# STRIGOLACTONE PROFILE OF SELECTED LEGUMES AND THEIR POTENTIAL FOR USE AS TRAP CROPS FOR *STRIGA*

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# DECLARATION

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

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# DEDICATION

I dedicate this work to my parents, Francis Kibet & Everlyn Ruto; sisters, Valentine Kibet & Jackline Jemutai; and friends who have supported me in this endeavor.

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# ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| DMBQ | 2,6 dimethoxy-1,4-benzoquinone |
| FAO | Food and Agricultural Organization |
| SSA | Sub-Saharan Africa |
| LC-MS-MS | Liquid Chromatography tandem Mass Spectrometry |
| Spp. | Species |
| M | Molar |
| QR | Quinone Reductase |
| ABA | Abscisic acid |
| RNA | Ribonucleic acid |
| DAP | Diammonium Phosphate |
| CCD | Carotenoid Cleavage Dioxygenase |
| MAX | MORE AXILLARY BRANCHES |
| KAI | KARRIKIN INSENSITIVE |
| HTL | HYPOSENSITIVE TO LIGHT |
| BecA – ILRI | Biosciences Eastern and Central Africa – International Livestock Research Institute |
| ANOVA | Analysis of Variance |
| SAS | Statistical analysis software |
| ICIPE | International Centre of Insect Physiology and Ecology |
| N | Nitrate |
| P | Phosphate |

# ABSTRACT

*Striga hermonthica*'s non-hosts stimulate parasite seed to germinate without getting infected because the non-hosts produce unique germination stimulants (strigolactones). This phenomenon – called suicidal seed germination is greatly used in *S. hermonthica* control. For improved efficiency of the suicidal seed germination method in *S. hermonthica* control, detailed analysis of comparative ability of potential trap crops to stimulate germination is critical. An additional and often ignored determinant of suitability of non-host intercrops is the extent to which the parasite can penetrate various non hosts and if such interaction can adversely affect the trap crop. This is critical because although the parasite is not able to effectively infect a non-host, it may still cause injuries that can expose the crop to other pathogens such as bacteria and fungi. In this study, suitability of Kenya's commonly used legumes (cowpea, pigeon pea, common bean, and garden pea) as potential intercrops in the control of *S. hermonthica* was determined. Firstly, their efficiency to induce germination of *S. hermonthica* seeds using germination assays was determined. Then, the amounts and types of strigolactones in their root exudates was assayed using a high performance liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS). Finally, the extent of interaction between the legumes and *S. hermonthica* was determined using histological analysis. There were significant differences in the induction of *S. hermonthica* germination by the legume root exudates, synthetic GR24, and water ($p=0.0001$). Analysis of strigolactones in root exudates of legumes revealed that the most abundant strigolactones were 2-epi-5-deoxystrigol and orobanchol with trace amounts of 2-epi-orobanchol and strigol. Expectedly, none of the legumes fully supported growth and development of *S. hermonthica* to enable the parasite complete its lifecycle. However, the extent of parasite penetration varied greatly in the different legumes. Cowpea and garden pea formed vascular connections with *S. hermonthica* parasite. However, the parasite did not grow beyond pigeon pea's endodermis, and in the common bean, the parasite barely attached on the host cortex. These findings suggest that all legumes tested are appropriate for use as intercrops because they induced high *S. hermonthica* seed germination. Additionally, confounding effects (mixed) on successful penetration of *S. hermonthica* in non-hosts will require further investigation. Knowledge from this study has provided fundamental insights on the importance of trap crops in induction of suicidal germination of *Striga hermonthica* seeds, which depletes the parasite's seedbank in soil.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background Information

Parasitic plants pose a significant threat to food production and ultimately food security. Most of the cultivated crop species have at least a parasite that can either be photosynthetically active (hemiparasite) or those that are entirely dependent on their host (holoparasite) (Spallek *et al*., 2013). Witchweed (*Striga* spp.) is an obligate hemiparasite belonging to the family *Orobanchaceae* (Bennet and Mathews, 2006; Mutuku *et al*., 2019). The genus *Striga* is composed of approximately 30-35 species most of which parasitize the grass species (*Poaceae*) (Spallek *et al*., 2013). Economically important *Striga* species include *Striga hermonthica* and *Striga asiatica*, which parasitizes monocots and *Striga gesneroides* that parasitizes dicots (Westwood *et al*., 2010).

*Striga* is the most economically important parasitic plant due to massive yield losses on cereal crops (Mutuku *et al*., 2020). *Striga* parasitizes essential cereal crops such as maize, sorghum, millet, wheat and rice (Scholes and Press, 2008). The parasite infests approximately two-thirds of the arable land in Africa making it the most severe biological constraint to food production. Crop fields that have been infested by *Striga* suffer losses of between 20 and 100% (Ejeta, 2007). In 2007, the Food and Agricultural Organization (FAO) estimated that *Striga* infestation in Africa causes annual losses of US $7 billion, which affects 300 million people (Ejeta, 2007). Infestation normally varies from light, moderate, and heavy, which is expected to rise if management approaches are not timely.

The lifecycle of *Striga* is highly synchronized with that of the host and only grows in the presence of a host (Runo and Kuria, 2018). Germination of *Striga* seeds require germination stimulants referred to as strigolactones that are produced by either host or non-host plants (Cardoso *et al*., 2011). The lifecycle of *Striga* involves a number of sequential stages namely germination, attachment to an appropriate host, formation of haustorium, penetration, establishment of vascular connections, siphoning of nutrients, flowering, and seed production (Yoshida *et al*., 2016). Following germination, the radicle tip of *Striga* makes contact with its host for a period of between 3 to 7 days. The roots of the host are sensed by the parasite either by direct contact or perception of diffusible phenolic compounds such as quinone and 2,6 dimethoxy-1,4-benzoquinone (DMBQ) originating from the lignin of the host plant root (Westwood *et al*., 2010).

The aforementioned phenolic compounds are referred to as haustorium-inducing factors, which are responsible for the formation of a haustorium (Kokla and Melnyk, 2018). Haustorium is a specialized organ used to penetrate the root cortex of host plants and act as a conduit for water and nutrients from the host (Goyet *et al*., 2019). Following the penetration of the cortex, haustorial cells undergo significant differentiation to form vessels that establish a bridge with the host xylem (Dorr, 1997). Vascular connections between the parasite and the host root is usually established 48 to 72 hours after contact (Mutuku *et al*., 2020). The formation of xylem-to-xylem connections makes *Striga's* cotyledons to enlarge and break from the seed coat within 24 hours. The xylem-to-xylem connections mark the onset of parasitism, whereby *Striga* begins to grow upwards and form adventitious roots that form other haustoria with the host plant and other nearby host plants (Westwood *et al*., 2010). The symptoms of *Striga* parasitism on host plants include stunted growth and drought-like characteristics (Spallek *et al*., 2013).

The management and control strategies for *Striga* are limited. The available control options include conventional cultural practices such as crop rotation, hand weeding, improving soil fertility, intercropping, and general sanitization (Jamil *et al*., 2021). Other control options include developing resistant cultivars, use of herbicide coated seeds, and direct application of chemicals in soil to reduce *Striga* seed levels (Kountche *et al*., 2019).

