) (Chapter 3). The high rate of gene flow between the West African and East African *S. hermonthica* populations, notwithstanding the wide geographic distance between them, could be explained by the overall arbitrary trans-Saharan trade network in Africa (Keyser 2014; Chimee 2018). This is because in subsistence agricultural systems, as mainly practiced in SSA (Sibhatu and Qaim 2017), *S. hermonthica*-contaminated crop seeds is dispersed mainly through trading activities. However, the dispersal of *S. hermonthica*-contaminated crop seeds by animal, wind or water is of little importance, due to the limitations in their spatial distances' coverage (Berner et al. 1994; van Delft et al. 1997). Thus, this could also explain the low genetic differentiation calculated between the West and East African *S. hermonthica* populations (F_{ST} = 0.012, P < 0.05). Because as the rate of gene flow is increased between two populations, so does the tendency of the two populations to attain equivalent allele frequencies, and therefore be considered a single effective population (Wright 1969; Slatkin 1987).

As *S. hermonthica* naturally exhibits a high rate of interbreeding, none of the *S. hermonthica* differentiation patterns showed a large/high genetic differentiation between the structured groups (Table 3.1). Interestingly, differentiation of the sampled *S. hermonthica* populations by phenotypic response to *Fos* isolates revealed a stronger population structure by intermediate genetic differentiation (F_{ST} = 0.143, P < 0.01), compared to the low genetic differentiation calculated between the West and East Africa sampling zones. This implied that in spite of the extent of gene flow among *S. hermonthica*, the genes responsible for the susceptibility response of *S. hermonthica* to *Fos* isolates were not affected as would be expected for obligate out-crossers, which should normally show a low genetic differentiation between structured groups (Charlesworth et al. 1993; Nordborg 2000). This evidence was also substantiated by the relatively smaller estimated variance within the structured *S. hermonthica* groups differentiated by phenotypic response to *Fos* isolates (87%), which when compared to the other differentiation patterns i.e., East and West Africa sampling zones (99%) and genetic

distance (91%), it indicated a reduction in genetic diversity, or put in another way, a relative tendency towards homozygosity (Charlesworth and Charlesworth 1995; Szczecińska et al. 2016). Hence, these findings, to an extent infer the stability of the susceptibility or non-susceptibility trait of *S. hermonthica* to *Fos* isolates. As such, this inference explains the association of simple sequence repeats (SSRs) loci (Y53 and E1009) to the *S. hermonthica* class with susceptibility to both Foxy-2 and FK3 (i.e., Group A), while the *S. hermonthica* class showing intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3 (i.e., made up of Groups B, C and D) was not associated to Y53 and E1009, nor to any of the utilized SSR markers in general. Rather, this latter *S. hermonthica* class demonstrated linkage disequilibrium (LD) decay (Table 3.2). LD decay can be influenced by various evolutionary forces e.g., mutation rate, selection, mating system, genetic recombination rate, hybridization, population genetic structure, genetic drift and population bottleneck (Gaut and Long 2003; Fox et al. 2019). Although, chapter 3 confirmed the insignificance of genetic drift and population bottleneck for explaining the population genetic structure that underlie the variable susceptibility of the sampled *S. hermonthica* to Foxy-2 and FK3. Instead, chapter 3 uncovered the mutational basis (i.e., underlying genomic variation) for the variable susceptibility of *S. hermonthica* to Foxy-2 or FK3. Through these mutations (which were in the form of point substitutions and insertion–deletion), the unique, protein-coding, nucleotide sequence at the E1009 locus of the *S. hermonthica* class with susceptibility to both Foxy-2 and FK3, was dissimilar to the *S. hermonthica* class showing intermediate susceptibility/non-susceptibility to either Foxy-2 or FK3 (Figs. 3.6 and 3.7).

Various evolutionary forces are known to be responsible for genomic variations that occur between natural populations of organisms. For instance, hybridization is an indispensable evolutionary force that causes genomic variations in natural populations (Thompson 2020; Moran et al. 2021). In the case of *S. hermonthica*, hybridization may occur through interspecific mating between *S. hermonthica* and other *Striga* spp. For example, *S. hermonthica* has been reported to hybridize with *S. aspera* to produce virulent and fertile hybrids, which bear viable seeds (Aigbokhan et al. 1998; 2000). Also, Aigbokhan et al. (2000), through a RAPD marker-assisted genetic investigation, detailed that F1 hybrids from *S. hermonthica* × *S. aspera* crosses showed closer similarity to their maternal parents, while F2 plants and backcrosses were mostly morphologically similar to *S. hermonthica*. They also suggested the existence of *S. hermonthica* × *S. aspera* hybrids in the wild, and that *S. hermonthica* were derived from *S. aspera*. This, therefore, implies that one may easily confuse *S. hermonthica* × *S. aspera* F2 plants and backcrosses, with pure *S. hermonthica* plants during field surveys. Hybrids arising from *S. hermonthica* crosses with other less common *Striga* spp. may manifest genomic

variation that are expressed through various phenotypic responses, wherein, variable phenotypic response to *Fos* isolates is not an exemption.

Given these genetic findings discussed above, together with the fact that the susceptibility pattern of the sampled *S. hermonthica* populations to Foxy-2 and FK3 was not consistent with the *S. hermonthica* sampling zones. For example, the *S. hermonthica* from Wad-Medani, Sudan (East Africa), was susceptible to both Foxy-2 and FK3, like almost all the sampled West African *S. hermonthica* populations; while the *S. hermonthica* from Bawku, Ghana (West Africa), was susceptible to Foxy-2 but not to FK3, unlike all the other sampled West African *S. hermonthica* populations (Fig. 3.2). Hence, these evidences confirm the superiority of genomic variation as a more realistic determiner of the variable susceptibility of *S. hermonthica* to *Fos* isolates, rather than the *S. hermonthica* sampling zone/origin. Therefore, in the context of genetic diversity in *S. hermonthica*, the inconsistent effectiveness of *Fos* isolates against differing *S. hermonthica* populations is better explained by genomic variation in *S. hermonthica*, rather than by the *S. hermonthica* sampling zone (i.e., East Africa and West Africa). Thus, the first of the study hypotheses is accepted.

6.3 Coinoculation of *Fos* and *B. subtilis* in a *S. hermonthica*-sorghum pathosystem did not synergistically optimize *S. hermonthica* management

In line with the well reported mycoherbicidal efficacy of *Fos* against *S. hermonthica* under both controlled and field environments (Kroschel et al. 1996; Rebeka 2013; Shayanowako et al. 2018), the ability of *Fos* to also specifically and significantly supress *S. hermonthica* incidence under controlled (climate chamber) environment were also presented in chapters 3 and 4. Though, it is worthy to re-emphasize that in the planting experiments, it was only FK3 that significantly suppressed the incidence of the sampled *S. hermonthica* from Sirinka, Ethiopia, but not Foxy-2 (Figs. 3.2, 4.5 and 4.7). This outcome demonstrated the critical challenge of *Fos* isolates inconsistent effectiveness in the biocontrol of *S. hermonthica* (Venne et al. 2009; Avedi et al. 2014), and which is the main research problem for the PhD study. This inconsistent effectiveness of the two *Fos* isolates (i.e., FK3 effectiveness and Foxy-2 non-effectiveness) against the sampled *S. hermonthica* population (from Sirinka, Ethiopia) were stably displayed by both the wild-type and transformed strains of the two *Fos* isolates (Fig. 4.7). In addition, the effectiveness of FK3 and non-effectiveness of Foxy-2 against the *S. hermonthica* population was unaltered by their application period during the planting experiments i.e., as pre-*Striga* seed conditioning or post-*Striga* attachment treatments application (Figs. 4.5 and 4.7). This, therefore, corresponded with an inference mentioned above regarding the stability of the susceptibility/non-susceptibility response of a given *S. hermonthica* population to *Fos* isolates.

On the other hand, *B. subtilis* (represented by isolate GB03) remarkably improved the aboveground biomass of *S. hermonthica*-infested sorghum in the study. Similarly, this effect was consistent for both the wild-type and transformed GB03 strains investigated, as well as for either when it was applied as pre-*Striga* seed conditioning or post-*Striga* attachment treatments (Figs. 4.5 and 4.7). Through this growth promoting effect by *B. subtilis*, the sorghum yield loss that is attributable to *S. hermonthica* infestation (as evident in the *S. hermonthica* positive control treatment), was adequately compensated. This interesting finding, however, contradicted Mounde et al. (2015), where they had reported the failure of various PGPR strains (precisely, isolates GB03 and Bsn5 of *B. subtilis*, isolate FZB42 of *B. amyloliquefaciens*, and isolate PsJN of *Burkholderia phytofirmans*) which were applied as post-*Striga* seed conditioning (pre-*Striga* attachment) treatments, to improve the biomass of *S. hermonthica*-infested sorghum. Hence, this implies that in spite of the higher extent of *S. hermonthica* infection of sorghum before *B. subtilis* was applied as a post-*Striga* attachment treatment (chapter 4), as oppose to the lower *S. hermonthica* infection extent of sorghum before *B. subtilis* was applied as pre-*Striga* attachment treatment (Mounde et al. 2015), the beneficial (growth promoting) impact of *B. subtilis* on the biomass/yield of *S. hermonthica*-infested sorghum was not restrained in chapter 4. The reason for this particular contradiction, in the biomass response of *S. hermonthica*-infested sorghum to *B. subtilis* in the study of Mounde et al. (2015), in contrast to chapter 4 of this thesis, was unclear.

Before now, the infection mode of *Fos* into *S. hermonthica* had been reported to occur by indirect mode i.e., rhizosphere-transmission through the “sorghum central cylinder–*S. hermonthica* haustorial” connecting tissues within sorghum root (Elzein et al. 2010; Ndambi et al. 2011). So that *Fos* eventually cause death to *S. hermonthica* by preliminarily colonizing *S. hermonthica* xylem vessels with its hyphae, followed by either degradation or blockage of the xylem vessels in young or matured *S. hermonthica* plants respectively (Ndambi et al. 2011). In chapter 4, *Fos* infection of *S. hermonthica* by direct mode was revealed. The process occurred through phyllosphere-transmission, whereby the germ tube of germinating *Fos* chlamydospore penetrated the *S. hermonthica* trichome (Fig. 4.8E). Through this newly identified anatomical pathway of *Fos* infection of *S. hermonthica*, it elucidates an alternative infection route of *Fos* into *S. hermonthica*, especially when *Fos* is applied as a post-*Striga* attachment treatment. Thus, this finding gives further insight that as *S. hermonthica* leaves and stems are highly pubescent (Mohamed et al. 2001), *Fos* application as post-*Striga* attachment treatment e.g., by foliar application (Marley et al. 1999) or basal application (Abbasher et al. 1998), could favourably lead to *Fos* infecting *S. hermonthica* by phyllosphere-transmission through *S. hermonthica* trichomes. Therefore, this infection route cannot be underestimated. Chapter 4 also revealed that both *Fos* and *B. subtilis* colocalized within

infected *S. hermonthica* shoot tissues, especially in flavonoid-abundant spots (Figs. 4.8K and 4.8L); their colocalization was further confirmed by PCR (see appendix 6). This discovery was in line with the proposed rhizosphere transmission of fungal and bacterial endophytes into the plant endosphere by van Overbeek and Saikkonen (2016). They suggested that prior endophytic fungal penetration of plant roots, facilitates endophytic bacteria entry and colonization of the plant endosphere, where they colocalize in the lumen of the xylem vessels by forming mixed biofilms. Interestingly, apart from corroborating this plant–endophytic (fungal, bacterial) model, chapter 4 gives further insight that the colonization of the bacterial agent in *S. hermonthica* endosphere is highly distributed in flavonoid-abundant regions (Fig. 4.8J). Though, the particular flavonoid type(s) present in the microbial colocalization regions within the infected *S. hermonthica* shoot tissues were not further characterised, as this was beyond the scope of the study. Flavonoids are important signaling compounds for initiating plant-microbe interactions (Straney et al. 2002; Mierziak et al. 2014). For instance, flavonoids act as carbon source or chemoattractants (chemotaxis) for initiating symbiotic rhizobacterial interaction with plants (Aguilar et al. 1998; Hassan and Mathesius 2012). Naringenin (in wheat roots), including luteolin and chrysin (in leguminous plants roots) are examples of flavonoids that stimulate or act as a specific signal for plant root colonizing bacteria such as *Azorhizobium* sp. and *Rhizobium* sp. (Hartwig et al. 1990; Webster et al. 1998). Also, other examples of flavonoids such as quercetin, galactoside and kaempferol, have been reported to promote spore germination and the hyphal growth of mycorrhizal fungi (Tsai et al. 1991; Poulin et al. 1997), while phytoalexins from legumes have been reported to induce sporal germination of the phytopathogenic fungi *F. solani* (Ruan et al. 1995). There are no available reports so far regarding the role of *S. hermonthica* flavonoids in signaling or stimulating *S. hermonthica*-*Fos* or *S. hermonthica*-*B. subtilis* interactions. It is unclear if *S. hermonthica* flavonoids play active role in facilitating the plant (i.e., *S. hermonthica*)–microbe (i.e., *Fos*, *B. subtilis*) interactions, as seen with other comparable instances previously reported.

Despite the colocalization of *Fos* and *B. subtilis* within infected *S. hermonthica*, the coinoculation of these fungal-bacterial agents negatively affected (reduced) the *Fos* mycoherbicidal efficacy against *S. hermonthica* incidence. This disadvantage was particularly obvious when the combined *Fos*-*B. subtilis* treatments were compared to the effective single FK3 treatment (Fig. 4.5). Several reports have described the antifungal activity of *B. subtilis* against various phytopathogenic *F. oxysporum* strains. For instance (but not limited to), the antifungal activity of *B. subtilis* against: Fusarium root rot of soybean (Zhang et al. 2009), Fusarium wilt of tomato (Ramyabharathi and Raguchander 2014), and Fusarium wilt of watermelon (Zhu et al. 2020). Hence, various *B. subtilis* metabolites which are important antagonists (antifungal agents) of *F. oxysporum* have also been identified e.g., macrolactins,

surfactins, fengycins, iturins, bacilysin and mersacidin (Caulier et al. 2019; Zhu et al. 2020). Although, this peculiar *B. subtilis* antagonistic activity against different *F. oxysporum* strains has not been a major concern for *Fos* under natural ecologies. Because in addition to *Fos* being a naturally occurring, soil-borne phytopathogen, with proven ability to control *S. hermonthica* under natural or artificial environments, *Fos* has also shown to be compatible with indigenous maize rhizosphere microbiota i.e., archaeal, bacterial and fungal communities (Musyoki et al. 2016; Zimmermann et al. 2016b). Nevertheless, knowledge of particular metabolites that are produced by common agriculturally important microbes (e.g., *B. subtilis*), but which antagonize *F. oxysporum*, will help improve the efficiency of utilizing *Fos* for *S. hermonthica* biocontrol. So that these metabolites and/or their microbial producers could be circumvented where necessary. It was an important finding from the study that the combined *Fos*-*B. subtilis* treatment application in the *S. hermonthica*-sorghum pathosystem, either as pre-*Striga* seed conditioning or post-*Striga* attachment treatments, did not prevent the activity of *B. subtilis* in improving the aboveground biomass of *S. hermonthica*-infested sorghum, (Figs. 4.5 and 4.7). Unfortunately, this combined microbial treatment application thwarted *Fos* suppressive activity against *S. hermonthica* incidence. Thus, because the *Fos*-*B. subtilis* coinoculation did not synergistically optimize the management of *S. hermonthica* in the *S. hermonthica*-sorghum pathosystem, therefore the second of the study hypotheses is rejected.