The use of trap crops offers a viable control strategy for *Striga*. Trap crops are described as non-host plants that stimulate the germination of *Striga* seeds but are not parasitized by the weed. Most of the plant varieties used as trap crops are legumes such as cowpea, desmodium, and groundnuts (Kureh *et al*., 2003). The role of trap crops is to stimulate a large proportion of the *Striga* seeds to germinate, which are then destroyed before they reach the reproduction stage (Yoneyama *et al*., 2009). The role of suicidal germination is to reduce the *Striga* seedbank in soil; thus, making it a viable management strategy. Suicidal germination entails the introduction of a germination stimulant in soil in the absence of an appropriate host (Rubiales *et al*., 2009). Trap crops release strigolactones into the rhizosphere in small concentrations, which induce subsequent germination of *Striga* seeds. Upon germination, *Striga* fails to find an appropriate host leading to its death; hence, the term suicidal germination (Runo & Kuria, 2018). Therefore, the use of trap crops constitutes an effective and cheaper alternative for controlling *Striga* because farmers can incorporate this control measure without much investment. Such cropping systems can be used in farmers' fields for successive seasons so as to deplete *Striga* seedbank in soil (Kountche *et al*., 2019). Depletion of *Striga* seedbank in soil has the influence of gradually decreasing infestations in farmers' fields; thus, improving food security.

## 1.2 Statement of the Problem

*Striga* control is challenging and therefore requires the use of integrated approaches. The concept of suicidal germination is relatively new, which requires further research on efficient non-host crops. As such, there is limited research on potential non-host crops, which are predominantly leguminous crops that induce germination of *Striga hermonthica* seeds in the absence of a host. A successful non-host crop that has been used in the suppression of the noxious weed is the forage legume in the genus Desmodium (Fabaceae), which releases allelopathic chemicals that inhibit the development of *Striga* in the field (Midega *et al*., 2014). *Clotalaria*, a non-host for *Striga hermonthica* has also been used as a trap crop for the induction of suicidal germination of parasitic seeds (Mwakha *et al*., 2020). However, the use of the aforementioned trap crops is not favorable amongst farmers given that they mostly serve as forage, thus lack significant economic importance in terms of returns. Therefore, it is imperative to assay the potential of Kenya's commonly grown legumes such as cowpea and common bean as agents for suicidal germination. Given that *Striga* losses in Sub-Saharan Africa amounts to US $7 billion and causes between 20% to 100% crop losses, the weed poses significant threat to food security (Ejeta, 2007). Continuous mono-cropping of cereal crops in successive seasons worsens the *Striga* scourge because it increases seedbank in soil (Mwakha *et al*., 2020).

## 1.3 Justification

*Striga* seeds only germinate when they get germination cues called strigolactones from host and non-host plants (Khosla and Nelson, 2016). Upon germination, the radicle of *Striga* grows towards the host plant and forms a haustoria, which is a specialized organ that is used to penetrate the host root (Yoshida *et al*., 2016). The formation of a xylem-to-xylem connection marks the beginning of siphoning of water and nutrients from the host plant by the parasite. A *Striga* plant emerges above the ground about 4-7 weeks after planting. At this stage, the parasite would have already damaged the host plant before emergence; thus, resulting to heavy crop losses (Jamil *et al*., 2021). Therefore, it is evident that post-emergence control strategies such as hand-pulling of *Striga* plants are inadequate in mitigating the *Striga* problem. Pre-attachment control strategies offer a viable solution because the approach focuses on controlling the parasite before it establishes a connection to its host (Fishman and Shirasu, 2021).

An approach to explore the pre-attachment control is to use trap crops that induce suicidal germination of *Striga* seeds. The implication of this is to reduce *Striga* seedbank in soil as well as reducing the frequency of attachment on host plants. Legume trap crops such as cowpea produce strigolactones that induce germination of *Striga* seeds but these trap crops are not parasitized by the weed (Kanampiu *et al*., 2018). It was imperative to conduct germination assays to ascertain the germination frequencies of *Striga* seeds induced by legume trap crops as well as determining the chemical composition of the legume root exudates. Further, a determination of the level of penetration of the parasite into the trap crops through histological analysis is essential in understanding host-parasite incompatibility that makes non-host plants not to be parasitized by *Striga*.

## 1.4 Null Hypotheses

i. There are no differences in efficiencies of induction of *Striga hermonthica* seed germination by root exudates of selected legumes, GR24, and water.
ii. There are no different profiles and quantities of strigolactones produced by the selected legumes.
iii. There is no non-host to parasite incompatibility between the selected legumes and *Striga hermonthica*.

## 1.5 Objectives

### 1.5.1 General Objective

To identify the strigolactones in selected legumes and assess their potential to induce suicidal germination of *Striga hermonthica* seeds *in vitro*.

### 1.5.2 Specific Objectives

i. To determine the efficiencies of stimulation of germination of *S. hermonthica* seeds by root exudates of selected legumes.
ii. To identify and quantify strigolactones produced in root exudates of selected legumes using Liquid Chromatography tandem Mass Spectrometry (LC/MS-MS).
iii. To determine the mechanism of incompatibility between *Striga hermonthica* and selected legumes through microscopic screening.

## 1.6 Significance of the Study

The knowledge gained from this study highlights the importance of trap crops to induce germination of *Striga hermonthica* seeds, which subsequently deplete parasitic weed seeds in soil. Trap crops with potential to induce high germination of *Striga hermonthica* seeds can be incorporated in the management of *Striga* through intercropping or crop rotation. Use of trap crops as a management strategy is easily applicable by farmers whose fields are affected by *Striga*. Additionally, companion cropping increases yield as well as boosting nutritional intake from the diverse crops that are harvested.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 *Striga* Biology

*Striga* plants have characteristic green opposite leaves with bright irregular flowers that can be pink, red, white or yellow (Spallek *et al*., 2013). The seeds produced by *Striga hermonthica* are usually very small and measuring 0.4 mm and weighing 0.7 μg (Spallek *et al*., 2013). Mechanisms of dispersal of *Striga* seeds is by wind, water, and animals. Once dispersed, *Striga* seeds have the potential of remaining viable in soil for up to 10 years (Runo and Kuria, 2018). A single capsule from the *Striga* plant produces approximately 700-1800 minute seeds. The length of life cycle from seed-germination until seed-production is approximately 4 months (Babiker, 2007).

*Striga* is a hemiparasitic weed and the seedlings cannot be sustained for long from the endosperm reservoir. For this reason, it is essential for the parasite to find a host root a few days after germination. However, the parasite requires germination stimulants, which are usually produced by both hosts and non-hosts (Gobena *et al*., 2017). Upon receiving these germination cues, the *Striga* seeds start to germinate by producing a radicle and the seedling has between 3 and 7 days to find a host otherwise the seedling will die (Cardoso *et al*., 2011).