6.4 Among the tested *Fusarium* exometabolites, DAS was the most promising candidate to antagonize *S. hermonthica*, but it is not produced by *Fos*

The ability of certain exometabolites, especially of *Fusarium* origin, to obstruct different stages in the life cycle of *S. hermonthica* have positioned them as potential biological tools for *S. hermonthica* biocontrol (Vurro et al. 2009; Cimmino et al. 2015; Triolet et al. 2020). In chapter 5, it was shown that among the tested highly-phytotoxic *Fusarium* exometabolites, DAS was unrivalled for completely inhibiting *S. hermonthica* seed germination *in vitro* (Table 5.3), and preventing *S. hermonthica* incidence *in planta* (Fig. 5.1). DAS also displayed broadscale efficacy of completely inhibiting the germination of diverse *S. hermonthica* populations (Table 5.4). Although, the bioherbicidal activity of DAS against *S. hermonthica* has not been previously reported; however, with *Orobanche ramosa* (branched broomrape), DAS was also reported to show a relatively stronger inhibitory potential of the seed germination, compared to other highly phytotoxic *Fusarium* exometabolites (Zonno and Vurro 2002). Earlier studies had presented the germination inhibiting potential of specific *Fusarium* exometabolites against *S. hermonthica* seeds, when applied either as post-*Striga* seed conditioning treatments (Zonno and Vurro 1999; Ahmed et al. 2001; Sugimoto et al. 2002; Idris et al. 2003), or as pre-

Striga seed conditioning treatment Kroschel and Elzein (2004). It is, however, noteworthy to state that in these previous studies, none of the tested exometabolites caused complete germination inhibition of *S. hermonthica* seeds at 1 µM, like DAS displayed in this study. Also, before now, assessment of the differential impact of a specific *Fusarium* exometabolite, when applied both as pre- or post-*Striga* seed conditioning treatment in a study, was lacking. Through this study, it was revealed that the *S. hermonthica* seed germination inhibition potential of DAS was more effective as a pre-*Striga* seed conditioning treatment (where it caused complete germination inhibition even at 1µM), than when it was applied as a post-*Striga* seed conditioning treatment (where it did not completely inhibit the germination at 1µM, but was nonetheless significantly active) (Fig. 5.3). The underlying basis for the higher potency of DAS as a pre-*Striga* seed conditioning treatment, rather than as a post-*Striga* seed conditioning treatment, was not investigated in this study. However, it was suspected that it may be connected to the disruption of essential metabolic pathways (e.g., synthesis of DNA, protein and hormones, including respiration) that normally occur during the seed preconditioning stage of parasitic Orobanchaceae (*Orobanche* sp., and *Striga* sp.), in preparation for germination (Joel 2000; Ejeta 2005). Thus, in ensuring a higher *S. hermonthica* biocontrol efficiency, this finding is in agreement with an earlier suggestion of applying *Striga*-bioherbicides (in this regard, DAS) as pre-*Striga* seed conditioning treatment (where applicable), rather than as a post-*Striga* seed conditioning treatment (chapter 4).

Up until now, metabolomic screenings of *Fos* exometabolites have only led to the identification of fusaric acid and dehydrofusaric acid (with their methyl esters) (Savard et al. 1997; Amalfitano et al. 2002), including beauvericin (Ndambi 2011), as *Fos* exogenous metabolites that are highly bioactive *Striga*-attacking agents. The findings presented in chapter 5 infer the non-production of DAS (Fig. 5.6), or essentially, trichothecene gene expression (Fig. 5.7) by *Fos*. On the one hand, this realization could be regarded an environmental advantage of *Fos* as a biocontrol agent i.e., it further supports the widely recognized biologically-safe status of *Fos*; but on the other hand, it suggests a limitation of *Fos* versatility as an efficient *S. hermonthica*-bioherbicide, particularly in relation to *Fos* inefficiency in blocking *S. hermonthica* seed germination. Hence, because DAS, which was the most potent/efficient candidate among the tested *Fusarium* exometabolites to antagonize *S. hermonthica*, is not part of the *Fos* exometabolome composition, therefore the third of the study hypotheses is rejected.

6.5 Study limitations, recommendations and future research directions

In uncovering the role of *S. hermonthica* genomic variation as a valid factor that underlies the inconsistent effectiveness of *Fos* isolates (Foxy-2 and FK3) against differing *S. hermonthica* populations, the study had utilized *S. hermonthica* populations that were sampled from ten locations, across seven countries in SSA (i.e., four countries in Western Africa and three countries in Eastern Africa), and they were genotyped with 22 SSR markers (chapter 3). Considering the difficulty of obtaining *S. hermonthica* seeds for research, which ranges from the rigours of field collection and preparation of pure seeds, to shipment/delivery (importation, custom clearance etc.), the number of *S. hermonthica* sampling locations/countries, together with the number of SSR markers utilized, was impressive. In comparison to previous studies that also researched on *S. hermonthica* genetic diversity using SSRs (e.g., Estep et al. 2011; Bozkurt et al. 2015; Joel et al. 2018), this PhD study has utilized the highest number of *S. hermonthica* sampling locations/countries, together with the highest number of SSR markers so far. However, it is undeniable that a larger set of *S. hermonthica* sampling populations from more locations/countries within the study regions would increase the robustness of verifying the linkage strength and stability of the marker–trait associations. Nevertheless, because of the validity of the molecular methods used for the population genetic analysis (see section 1.8.1), the results obtained from the study were not undermined, but well-grounded/valid.

As it was revealed from the study that genomic variation in *S. hermonthica* is superior over *S. hermonthica* sampling zone/origin for influencing the variable susceptibility pattern of *S. hermonthica* to contrasting *Fos* isolates, it therefore suggests that before any *S. hermonthica* biocontrol program whereby *Fos* is intended for use, the susceptibility of the given *S. hermonthica* population to different *Fos* isolates could be first ascertained through pilot experiments. This would be a guiding step for selecting a particular *Fos* isolate to effectively combat the *S. hermonthica* population. Hence, this represents a potentially proactive measure to avoid wastage of time, effort and resources during *S. hermonthica* biocontrol. Another possible proactive measure could be to develop specific molecular markers from the two SSR loci discovered to be associated with the *S. hermonthica* class with susceptibility to both Foxy-2 and FK3 (i.e., EST-SSR locus Y53, and SSR locus E1009). Though, bioinformatic analysis of their nucleotide sequences was only further investigated with E1009 (> 200 bp), but not Y53 (mean 86 bp), because of its relatively short fragment size which was unsuitable for the utilized sanger nucleotide sequencing. In case of future bioinformatic interest on Y53, suitable next-generation, short-read sequencing methods (i.e., massive parallel sequencing) could be utilized for sequencing Y53. These associated loci represent promising candidates for developing a marker-assisted identification procedure of a *S. hermonthica* population with this

desired trait i.e., susceptibility to contrasting *Fos* isolates (e.g., Foxy-2 and FK3). In this regard, the relatively high LD realized between SSR loci pairs in the *S. hermonthica* class susceptible to both Foxy-2 and FK3, opens up the way further LD mapping. This would give deeper insights into the genes underlying this trait (Weir 2008; de Souza et al. 2018). Thus, it is also a means for understanding the biological relevance of the identified associated loci in *S. hermonthica*.

The colocalization of *Fos* and *B. subtilis* mainly in flavonoid-rich spots of diseased *S. hermonthica* shoot (chapter 4), is an interesting discovery that leads to various important questions. Some examples of these questions are as follows:

- What characteristic flavonoid(s) do *S. hermonthica* produce that attract *Fos* and/or *B. subtilis* infection?
- Are these characteristic flavonoids uniquely produced by *S. hermonthica*, but not by the cereal host plant?
- What are the functional roles of these flavonoids to *S. hermonthica?*
- Does *Fos* and/or *B. subtilis* exploit these flavonoids as nutrient source, which could lead to disease/death of *S. hermonthica* when the flavonoids are consumed*?*
- Between *Fos* or *B. subtilis*, which microbe is principally attracted to these *S. hermonthica* flavonoids, thereby influencing its preliminary infection of *S. hermonthica*; while the infection channel serves as entry route for the other microbe into *S. hermonthica*?

Proper understanding of these questions would give better insight into the biological interactions that occur in the host (*S. hermonthica*)–pathogen/microbe (*Fos*, PGPR) pathosystem, especially in terms of optimizing *S. hermonthica* management through a *Fos*-PGPR bioherbicide cocktail. Furthermore, the newly identified, phyllosphere-transmitting, direct infection route of *Fos* into *S. hermonthica* (through the trichome), is a fascinating discovery that presents a novel paradigm to the infection mechanism occurring under the *S. hermonthica–Fos* bipartite interaction. This is in addition to the previously known indirect rhizosphere-transmission (Elzein et al. 2010; Ndambi et al. 2011). Through this novel discovery, it opens up a new research direction that is targeted at exploiting this alternative *Fos* infection route into *S. hermonthica,* for better combating the witchweed by biological control. For example, the mode by which *Fos* discharges its deteriorating role on *S. hermonthica* following its phyllosphere transmission could be investigated through current tracer-based imaging/mapping technologies (e.g., dye or isotope labelling, fluorescent gene reporting), as well as omics-based technologies (e.g., genomics, transcriptomics, proteomics and metabolomics). This, therefore, will elucidate the biological processes that occur after *Fos*

trichome entry into *S. hermonthica*, such as *Fos* hyphal growth towards *S. hermonthica* xylem vessels and/or *Fos* release of lytic exudates for local *S. hermonthica* tissue degradation.

Furthermore, apart from the outstanding biopesticidal potential of DAS against *S. hermonthica* (chapter 5), the ability of DAS to markedly enhance the rhizosphere microbial community abundance over a short period (i.e., 2 weeks), which also coincided with the complete degradation of DAS in the soil substrate, are additional advantages (i.e., pilot indicators of its ecological safety) that support the promising potential of DAS as an efficient biopesticide against *S. hermonthica* under natural conditions. Nevertheless, before field assessment of DAS efficacy against S. *hermonthica* is performed, knowledge of DAS specific mode of action in inhibiting *S. hermonthica* seed germination is indispensable. This important mystery was uncovered in the study, because it was not within the purview of the study aims. A clear understanding of this important question will illuminate DAS specificity of action against the target weed, *S. hermonthica*, in contrast to non-target organisms. Thus, solving this conundrum would be a major boost towards assuring the ecological safety of DAS (at low concentration i.e., ≤ 20 µM) for *S. hermonthica* control, before embarking on field assessment.

Lastly, the cost of procuring isolated/purified fungal exometabolites, is undoubtedly, an important limitation that hampers the interest of using fungal toxins as biopesticides to combat *S. hermonthica* (either for research, practical or actual field use). This limitation which centres on affordability and sustainability, would even be worse-off for low-income farmers who suffer the most from the menace of *S. hermonthica*. Hence, cheaper means of obtaining fungal exometabolites of interest at a large/commercial scale from the microbial producers i.e., “biomanufacturing”, could be developed through collaborative biotechnological techniques of genetic engineering and exometabolomics/metabolic footprinting (Mapelli et al. 2008; Zhang et al. 2017; Amer and Baidoo 2021). Alternatively, in the context of DAS, direct utilization of DAS-producing microbes for *S. hermonthica* biocontrol may even be a preferable option for low-income farmers, especially because this would be more affordable and sustainable. But as *Fos* was confirmed to be a non-producer of DAS from the study, it therefore paves the way for further research that focuses on the possibility of including other ubiquitous, soil-borne, non-phytopathogenic *Fusarium* sp. that are confirmed DAS producers e.g., *F. venenatum* (Miller and MacKenzie 2000; King et al. 2018), in an integrated biocontrol system with *Fos* (i.e., a bioherbicide cocktail). Further investigations will reveal whether a bioherbicidal cocktail of *F. venenatum* and a *Fos* isolate (with known pathogenicity towards the given *S. hermonthica* population) will successfully improve the efficiency of combating *S. hermonthica*, based on the fundamental assumptions given below (Fig. 6).

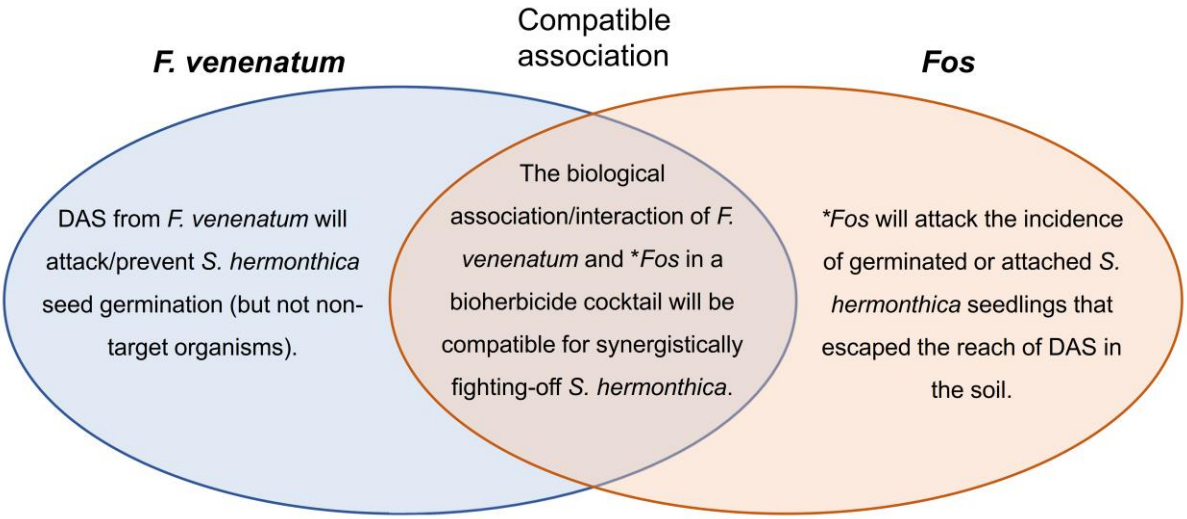

Fig. 6: Three fundamental assumptions to be fulfilled for a *F. venenatum* and *Fos* bioherbicidal cocktail to successfully optimize *S. hermonthica* control. **Fos* – *Fos* isolate with known pathogenicity towards the given *S. hermonthica* population. (Proposed by Williams O. Anteyi).

If trial results from efficacy assessments of the bioherbicidal potency of a *Fos-F. venenatum* cocktail is in the affirmative with the stated assumptions for *S. hermonthica* control, then this strategy holds great value for eradicating *S. hermonthica* which is infamous for causing billions of (US) dollars' worth of crop losses annually in SSA and threatening the livelihoods of millions of households in this region.