*Striga* has developed particular characteristics that promote parasitism. Parasitic plants such as *Striga* has a particular life cycle, which can be described using three main stages. These stages are identification of an appropriate host, establishing access to the host for purposes of acquiring water and minerals, and full establishment of parasitism until maturity (Spallek *et al*., 2013). The evolution of *Striga* as a parasite has enabled it to develop effective strategies for survival and success. One strategy of survival is the dispersal of numerous amount of *Striga* seeds (Atera and Itoh, 2011).

*Striga* seeds require a period of conditioning that is characterized by high temperature and moisture for a period of 7 to 14 days (Cardoso *et al*., 2011). Failure to be exposed to such conditions results to a secondary dormancy. Strigolactones are efficient in inducing germination of *Striga* seeds, negative regulation of shoot branching, and the induction of hyphal branching of arbuscular mycorrhizal fungi (Pandey *et al*., 2016). Strigolactone concentration of as low as $10^{-6}$ M is required to initiate germination of *Striga* seeds (Tsuchiya *et al*., 2018). It is noteworthy that the first strigolactone was isolated from the root exudates of cotton (*Gossypum hirsutum*) (Cook *et al*., 1966). As such, the use of non-host plants offers a promising strategy in the management of *Striga*. The use of *Desmodium uncinatum* in intercrop systems has proved to be effective in some parts of Africa (Khan *et al*., 2006).

Haustoria development is initiated after the radicle tip of *Striga* makes contact with the host root (Kokla and Melnyk, 2018). Upon making contact, the radical of *Striga* stops growing and forms a haustorium that is used to penetrate the root cortex of host plants (Goyet *et al*., 2019). After a period of 12 hours, a reorganization of *Striga's* meristem begins (Bandaranayake *et al.*, 2010). Haustoria inducing factor such as 2, 6-dimethoxy-p-benzoquinone (DMBQ) is usually produced by host cell wall and penetrates the parasite's cells (Yoshida *et al*., 2016). Quinone reductase (QR1) then reduces DMBQ to form a semiquinone intermediate that induces the process of haustorium development. A second quinone reductase (QR2) serves to regulate DMBQ for detoxification purposes (Bandaranayake *et al*., 2010). Therefore, it is essential to have a balance between the detoxification of DMBQ and haustoria inducing factors so as to optimize haustoria formation (Kokla and Melnyk, 2018). Apart from chemotropism, thigmotropism also plays an important role in haustoria formation (Kokla and Melnyk, 2018). In the 24-hour period of establishing contact with the host root, a hypertrophic growth phase sets in, which is characterized by elongation of distal cells into the epidermis. Subsequent cell divisions occur making it possible for these cells to reach the cortex of the host root (Yoshida *et al*., 2016).

Continual growth of the haustoria reaches the host endodermis whereby there is a rearrangement of palisade cells, which ultimately establishes vascular connections (Wada *et al*., 2019). Vascular connections between the parasite and the host root is usually established 48 to 72 hours after contact (Mutuku *et al*., 2020). There are no phloem-to-phloem connections between host and *Striga*. The establishment of xylem-to-xylem connection makes *Striga's* cotyledons to enlarge and break from the seed coat within 24 hours (Yoshida *et al*., 2016). In most non-host plants, penetration of *Striga hermonthica* is possible but only during the early stages. However, infection by the parasite is arrested at the cortex in *Lotus japonicus* and stele in Arabidopsis and cowpea but the vegetative structure does not proceed beyond the six-leaf-pair stage (Yoshida and Shirasu, 2009).

Formation of xylem-to-xylem connections marks the establishment of parasitism whereby *Striga* begins to grow upwards and form adventitious roots. The adventitious roots are able to form other haustoria with the host plant or other host plants (Westwood *et al*., 2010). Naturally, a single host plant is normally parasitized by several *Striga* plants, which act as a metabolic sink by taking significant amount of photoassimilates and nutrients (Yoshida *et al*., 2016). A *Striga* plant that has infected a host plant normally has twice the amount of nitrogen (Frost *et al*., 1997). Significant reduction in nitrogen levels in the host plant has a negative impact on its physiology, which leads to a lower rate of photosynthesis that is synonymous to plants affected by *Striga*. The negative impact on photosynthesis leads to elevated levels of abscisic acid (ABA), which affects stomatal conductance in response to stress levels in the host plant (Frost *et al*., 1997). Additionally, the parasite also has an influence on other hormones in the host plant such as cytokines and gibberellic acid. The way *Striga* manipulates host plant hormone homeostasis is not yet clear but it may have a contribution on the advancement of parasitism.

The emergence of *Striga* plants from the soil marks the beginning of the parasite's photosynthesis (Mwangangi *et al*., 2021). However, *Striga* plants remain host-dependent owing to negative carbon gain and degenerate palisade layer, which implies a lower number of chloroplasts per cell (Wickett *et al*., 2011). This is supported by transcriptomic data from the RNA isolated from *Striga hermonthica* tissue that has emerged above ground, which shows a low expression of chlorophyll biosynthesis and genes responsible for photosynthesis (Wickett *et al*., 2011). High transpiration rates witnessed in *Striga* is due to the transpiration pull, which serves to obtain host's photoassimilates. For this reason, it is challenging for *Striga* to survive in humid areas (Frost *et al*., 1997). Additionally, the stomata of *Striga* exhibits a high conductance and high transpiration rates with little response to stomatal closure induced by darkness (Mutuku *et al*., 2020). Host plants showcase no significant water depletion until the late stages of infection when symptoms such as stunted growth are exhibited (Waweru *et al*., 2019).

Given that the symptoms caused by *Striga* on the host plants appear before the parasite emerges above ground, control strategies such as hand weeding and use of herbicides prove ineffective. However, the use of such control strategies serves the purpose of preventing the reproduction of *Striga* through seed dispersal (Sibhatu, 2016). The flower colors of *Striga* varies between species and include blue and pink (*Striga hermonthica* and *Striga gesneroides*) and white, yellow, and red (*Striga asiatica*). After pollination, a single *Striga* plant is capable of producing between 50,000 and 500,000 seeds (Mwangangi *et al*., 2021). When the pods of *Striga* crack, the *Striga* seeds are dispersed and often require approximately 6 months before finding a suitable host so as to prevent germination during the latter stages of the rainy season (Brun *et al*., 2018).

## 2.2 *Striga* Distribution and Host Range

*Striga* is described as an ‘Old World’ parasite and a number of species have been identified as parasites to cereal crops mainly in Africa and Asia (Spallek *et al*., 2013). An estimate of 80% of *Striga* species are prevalent in Africa whereby they are common in open grasslands and savannahs (Cochrane and Press, 1997). *Striga* infestation is conspicuous in infertile soils and continued monoculture has contributed to this phenomenon in some parts of

Africa (Kamara *et al*., 2009). The existence of germination cues such as strigolactones derived from hosts as well as high temperature and humidity is needed to break seed dormancy in *Striga* (Ejeta and Gressel, 2007). Approximately 50 million hectares of land in Africa is infested with *Striga*, which affects at least 25 African countries (Westwood *et al*., 2010).

Socioeconomic challenges are not easy to determine but the *Striga* problem affects close to 100 million people in Africa leading to losses of 1 billion $US annually (Waruru, 2013). Cereal crops that are mostly affected by *Striga* infestation include maize, sorghum, millet, upland rice, and cowpeas, which represent the major staple crops for most subsistence farmers. The losses arising from *Striga* can range from 20% to complete crop loss in the affected areas (Atera and Itoh, 2011). *Striga* infestation is characterized by severe symptoms such as leaf lesions, chlorosis, necrosis, and leaf desiccation (Runo and Kuria, 2018).

There are three *Striga* species of economic importance namely *S. hermonthica*, *S. asiatica*, and *S. gesneroides*. *Striga hermonthica* is widespread in Sub-Saharan Africa and parasitizes maize, sorghum, and millet (Atera and Itoh, 2011). *Striga asiatica* distribution ranges from South Africa to East Africa and from the Arab Peninsula to East Asia. *Striga asiatica* found in India and Pakistan have white flowers whereas those in Thailand and Indonesia have yellow flowers. *Striga asiatica* found in Africa has red flowers but its infestation is less compared to that in Asia. *Striga asiatica* was at first restricted to South and Central Africa but later spread to other parts of Africa (Spallek *et al*., 2013). The region around Tanzania acts as a border for *S. hermonthica* and *S. asiatica* whereby the former is common in Tanzania, Malawi, Mozambique, and Madagascar (Parker, 2009). *Striga asiatica* was unintentionally introduced in the United States in the 1950s but concerted efforts costing $250 million were used to eradicate the parasite. Ethylene is known to induce the germination of *Striga* seed and has been extensively used in the United States to induce the germination of *S. asiatica* seeds. Soil with a high clay content is infused with ethylene, which is absorbed up to a depth of 30 cm (Bebawi & Eplee, 1986). As a result, this leads to a germination frequency of approximately 90% of *Striga* seeds. A 92% depletion of *Striga* seedbank is plausible with a single application of ethylene. The use of this management strategy in Africa has been hampered by the lack of advance control strategies, but holds potential in Africa countries (Bebawi & Eplee, 1986). *Striga gesneroides* is common in the African continent. *Striga gesneroides* parasitizes cowpea in West Africa and tobacco in Zimbabwe. Presently, *Striga* is found in most parts of Sub-Saharan Africa but its prevalence is high in areas with low rainfall and low soil fertility (Mwangangi *et al*., 2021). *Striga* distribution in Africa is categorized as heavy, moderate, and light infestation as shown in figure 2.1 (Ejeta, 2007). *Striga hermonthica* is the most common in this region owing to its obligate out-crossing behavior and large plant stature. The outcrossing nature increases genetic variability of *Striga hermonthica* making it difficult to find completely resistant host plants, which subsequently increases its prevalence (Yoshida and Shirasu, 2009).

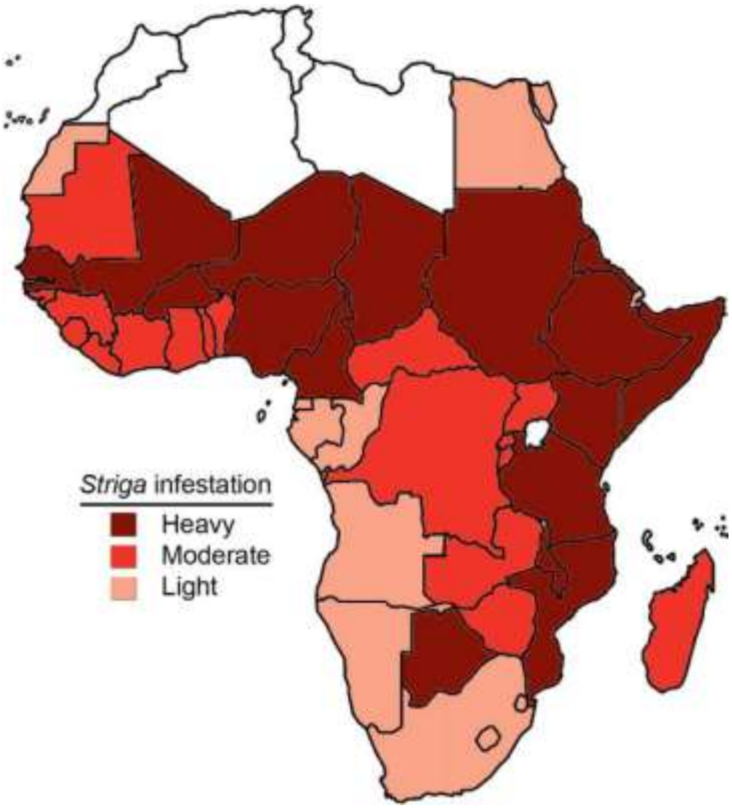


**Figure 2.1:** ***Striga*** **distribution in Africa**

### 2.3 Economic Importance of *Striga*

*Striga* offers significant constraints to crop production in Africa but sufficient data on yield losses and spread is not available except for a few reports. However, yield losses arising from *Striga* is above 50% but infestation can be light, moderate, and heavy. Areas in Africa with severe *Striga* infestation range from 30 to 50 million hectares (Ejeta, 2007). Degree of infestation is on the rise especially in Eastern and Western Africa, which significantly reduces crop yield and subsequent economic benefit. The Food and Agricultural Organization (FAO) estimates that *Striga* infestation in Africa causes annual losses of US$ 7 billion, which affects 300 million people (Ejeta, 2007).

*Striga*, is the most economically important parasitic weed owing to the large amounts of yield losses in cereal crops caused by the parasite (Kanampiu *et al*., 2018). There are approximately 28 species of witchweed that belong in the family Orobanchaceae, but only a number have negative impact on agricultural production (Gethi *et al*., 2005). The significant *Striga* species are *Striga hermonthica* and *Striga asiastica*, which affect cereal crops whereas *Striga gesneriodes* affects legumes. The losses that ensue from these parasitic plants are dependent on their density, type of host, soil fertility, and rainfall patterns (Atera *et al*., 2012). *Striga hermonthica* causes significant damage as a root parasite by obtaining nutrients and water from the host plant. The loss in cereal production as a result of *Striga* infestation is approximately 7 billion dollars in Sub-Saharan Africa (Ejeta, 2007). Losses in Ethiopia, Mali, and Nigeria are estimated at 75 million, 87 million, and 1.2 billion dollars in a year respectively. Sudan is also affected by *Striga* infestation especially in regions with low rainfall levels resulting in nearly 70-100% yield losses (Sibhatu, 2016).

The infestation of crop fields by *Striga* in Africa leads to losses of up to 30-50%. Studies conducted in Nigeria indicate that *Striga* infestation poses a significant threat to agricultural production coupled with low soil fertility (MacOpiyo *et al*., 2010). In Kenya, there is a significant threat to production as a result of *Striga hermonthica*, which causes losses of 1.15, 1.10, and 0.99 tons per hectare of maize, sorghum, and millet respectively (MacOpiyo *et al*., 2010). In maize, this loss represents approximately 12.3% loss of maize production per year in Kenya. This is a significant loss to cereal production in the country, which mostly affects small-scale farmers. Therefore, the menace caused by *Striga* requires an innovative biotechnological approach to salvage this agricultural scourge (Traore *et al*., 2011).