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Summary

Cereals are a major staple that is crucial for food security in sub-Saharan Africa (SSA). Sadly, the obligate hemiparasitic witchweed, *Striga* spp., especially *Striga hermonthica* (Delile) Benth., is a major biotic constraint to cereal production in SSA, causing enormous crop yield losses estimated at US$10 billion annually. *Fusarium oxysporum* f. sp. *strigae* (*Fos*) is the most renowned fungal biological control agent (BCA) for specifically and significantly tackling *S. hermonthica* under agricultural systems. Field surveys, however, have revealed the inconsistent effectiveness of *Fos* isolates against *S. hermonthica* in differing zones of SSA (i.e., West Africa, East Africa). This daunting phenomenon is a critical challenge that affects *Fos* reliability and deters its use for *S. hermonthica* management. The inconsistent effectiveness of *Fos* against *S. hermonthica* was presumably ascribed to the interactions that occur between the differing location-specific ecological factors of the pathosystem i.e., abiotic (climate, moisture, or soil physico-chemistry) or biotic (*S. hermonthica, Fos* isolate, or the plant microbiome). Without doubt, the diversity of a host or pathogen is a primary determinant of the innate susceptibility or virulence of the host or pathogen, respectively. In terms of *S. hermonthica* diversity, genomic variation of individuals, or regional genetic variation of the sampling zone, were the two major forces suspected. However, the important determiner out of the two forces was unknown. Besides, despite the suppression/death that *Fos* causes to *S. hermonthica*, the physiological damage *S. hermonthica* initiates to an infested cereal crop is mostly irreversible. Hence, in examining strategies for circumventing the main problem of *Fos* inconsistent effectiveness against *S. hermonthica*, and the physiological consequences of *S. hermonthica* on the host cereal crop, the integration of other (non-*Fos* inoculum) BCA were suggested as possible means for improving the efficiency of *S. hermonthica* biocontrol. For example, by utilizing a bioherbicide cocktail of *Fos* and plant growth promoting rhizobacteria (PGPR), or *Striga* seed germination-inhibiting fungal toxins. Apart from the popular reputation of PGPR in enhancing crop health and growth, certain PGPR strains (especially *Bacillus subtilis* isolate GB03) have been earlier reported for their highly-promising potential of antagonizing *S. hermonthica* development. Similarly, certain fungal extracellular metabolites (exometabolites), especially of *Fusarium* origin, were reported to completely inhibit *S. hermonthica* seed germination *in vitro* at very low concentrations (≤ 1 mM). Unfortunately, knowledge of the microbe (*Fos*)–microbe (PGPR) interaction, their localization and ecological niche, for enabling their expected synergistic impact of simultaneously suppressing *S. hermonthica* and enhancing the *Striga*-infected cereal crop biomass, was unknown. Also, it was unknown if highly potent/efficient *Striga* seed germination-inhibiting fungal exometabolites will consistently suppress *S. hermonthica in planta*. Thus, in the context of genetic diversity in *S. hermonthica*, the PhD study focused on gaining (molecular) insights into the inconsistent

effectiveness of *Fos* against *S. hermonthica*; including the examination of some strategies for improving *S. hermonthica* biocontrol efficiency, precisely by integrating PGPR, or *Striga* seed germination-inhibiting *Fusarium* exometabolites, into a *S. hermonthica* biocontrol system.

The first research examined the molecular genetic basis, underlying the variable susceptibility of *S. hermonthica* populations sampled from differing zones of SSA (West Africa, East Africa) to contrasting *Fos* isolates (Foxy-2, FK3). Regardless of sampling zone, the *S. hermonthica* populations displayed divergent susceptibility patterns to the *Fos* isolates i.e., a *S. hermonthica* class was susceptible to both Foxy-2 and FK3, while the other class was susceptible to either Foxy-2 or FK3. This manifestation correlated with nucleotide mutations at certain loci. Thus, genomic variation in *S. hermonthica* is a superior determinant of the inconsistent effectiveness of *Fos* isolates, rather than the *S. hermonthica* sampling zone. The second research examined the impact of coinoculating *Fos* and a PGPR (*B. subtilis* isolate GB03) into a *S. hermonthica*-sorghum parasitic system. Notwithstanding the colocalization of *Fos* and GB03 in common ecological niches of diseased *S. hermonthica* shoot (mainly in flavonoid-rich regions), GB03 thwarted *Fos* suppressive activity against *S. hermonthica*. Interestingly, a novel, alternative *Fos* entry route into *S. hermonthica* (through the trichome) was discovered. The coinoculation of *Fos* and GB03 presented no added advantage for *S. hermonthica* control. Finally, the third research screened a set of highly phytotoxic *Fusarium* exometabolites against *S. hermonthica* seed germination (*in vitro*) and incidence (*in planta*). This was to identify the most potent/efficient *Fusarium* exometabolite for *S. hermonthica* biocontrol. Among the tested exometabolites, diacetoxyscirpenol (DAS) was the most potent/efficient to completely suppress *S. hermonthica* both *in vitro* and *in planta*. *F*os, however, did not produce DAS, due to underexpression of key genes necessary for *Fusarium* trichothecene biosynthesis. In conclusion, owing to the obligate outcrossing mating system in *S. hermonthica*, genomic variation is an inevitable phenomenon. This, therefore, plays a crucial role in the variable susceptibility of *S. hermonthica* to *Fos*. The newly discovered *Fos* (direct) entry route into *S. hermonthica* (trichome entry), elucidates a novel paradigm to the infection mechanism occurring under the *S. hermonthica* (host)–*Fos* (pathogen) interaction, in addition to the previously reported indirect, rhizosphere-transmission. Thus, this novel phyllosphere-transmission, paves the way for further research that exploit this alternative *Fos* infection route for better *S. hermonthica* biocontrol. Lastly, considering the potency and broadscale efficacy against diverse *S. hermonthica* populations, the exometabolite DAS could serve as a new agent for a more efficient *S. hermonthica* biocontrol. Though, further examination of its specific mode of action against the target weed (*S. hermonthica*), as opposed to non-target organisms, is required.

Zusammenfassung (German Summary)

Getreide ist ein wichtiges Grundnahrungsmittel, das für die Ernährungssicherheit in Afrika südlich der Sahara (SSA) von entscheidender Bedeutung ist. Leider ist das obligate halbparasitäre Sommerwurzgewächs, *Striga* spp., insbesondere *Striga hermonthica* (Delile) Benth., eine große biotische Einschränkung für die Getreideproduktion in SSA und verursacht enorme Ernteverluste, die auf 10 Milliarden US-Dollar jährlich geschätzt werden. *Fusarium oxysporum* f. sp. *strigae* (*Fos*) ist der bekannteste pilzliche biologische Bekämpfungswirkstoff (BCA) zur spezifischen und signifikanten Bekämpfung von *S. hermonthica* in landwirtschaftlichen Systemen. Felduntersuchungen haben jedoch gezeigt, dass die Wirksamkeit von *Fos*-Isolaten gegen *S. hermonthica* in verschiedenen Zonen der SSA (d. h. Westafrika, Ostafrika) uneinheitlich ist. Dieses entmutigende Phänomen ist eine kritische Herausforderung, die die Zuverlässigkeit von *Fos* beeinträchtigt und seine Verwendung für die Bekämpfung von *S. hermonthica* verhindert. Die uneinheitliche Wirksamkeit von *Fos* gegen *S. hermonthica* wurde vermutlich auf die Wechselwirkungen zurückgeführt, die zwischen den verschiedenen standortspezifischen ökologischen Faktoren des Pathosystems auftreten, d. h. abiotischen (Klima, Feuchtigkeit oder physikalisch-chemische Eigenschaften des Bodens) oder biotischen (*S. hermonthica*, *Fos*-Isolat oder das Pflanzenmikrobiom). Zweifelsohne ist die Diversität eines Wirts oder Krankheitserregers eine der wichtigsten Determinanten für die angeborene Anfälligkeit bzw. Virulenz des Wirts oder Krankheitserregers. In Bezug auf die Diversität von *S. hermonthica* waren die genomische Variation der Individuen oder die regionale genetische Variation des Probenahmegebiets die beiden vermuteten Hauptfaktoren.