## 2.4 *Striga* Management and Control Methods

*Striga* poses a significant problem in regions that have low soil fertility and moisture. The parasite infestation is heightened in areas that has a high population pressure on land, lack of fallow lands, and a decreased use of fertilizers (Mwangangi *et al*., 2021). It poses a major economic problem to small-scale farmers who rely on cereal farming in Africa. Production of many seeds and a prolonged viability of seeds in soil makes control of the parasite challenging (Kountche *et al*., 2019). A number of approaches have been used in the control of *Striga* such as using resistant or tolerant lines, using clean seeds, crop rotation, intercropping, use of fertilizers, soil fumigation using ethylene, hand weeding, using post emergence herbicides, push and pull technology, and biological agents (Babiker, 2007). The use of conventional hand and mechanical weeding is ineffective in the control of *Striga* since the parasite exerts its damage subterranean. However, these conventional control methods are still in use up to date. On the other hand, small-scale farmers find these control methods as being expensive, uneconomical, and labor intensive (Babiker, 2007).

### 2.4.1 Hand-weeding and Sanitation

This is the most commonly used control measure for *Striga* but it is only important in preventing seed dispersal. Weeding as a control method does not increase the yield of already infected plants (Kanampiu *et al*., 2018). Pulling of these parasitic weeds should be done 2-3 weeks after flowering to prevent seeding (Sibhatu, 2016). Sanitation entails the isolation of the infested areas by setting up perimeters, which prevent seed dispersal by wind or animals. Crop stubbles should be uprooted and subsequently burnt to destroy the seeds. Hand-weeding is not an effective approach in *Striga* control because it is labor intensive; hence, high level infestation requires other approaches (Sibhatu, 2016).

### 2.4.2 Intercropping practice

Intercropping is an essential strategy used in weed control given that chemical use can be challenging once the crops have emerged (Midega *et al*., 2014). A study conducted by Bilalis *et al*. (2010) indicated that intercropping maize with legumes led to a significant reduction in weed density by limiting the amount of light available for weeds. Intercropping maize with fodder leguminous *Desmodium uncinatum* and *Desmodium intortum* led to a reduction in *Striga* infestation and subsequently increased agricultural produce (Hailu *et al*., 2018). Studies conducted in Kenya showed that intercropping cowpea with maize has the potential of reducing *Striga* infestation (Odhiambo and Ransom, 1993). Additionally, intercropping of finger millet with *Desmodium intortum* reduces *Striga hermonthica* infestation in intercrops compared to monocrops (Fasil *et al*., 2005). Intercropping sorghum and cowpeas reduced *Striga* infestation in intercrops compared to sole crops. A number of legumes such as cowpea, green grams, and peanut have the potential of reducing *Striga* infestation. They do this by inducing suicidal germination, which interferes with the germination of the witchweed. Nitrogen fixation carried out by legumes is also responsible for a reduction in *Striga* densities (Franke *et al*., 2018).

### 2.4.3 Use of fertilizer

Given that *Striga* thrives in areas with low soil fertility, a control method that improves soil fertility is viable. For instance, the use of organic materials from crops and organic manure improves soil fertility, which subsequently reduces *Striga* infestation (Mwangangi *et al*., 2021). The study conducted by Kamara *et al*. (2009) in North East Nigeria indicated that the application of nitrate fertilizer reduced *Striga* infestation. Germination of *Striga hermonthica* seeds is dependent on stimulants produced by host plants, which is determined by the nutrient status of the soil. Low levels of nitrate and phosphate makes host plants to secrete more germination stimulants in the rhizosphere whereas an elevated level of nitrate and phosphate reduces the secretion (Kamara *et al*., 2009).

It is noteworthy that research studies have indicated that the effect of N on strigolactone production is less compared to that of P. For instance, diammonium phosphate (DAP) fertilizer contains 18% N and 46% P; thus, high P levels causes a low production of strigolactones. According to Sun *et al*. (2014), the level of strigolactones, namely 2’-epi-5-deoxystrigol, orobanchol, and orobanchyl acetate increased by 63-, 33-, and 18-fold under low phosphate, which was significantly higher compared to low nitrate conditions (Sun *et al*., 2014). However, the high cost of mineral fertilizers is problematic especially to small-scale farmers who cannot afford it as a control strategy for *Striga*. Therefore, it is necessary to investigate on the possibility of using fertilizers at minimal levels in a process referred to as micro-dosing. Micro-dosing of DAP fertilizers has shown to be cost effective to small-scale farmers in reducing the prevalence of *Striga hermonthica* in Sub-Saharan Africa (Kamara *et al*., 2009).

### 2.4.4 Genetic Resistance

Genetic resistance in relation to *Striga* is the ability of host plants to stimulate germination of *Striga* seeds but avert excessive attachment of the seedlings (Adewale *et al*., 2020). The use of resistant crop cultivars is economically viable in management of *Striga*. However, resistant crop cultivars are not present in all host plants (Kavuluko *et al*., 2020). Cultivation of resistant crops such as rice, sorghum, and some maize genotypes has the effect of reducing *Striga* infestation; thus, increasing agricultural yield. Such cereals have been found to be resistant to *Striga* infestation and studies conducted in infested areas show high crop yields when using resistant genotypes (Sibhatu, 2016).

### 2.4.5 Biological Control

Biological control of *Striga* infestation entails the use of living organisms for suppression purposes (Shayanowako *et al*., 2018). For instance, herbivorous insects such as defoliators, gall forming, shoot borers, miners, inflorescence feeders, and fruit feeders feed on different parts of the *Striga* plant. These insects are classified according to their point of damage in *Striga* plants which include defoliators, gall forming, shoot borers, miners, inflorescence feeders, and fruit feeders. Fungi such as *Fusarium nygamai* and *Fusarium semitectum* have the ability to control the prevalence of *Striga* plants (Nzioki *et al*., 2016).

### 2.4.6 Chemical Control

A number of chemicals such as herbicides, fumigants (methyl bromide) and germination stimulants (ethylene) can be used in the management of *Striga*. Herbicides such as

Imazapyr and prithiobac are used to coat maize seeds as a control measure of the parasitic plant. The advantage of using herbicides is that they remain in the rhizosphere for a longer period (Baiyegunhi *et al*., 2018). Testing of the herbicide-coated seeds in different locations revealed that it increased crop yield by 3 to 4-fold (Kanampiu *et al*., 2003).

## 2.5 Suicidal Germination of *Striga hermonthica*

Trap crops are responsible for causing suicidal germination of witchweed, which subsequently reduces the seed bank. Some of the plant varieties that act as trap crops include cowpea and groundnuts (Kureh *et al*., 2003). The effect of this is to reduce seed bank in soil when intercropped with maize (Midega *et al*., 2014; Kountche *et al*., 2019). The role of catch crops is to stimulate a large proportion of the *Striga* seeds to germinate, which are then destroyed before they reach the reproduction stage (Yoneyama *et al*., 2009).

Suicidal germination is a control method of *Striga*, which functions by reducing the seed bank in soil. Suicidal germination entails the introduction of a germination stimulant in the soil in the absence of an appropriate host (Rubiales *et al*., 2009). The germination stimulants occur naturally in host as well as non-host plants. Some of the germination stimulants include strigol, orobanchol, and sorgolactone, which are collectively referred to as strigolactones. The natural occurring stimulants are difficult to produce on a large scale; hence, proving ineffective in the control of parasitic weeds (Kountche *et al*., 2019). As a result, a number of strigolactone analogues with simpler structures have been prepared with the ability of retaining germination activity. Notable strigolactone analogues include GR24 and GR7, but they are susceptible to hydrolysis due to the presence of the enol ether bridge in their chemical structure (Pandey *et al*., 2016). Research initiatives are focused on developing strigolatones with a high germination efficiency, which can serve to deplete *Striga* seed bank (Traore *et al*., 2011).

Strigolactones play an important role as a control strategy against *Striga*. Trap crops such as leguminous crops produce strigolactones from root exudates, which serve as germination stimulants for parasitic weeds (Khosla and Nelson, 2016). However, the germination is termed as suicidal because the parasitic plant fails to secure a suitable host. Trap crops are resistant to the parasitic plant; thus, the *Striga* dies off for lack of a host (López-Ráez *et al*., 2009).

Synthetic germination stimulants can also be used to induce suicidal germination of *Striga*. Some of the commonly used synthetic strigolactone analogues include GR24 and Nijmegen, which function at low concentrations (Kountche *et al*., 2019). The synthetic strigolactones are used to treat soil before planting the seeds, which would induce suicidal germination of *Striga*; thus, reducing the seed bank (Kountche *et al*., 2019). A disadvantage of this approach in the control of *Striga* is that synthetic strigolactones are expensive; hence, making it difficult for small scale farmers to acquire them. It is important for these synthetic strigolactones to be more stable compared to natural strigolactones to boost its effectiveness (López-Ráez *et al*., 2009).

## 2.6 Incompatibility of Legume Trap Crops to *Striga hermonthica*

The use of trap crops is an effective method in mitigating cereal losses caused by *Striga*. Examples of such trap crops include cotton, cowpea, groundnut, soybean, and desmodium. Trap crops function by inducing germination of *Striga* seeds but are not parasitized; thus, reducing the *Striga* seedbank in soil (Mwakha *et al*., 2020). Intercropping is easily applicable in farmers' fields since it ultimately boosts crop production. However, lack of awareness of the *Striga* lifecycle makes them reluctant to adopt pre-emergence control strategies (Fishman and Shirasu, 2021). Rotation of legume crops entail two clear mechanisms namely suicidal germination and improvement of soil fertility. An increase in the soil organic and inorganic matter serves to suppress the prevalence of *Striga* (Sibhatu, 2016).

Non-host resistance describes a scenario whereby all members of a particular species are resistant to a specific parasitic species. Under natural conditions *Striga hermonthica* and *Striga asiatica* parasitizes gramineous species but do not parasitize dicotyledonous plants (Midega *et al*., 2014). There are limited studies that describe the interaction between *Striga hermonthica* and *Striga asiatica* with dicotyledonous species. However, *S. hermonthica* and *S. asiatica* are able to penetrate the roots of cowpea but the infection is arrested at the root cortex (Hood *et al*., 1998). This particular phenotype resembles that of the wild relative of maize *Tripascum dactyliodes* that showcases resistance to *Striga hermonthica*, which is characterized by the immature development of a hyaline body. The hyaline body is a distinctive tissue for the parasitic plant haustorium with densely stained cells that circles the parasite vascular core (Gurney *et al*., 2003). The hyaline body is normally less developed compared to those found in the natural hosts.

Research in International Centre of Insect Physiology and Ecology (ICIPE) led to the discovery of a companion crop that harbors chemicals that suppress *Striga*. Desmodium is a leguminous plant that is mainly used as a fodder crop but can also be intercropped with maize, sorghum, millet, and rice to reduce *Striga* seedbank in soil (Midega *et al*., 2017). There are two desmodium species namely *Desmodium uncinatum* that has a silver leaf and *Desmodium intortum* with a green leaf (Khan *et al*., 2008). The mechanism of suppressing *Striga* entails production of allelo-chemicals that have two distinct effects on *Striga*. First, flavones are the main exudates produced by desmodium, which stimulate the germination of *Striga* seeds. Second, other phytochemicals produced by desmodium root exudates inhibit the growth and development of the *Striga* radicle (Khan *et al*., 2008). As such, the two effects produced is referred to as allelopathy, which leads to suicidal germination of *Striga* (Hooper *et al*., 2015). Desmodium is a perennial plant that lives for more than two years; thus, the chemicals persist in soil for a long time and significantly reduces *Striga* seedbank in soil (Khan *et al*., 2008).

### 2.7 Strigolactones as Germination Stimulants

Plants produce a wide array of chemicals as well as primary and secondary metabolites. Strigolactones are classified as secondary metabolites that are released in the rhizosphere and act as signaling molecules (Lopez-Raez *et al*., 2009). Recent spectrometric techniques have made it possible to analyze and quantify strigolactones. The hormones act as germination stimulants of parasitic weeds in the family Orobanchaceae (Mutuku *et al*., 2020). Strigolactones also trigger the formation of a symbiosis between arbuscular mycorrhizal fungi and its host, which promotes nitrogen fixation (Khosla and Nelson, 2016). Further, strigolactones are responsible for the inhibition of shoot branching, which controls plant architecture. It is noteworthy that the name strigolactone originates from their initial identified role of stimulating germination of seeds of the parasitic weed *Striga* and the presence of a lactone ring in the chemical structure (Cook *et al*., 1966).

Strigolactones constitute a novel class of plant hormones that have become increasingly important in plant science. These chemicals produced by plant are used to communicate within the plant system and with other plants. Of importance, the parasitic seeds of *Striga* are dependent on these allelo-chemicals to germinate. These germination stimulants are usually present in the rhizosphere of host and non-host plants and often trigger germination (Tsuchiya *et al*., 2018). Additionally, these allelo-chemicals are involved in the formation of a specialized organ called the haustorium, which is responsible for the establishment of vascular connections between the host and parasite (Mutuku *et al*., 2020). As a result, following the establishment of parasitism, the crop yield is significantly affected, which causes a serious problem in food production.

Witchweeds (*Striga* spp.) belonging to the family Orobanchaceae are considered as one of the most devastating weeds, which parasitize the roots of their respective host plants (Parker, 2009). *Striga* is regarded as a hemiparasite because they have a functional chloroplast, but for them to survive they have to establish parasitism with host roots

(Yoneyama *et al*., 2010). The lifecycle of *Striga* is highly synchronized with that of its host and involves a number of stages. Phase I is the pre-incubation period whereby *Striga* seeds are exposed to suitable and moist temperatures. Phase II involve the perception of germination stimulants termed strigolactones that are released from host plant roots and act on seeds that are in close proximity. Upon germination, the radicle of the *Striga* seeds grows a few millimeters towards the host root and must establish an attachment before exhausting resources from its endosperm. Phase III entails the formation of a haustoria whereas Phase IV is whereby the haustoria adheres and penetrates the epidermis and cortex of the host plant and subsequently makes connections with the vascular system. Phase V entails siphoning of water and mineral salts from the host. Phase VI is whereby the parasite develops tubercles whilst underground for several months and Phase VII represents emergence above the ground, which is signified by flowering of shoots (Yoneyama *et al*., 2010).