Es war jedoch nicht bekannt, welcher der beiden Faktoren ausschlaggebend ist. Außerdem sind die physiologischen Schäden, die *S. hermonthica* an einer befallenen Getreidepflanze verursacht, trotz der Unterdrückung/Tötung von *S. hermonthica* durch *Fos* meist irreversibel. Bei der Untersuchung von Strategien zur Umgehung des Hauptproblems der unzureichenden Wirksamkeit von *Fos* gegen *S. hermonthica* und der physiologischen Folgen von *S. hermonthica* für die Wirtsgetreidekulturen wurde daher die Integration anderer BCA (ohne *Fos*-Inokulum) als mögliches Mittel zur Verbesserung der Effizienz der Biokontrolle von *S. hermonthica* vorgeschlagen. Zum Beispiel durch die Verwendung eines Bioherbizid-Cocktails aus *Fos* und pflanzenwachstumsfördernden Rhizobakterien (PGPR) oder von Pilztoxinen, die die Keimung von *Striga*-Samen hemmen. Abgesehen von dem guten Ruf, den PGPR bei der Förderung der Gesundheit und des Wachstums von Nutzpflanzen genießen, wurde bereits früher über das vielversprechende Potenzial bestimmter PGPR-Stämme (insbesondere des Isolats GB03 von *Bacillus subtilis*) zur Bekämpfung der Entwicklung von *S. hermonthica* berichtet. Ebenso wurde berichtet, dass bestimmte extrazelluläre Metaboliten von Pilzen

(Exometaboliten), insbesondere von *Fusarium*, die Keimung von *S. hermonthica*-Samen *in vitro* bei sehr niedrigen Konzentrationen (≤ 1 mM) vollständig hemmen. Leider war das Wissen über die Interaktion zwischen Mikroben (*Fos*) und Mikroben (PGPR), ihre Lokalisierung und ökologische Nische nicht bekannt, um die erwartete synergistische Wirkung der gleichzeitigen Unterdrückung von *S. hermonthica* und der Verbesserung der Biomasse von *Striga*-infizierten Getreidepflanzen zu ermöglichen. Es war nicht bekannt, ob hochwirksame/effiziente, die Keimung von Striga-Samen hemmende, Pilzexometaboliten, dauerhaft *S. hermonthica in planta* unterdrücken können. Vor dem Hintergrund der genetischen Vielfalt von *S. hermonthica* konzentrierte sich die Doktorandenstudie daher auf die Gewinnung von (molekularen) Erkenntnissen über die uneinheitliche Wirksamkeit von Fos gegen *S. hermonthica*, einschließlich der Untersuchung einiger Strategien zur Verbesserung der Biokontroll-Effizienz von *S. hermonthica*, insbesondere durch die Integration von PGPR oder *Striga*-Samenkeimung hemmenden *Fusarium* Exometaboliten in ein *S. hermonthica*-Biokontrollsystem.

Die erste Studie untersuchte die molekulargenetische Grundlage, die der unterschiedlichen Anfälligkeit von *S. hermonthica*-Populationen aus verschiedenen Zonen in SSA (Westafrika, Ostafrika) gegenüber unterschiedlichen *Fos*-Isolaten (Foxy-2, FK3) zugrunde liegt. Unabhängig von der Probenahmezone zeigten die *S. hermonthica*-Populationen unterschiedliche Empfindlichkeitsmuster gegenüber den *Fos*-Isolaten, d. h. eine *S. hermonthica*-Klasse war sowohl für Foxy-2 als auch für FK3 empfänglich, während die andere Klasse entweder für Foxy-2 oder für FK3 empfänglich war. Diese Erscheinung korrelierte mit Nukleotidmutationen an bestimmten Loci. Somit ist die genomische Variation in *S. hermonthica* ein entscheidenderer Faktor für die uneinheitliche Wirksamkeit von *Fos*-Isolaten als die *S. hermonthica*-Probenahmezone. Die zweite Studie untersuchte die Auswirkungen der Ko-Inokulation von *Fos* und einer PGPR (*B. subtilis* Isolat GB03) in einem parasitären *S. hermonthica*-Sorghum-System. Ungeachtet der Kolokalisierung von *Fos* und GB03 in gemeinsamen ökologischen Nischen des erkrankten *S. hermonthica*-Sprosses (hauptsächlich in flavonoidreichen Regionen) vereitelte GB03 die suppressive Aktivität von *Fos* gegen *S. hermonthica*. Interessanterweise wurde ein neuer, alternativer *Fos*-Eintrittsweg in *S. hermonthica* (durch das Trichom) entdeckt. Die Ko-Inokulation von *Fos* und GB03 brachte keinen zusätzlichen Vorteil bei der Bekämpfung von *S. hermonthica*. In der dritten Forschungsarbeit schließlich wurde eine Reihe hochgradig phytotoxischer Exometaboliten von *Fusarium* gegen die Keimung von *S. hermonthica*-Samen (*in vitro*) und das Auftreten (*in planta*) untersucht. Damit sollte der wirksamste/effizienteste Exometabolit von *Fusarium* für die Biokontrolle von *S. hermonthica* ermittelt werden. Unter den getesteten Exometaboliten war Diacetoxyscirpenol (DAS) der wirksamste, um *S. hermonthica* sowohl *in vitro* als auch *in*

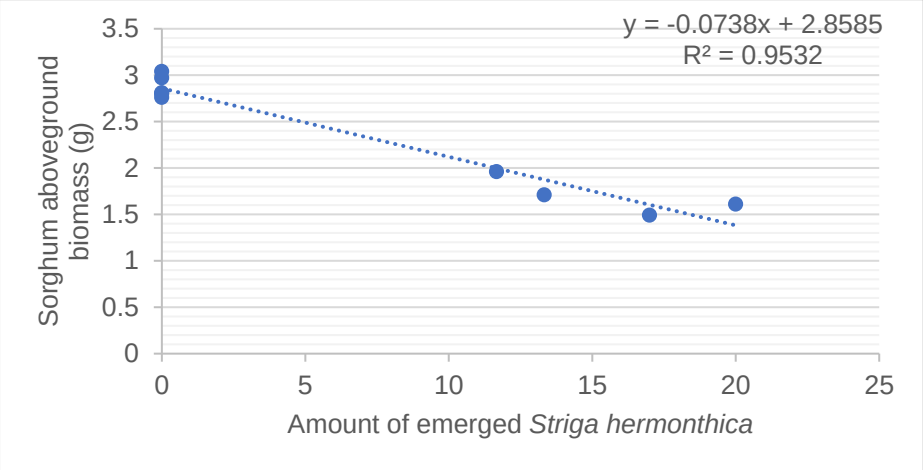
planta vollständig zu unterdrücken. *Fos* produzierte jedoch kein DAS, was auf die Unterexpression von Schlüsselgenen zurückzuführen ist, die für die Biosynthese von *Fusarium*-Trichothecen notwendig sind. Zusammenfassend lässt sich sagen, dass aufgrund des obligaten Auskreuzungssystems in *S. hermonthica* genomische Variation ein unvermeidliches Phänomen ist. Dies spielt daher eine entscheidende Rolle bei der variablen Anfälligkeit von *S. hermonthica* gegenüber *Fos*. Der neu entdeckte (direkte) Eintrittsweg von *Fos* in *S. hermonthica* (Trichom-Eintritt) stellt ein neues Paradigma für den Infektionsmechanismus dar, der im Rahmen der Interaktion zwischen *S. hermonthica* (Wirt) und *Fos* (Erreger) abläuft, zusätzlich zu der zuvor berichteten indirekten Rhizosphären-Übertragung. Diese neuartige Phyllosphären-Übertragung ebnet somit den Weg für weitere Forschungen, die diesen alternativen *Fos*-Infektionsweg für eine bessere Biokontrolle von *S. hermonthica* nutzen. Schließlich könnte der Exometabolit DAS in Anbetracht seiner Potenz und breiten Wirksamkeit gegen verschiedene *S. hermonthica*-Populationen als neuer Wirkstoff für eine effizientere Biokontrolle von *S. hermonthica* dienen. Allerdings ist eine weitere Untersuchung seiner spezifischen Wirkungsweise gegen das Zielunkraut (*S. hermonthica*) im Gegensatz zu Nicht-Zielorganismen erforderlich.

Appendices

Appendix 1: Linear regression (A) and Pearson correlation coefficient (B) of sorghum aboveground biomass and *S. hermonthica* incidence. (α = 0.05).