Haustoria formation is essential for establishing parasitism. Haustoria is a specialized multicellular organ that develops at the interface of the parasite and host root, which subsequently penetrates the host’s epidermis and cortex in order to establish a xylem-xylem connection (Kokla and Melnyk, 2018). In parasitic plants belonging to the family Orobanchaceae, root hair-like structures usually form on the haustoria to aid in establishing connection to the host. In *Striga* plants, haustoria formation occurs either at the meristematic tip of the primary root usually referred to as terminal haustoria or on the side of the growing root usually referred to as lateral haustoria (Yoshida *et al*., 2016). The formation of haustoria is through the perception of secondary metabolites referred to as haustorium inducing factors. Haustorium inducing factors that have been identified are phenolic derivatives which include flavonoids and quinones (Goyet *et al*., 2019). An important role of haustorium inducing factors is to determine host specificity so as to prevent spontaneous formation of haustorium on plant’s own roots. A number of haustorium inducing factors have been studied by following the pathway of 2, 6-dimethoxy-*p*-benzoquinone (DMBQ). DMBQ is a byproduct of lignin oxidation and decarboxylation of phenolic acids, which is present in plant cell walls (Wada *et al*., 2019). Upon release from the host cell wall, DMBQ infiltrates the parasite cells. Consequently, quinine reductase QR1, which is NADPH-dependent reduces DMBQ to form an unstable semiquinone intermediate, which is responsible for the formation of haustorium (Yoshida and Shirasu, 2012).

### 2.7.1 Chemical Structure of Strigolactones

Strigolactones are considered as ancient plant signaling molecules (Cook *et al*., 1966). Natural strigolactones contain a characteristic tricyclic rings (ABC part) that is connected to a butenolide (D ring) via an enol ether bridge as shown in figure 2.2 (Yoneyama *et al*., 2010). Studies on the activity of the structure of strigolactones indicate that the germination stimulation of strigolactones is situated at the C-D ring moiety (Zwanenberg *et al*., 2009). The difference in these strigolactones is in the substitutions that occur in the A and B rings. Such substitutions have an effect on germination stimulation as well as the stability of strigolactones (Tsuchiya *et al*., 2018).

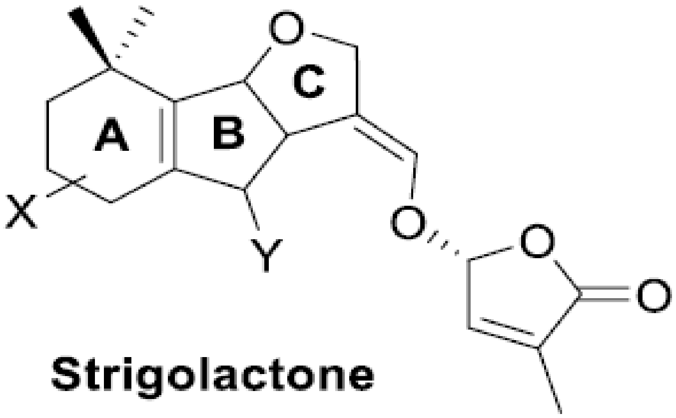


**Figure 2.2: Basic structure of a strigolactone**

Natural strigolactones induce over 80% of germination of Orobanchaceae seeds at concentrations of ≤ 1 nM (Xie *et al*., 2009). Among the natural strigolactones, the three monohydroxy strigolactones, 2’-epiorobanchol, orobanchol, and sorgomol are the most active strigolactones, which induce over 80% germination (Xie *et al*., 2009). The C-2’-(R)-configuration present in most natural strigolactones is an important features responsible for inducing high germination. Regarding strigolactone stereoisomers, C-2’-(R)-isomers are more active compared to their C-2’-(S)-isomers owing to the positive effect of 4-hydroxyl group on stimulation of germination (Xie *et al*., 2007). There are different characterized natural strigolactones, some of which are shown in figure 2.3 (Yoneyama *et al*., 2010). Improvement in chromatography and mass spectrometry has made it possible for the identification of novel natural strigolactones. However, germination assays have a high sensitivity compared to mass spectrometry, which is an indication that many novel strigolactones have not yet been characterized (Al-Babili and Bouwmeester, 2015).