(A)

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.976309874							
R Square	0.95318097							
Adjusted R Square	0.946492538							
Standard Error	0.148376701							
Observations	9							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	3.137490482	3.137490482	142.5118556	6.58381E-06			
Residual	7	0.154109518	0.022015645					
Total	8	3.2916						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.858530635	0.065274747	43.79228972	8.45412E-10	2.704180385	3.012880885	2.704180385	3.012880885
X Variable 1	-0.073818963	0.006183615	-11.93783295	6.58381E-06	-0.088440889	-0.059197037	-0.088440889	-0.059197037



(B)

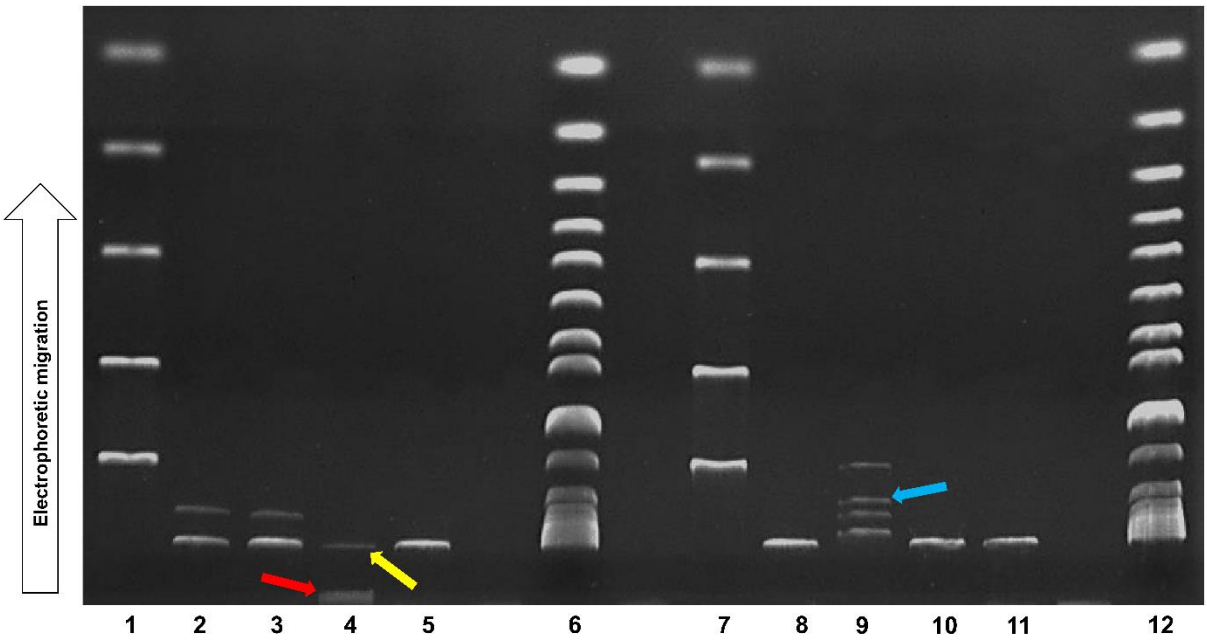
Mean from 6 replications			r = -0.98	
Striga emergence (X)	Sorghum biomass (Y)			
0	2.81			
0	3.04			
0	2.8			
0	2.76			
0	2.97			
11.67	1.96			
13.33	1.71			
17	1.49			
20	1.61			

Appendix 2: Meaning of letter codes in nucleotide and protein sequence data (IUPAC Single Letter Codes).

DNA/RNA				**Amino acid**		
Single-letter code	**Name**	**Remarks**		**Single-letter code**	**Name**	**Three-letter code**
A	Adenine	Purine		A	Alanine	Ala
G	Guanine	Purine		C	Cysteine	Cys
C	Cytosine	Pyrimidine		D	Aspartic Acid	Asp
T	Thymine	Pyrimidine		E	Glutamic Acid	Glu
U	Uracil	Pyrimidine		F	Phenylalanine	Phe
R	Purine	A or G		G	Glycine	Gly
Y	Pyrimidine	C or T/U		H	Histidine	His
M		A or C		I	Isoleucine	Ile
K		G or T		K	Lysine	Lys
S	Strong	C or G		L	Leucine	Leu
W	Weak	A or T		M	Methionine	Met
H	Not G	A or C or T		N	Asparagine	Asn
B	Not A	C or G or T		P	Proline	Pro
V	Not U/T	A or C or G		Q	Glutamine	Gln
D	Not C	A or G or T		R	Arginine	Arg
N	Ambiguous	A or C or G or T		S	Serine	Ser
				T	Threonine	Thr
				V	Valine	Val
				W	Tryptophan	Trp
				Y	Tyrosine	Tyr

Adapted from: Molecular Evolutionary Genetics Analysis - MEGA software. MEGAX-Help. Part III: Input Data Types and File Formats.
https://www.megasoftware.net/web_help_10/index.htm#t=IUPAC_Single_Letter_Codes.htm

Appendix 3: Verification of plasmids by diagnostic restriction digestion.



Gel electrophoretic image of plasmids (pFPL-Ch and pFPL-Rh) restriction digest. pFPL-Ch (Left-side). pFPL-Rh (Right side). 1 and 7 – Easy ladder I (Bioline GmbH, Luckenwalde, Germany). 6 and 12 – 1kb plus DNA ladder (Life technologies GmbH, Darmstadt, Germany).

pFPL-Ch (Left-side)	**pFPL-Rh (Right side)**
2: HindIII – 2 sites	8: HindIII – 1 site
3: FspI – 2 sites	9: FspI – 3 sites. Blue arrow is uncut plasmid fragment
4: KpnI – Undigested control (no site). Red arrow is nicked DNA. Yellow arrow is supercoiled DNA	10: KpnI – 1 site
5: AflII – 1 site.	11: AflII – 1 site.

Appendix 4: Detailed methodology for PEG-mediated transformation of *Fusarium oxysporum* f. sp. *strigae* (*Fos*).

Foxy-2 and FK3 mycelia from 5-day grown PDA cultures were separately transferred to 150 mL complete medium (0.6% yeast extract [Carl Roth GmbH], 0.3% casein hydrosylate enzymatic [MP Biomedicals, LLC, Solon, Ohio, USA], 0.3% casein hydrosylate acid [MP Biomedicals, LLC], 1% D-saccharose [Carl Roth GmbH]). This was incubated for 36 h, at 130 rpm and 28°C. Then, the mycelia were isolated with 3 layers of UV sterilized, Whatman™ lens cleaning tissue (GE Healthcare UK limited, Amersham, Buckinghamshire, UK), and washed thrice with 0.7 M sodium chloride solution (Carl Roth GmbH). Three g mycelia were transferred to 50 mL centrifuge tubes. Six mL of 2% (w/v) Driselase™ Basidiomycetes sp. (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 0.7 M sodium chloride solution were added to the mycelia in the centrifuge tubes. The mixture was shaken for 3.5 h at 50 rpm and 28°C, then filtered with 3 layers of sterilized Whatman™ lens cleaning tissue for 15 min at 4000 rpm and 4°C to purify the protoplasts. Protoplasts were washed twice with sorbitol-TC solution (1.2 M D-Sorbitol [Sigma-Aldrich Chemie GmbH], 10 mM Tris hydrochloride buffer pH 7.5, 50 mM calcium chloride [Carl Roth GmbH]), and thereafter suspended in 3 ml sorbitol-TC solution.

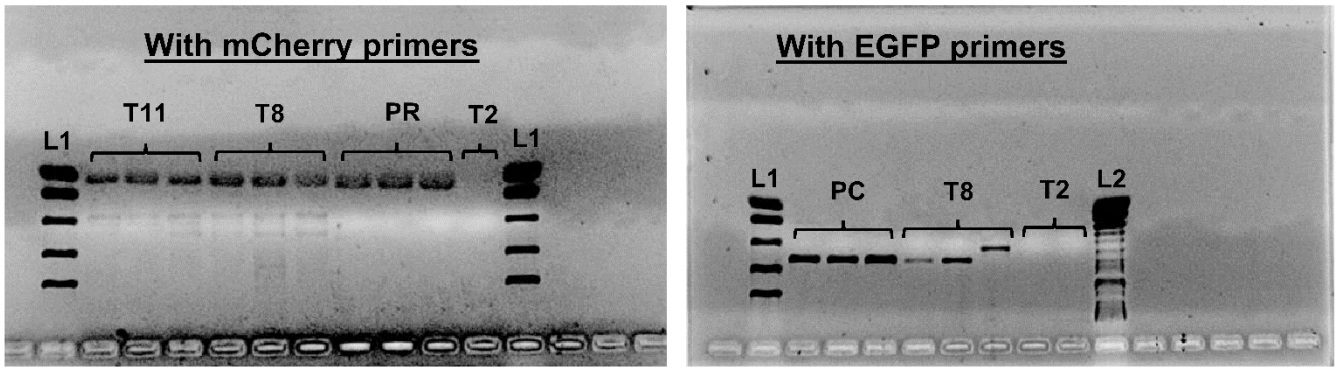
For the transformation step, 20 µL pFPL-Rh pDNA was added to 150 µL protoplast suspension. With gentle shaking, it was made up to 300 µL using sorbitol-TC solution. Then, it was instantly placed on ice for 30 min, and 2 mL PEG-TC solution (6% polyethylene glycol 6000 [Carl Roth GmbH], 10 mM Tris hydrochloride buffer pH 7.5, 50 mM calcium chloride) was added dropwise. It was re-placed on ice for 20 min, and 20 mL sorbitol-TC solution was added, followed by centrifugation for 15 min at 4000 rpm and 4°C. The pellet (transformed protoplasts) was suspended in 3 mL liquid regeneration medium (0.1% yeast extract, 0.1% casein hydrosylate enzymatic, 34.2% D-saccharose), and incubated for 16 h at 28°C. 20 mL freshly prepared, semi-cooled, molten, solid regeneration medium (liquid regeneration medium + 1.6 % agar [Carl Roth GmbH]) was mixed with the transformed protoplasts, and poured into petri dishes to solidify. Then, it was overlaid with 15 mL of 0.8% (w/v) agar in ddH_2O (Hyg-B/Kan supplemented), and incubated at 28°C for 1 week. Afterwards, hyphae of Fos transformants grew through the liquid overlay. Transformed Fos was transferred to grow further on solidified-complete medium (1.6% [w/v] agar in complete medium, supplemented with Hyg-B/Kan). The transformation procedure of Fos was done in 2 experimental repetitions, with each transformation procedure having 3 replicates. In addition, a no template control (NTC) where ddH_2O was added to the prepared protoplast in place of pDNA.

Appendix 5: Detailed methodology for "mixed salts" mediated transformation of *Bacillus subtilis* (GB03 isolate).

A colony of GB03 from an overnight, streaked LB agar culture was transferred into 50 mL LB broth, and shaken overnight for 16 h, at 75 rpm and 37ºC. A solvent mixture of 90.8 mL ddH_2O and 9.2 mL K-P-Na-citrate solution (1.232 g dipotassium phosphate [Carl Roth GmbH], 600 mg monopotassium phosphate [Carl Roth GmbH], 100 mg trisodium citrate dihydrate [Carl Roth GmbH], all dissolved in 10 ml ddH_2O) was prepared. Then, 20% (w/v) dextrose (BDH Chemicals LTD, Poole, England), 40% (w/v) L-glutamic acid potassium salt monohydrate (Sigma-Aldrich GmbH), 0.22% (w/v) ammonium iron (III) citrate (Merck KGaA, Darmstadt, Germany), 0.5% (w/v) L-Tryptophan (Sigma-Aldrich GmbH), and 24.7% (w/v) magnesium sulphate heptahydrate (Carl Roth GmbH) were added to the solvent mixture to produce 'mixed salts' medium. Forty % (v/v) mixed salts medium was added to the overnight GB03 culture, and incubated for 2 h, at 75 rpm and 37ºC, to obtain competent cells.

In transforming GB03, 400 µL of the competent GB03 cell culture was aliquoted into 2 mL Eppendorf tube, and 600 ng pFPL-Ch pDNA was added to the tube. Then, the tubes were incubated for 1 h, at 1450 rpm and 37ºC. Afterwards, 100 µL expression mix (5% (w/v) yeast extract, 10% (w/v) casamino acid [MP Biomedicals, LLC], and (w/v) 0.5% L-Tryptophan, all dissolved in 10 mL ddH_2O) was added to the tube, and further incubated for 1 h at 1450 rpm and 37ºC. Thereafter, 100 µL content from the tube was plated on LB agar (Hyg-B/Kan supplemented). As from 2 to 3 days, transformed GB03 colonies were growing on the media. Similarly, GB03 transformation was done in 2 experimental repetitions, with each transformation procedure having 3 replicates, in addition to a no template control (NTC).

Appendix 6: PCR of fluorescent genes segment of the microbial transformants.



Gel electrophoretic images of PCR products confirming the localization of FK3 and GB03 transformants in infected *S. hermonthica* shoot from the post-Striga attachment treatment application planting trials.

L1 – Easy ladder I (Bioline GmbH, Luckenwalde, Germany).

L2 – 1 kb plus DNA ladder (Life technologies GmbH, Darmstadt, Germany).

PR – pDNA of pFPL-Rh.

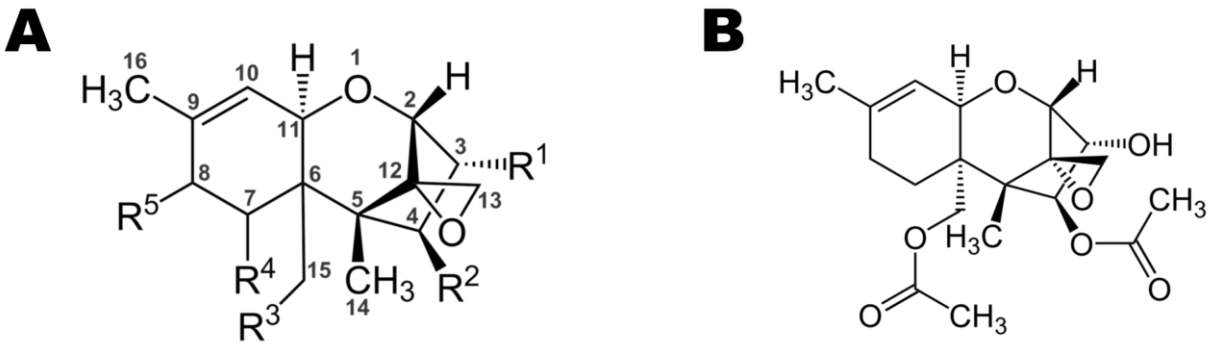
PC – pDNA of pFPL-Ch.

T11 - *S. hermonthica* shoot from treatment 11 “Striga + FK3[T]”.

T8 – *S. hermonthica* shoot from treatment 8 “Striga + FK3[T] + GB03[T]”.

T2 – *S. hermonthica* shoot from treatment 2 “Positive control”.

Appendix 7: Chemical structures of trichothecene core and diacetoxyscirpenol (DAS).



Chemical structures of (A) trichothecene core, and (B) diacetoxyscirpenol (DAS). The chemical structures were adapted from Fk (2005) and Yikrazuul (2009) respectively.

Fk (2005). Chemical structure of Trichothecenes. https://en.wikipedia.org/wiki/Trichothecene#/media/File:Trichothecenes.png

Yikrazuul (2009). diacetoxyscirpenol; Anguidine; diacetoxyscirpenol; Anguidin; 4,15-Diacetoxyscirpenol. https://en.wikipedia.org/wiki/Diacetoxyscirpenol#/media/File:Diacetoxyscirpenol.svg