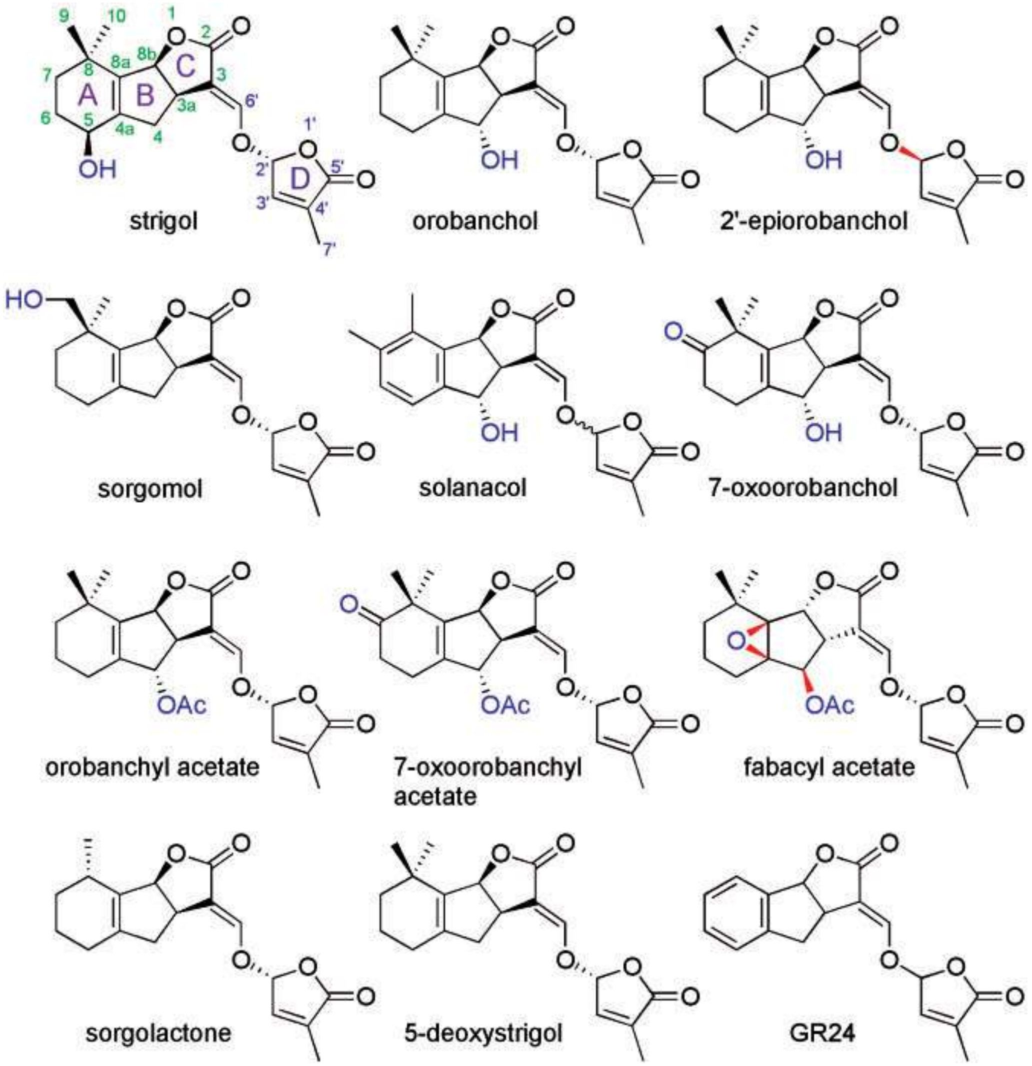


**Figure 2.3: Structures of eleven natural strigolactones and the synthetic analog GR24**

### 2.7.2 Strigolactone Biosynthesis and Mode of Action

A key precursor of the strigolactone biosynthetic pathway is carlactone, which is obtained from all-trans β-carotene (Al-Babili and Bouwmeester, 2015). Strigolactones are obtained from the β-carotene through stages of isomerization**,** cleavage**,** oxidation, and cyclization, which ultimately form the four characteristic rings of strigolactones (Alder *et al*., 2012).

There are a series of four enzymes involved in these steps. First, the DWARF27 (D27), which is a carotenoid isomerase converts all-trans-β-carotene to 9-cis-β-carotene. Second, the carotenoid cleavage dioxygenase 7 (CCD7) cleaves the 9-cis-β-carotene to form 9-cis-β-apo-10′-carotenal. Third, the carotenoid cleavage dioxygenase 8 (CCD8) converts the 9-cis-β-apo-10′-carotenal to form carlactone, which contains the A and D rings (Yoneyama, 2019). Carlactone is subsequently oxidized by MORE AXILLARY BRANCHES 1 (MAX1), which entail further ring closures and functionalization that gives rise to strigolactones and strigolactone-like compounds as shown in figure 2.4 (Waters *et al.*, 2012).

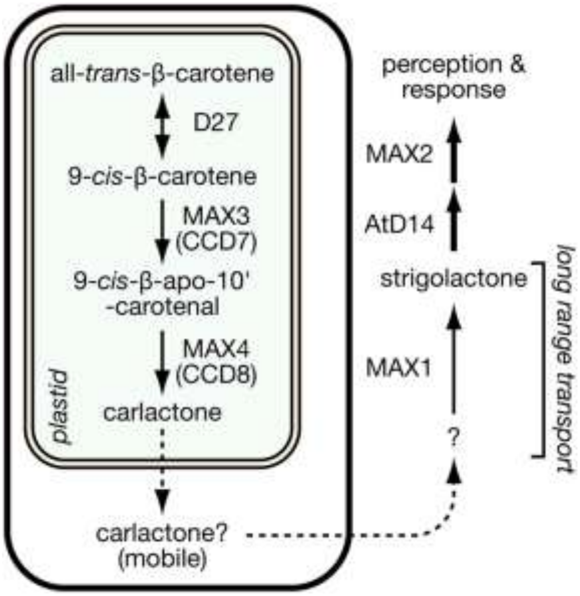


**Figure 2.4: Strigolactone biosynthesis and signaling pathway**

Signaling of strigolactones involves the interaction between strigolactones and a protein receptor. Recent studies of protein structures have shed more light on signal perception of strigolactones especially in the inhibition of shoot branching (Hamiaux *et al.*, 2012). With the aid of genetic approaches, strigolactone receptors have been identified in a number of vascular land plants such as petunia (DAD2), rice (D14), and Arabidopsis (AtD14) (Hamiaux *et al*., 2012; Waters *et al*., 2012; Zhao *et al*., 2013). The aforementioned proteins belong to a group of α/βhydrolase enzyme family. AtD14 is also referred to as KARRIKIN INSENSITIVE 2 (KAI2), which are smoke compounds present in Arabidopsis. The function of D14 proteins, which belong to the α/βhydrolase family is hormone signaling, for example between gibberellin and the receptor GIDI. The three receptor proteins namely DAD2, D14, and KAI2 are highly superimposable, which is an indication that they are orthologs (Murase *et al*., 2008). The hydrolytic activity of DAD2 protein is evident in its crystal structure whereby the α/βhydrolase pocket contains a catalytic triad of Ser-His-Asp that accommodates strigolactone. In an experimental study, the protein receptor was incubated with GR24 in a 1:20 ratio. After an 18-hour period, all the GR24 had been hydrolyzed forming a tricyclolactone and a hydroxyl butenolide (Zhao *et al*., 2013). The hydrolytic detachment is an indication that strigolactones are required in signal transduction (Kholsa and Nelson, 2016). In spite of the progress made in understanding the strigolactone biosynthesis, perception, and signaling, there are a number of unclear issues regarding strigolactones. For instance, the enzymatic activity of strigolactone receptor has been preserved throughout evolution, which is an indication that it plays an important role (Uraguchi *et al*., 2018).

The function of the strigolactone receptor is not yet clear given that the hydrolysis products of strigolactone namely the ABC-part and D-OH parts are also inactive. This raises the question that the enzymatic activity is essential in strigolactone reception and signaling

(Tsuchiya *et al*., 2018). Additionally, the downstream processes involved in strigolactone perception are still unclear. There are some downstream transcription factors that have been identified, which are transcriptionally regulated after the application of strigolactones. However, these transcription factors are only regulated for a short period of time in comparison with other plant hormones. This indicates that non-transcription factors are also involved in mediating the response to strigolactones. Therefore, further research in both transcription and non-transcription function is needed in strigolactone reception and signaling (Zwanenburg *et al*., 2009).

Attempts to identify a protein receptor in *Striga hermonthica* uses the expression in Arabidopsis (Toh *et al*., 2015). An example of α/βhydrolase gene found in the model plant Arabidopsis is HYPOSENSITIVE TO LIGHT (HTL), which plays a role in germination. As such, since *Striga* requires strigolactones for the induction of germination, it can be inferred that HTL homologs may have a similar role in parasitic plant species (Kholsa and Nelson, 2016). In contrast, the Arabidopsis HTL (AtHTL) gene binds to karrikin, which is a smoke signaling molecule. However, *Striga* does not germinate in response to karrikin but is only sensitive to picomolar concentrations of strigolactones (Awad *et al*., 2006). In this regard, the strigolactones receptors in *Striga* may have a resembling role with that of AtHTL. Therefore, it is hypothesized that *Striga* carries orthologs that have obtained novel functions in the course of evolution so as to detect strigolactones and subsequently stimulate germination (Toh *et al*., 2015).

## 2.8 Principles of LC-MS/MS

Liquid chromatography tandem mass spectrometry is a powerful analytical technique that combines the resolving power of liquid chromatography and detection specificity of mass spectrometry. Liquid chromatography physically separates the samples of interests, which are then introduced into the mass spectrometer. The function of the mass spectrometer is to create and detect charged ions (Kumar and Vijayan, 2014). Data from LC-MS/MS provides information about molecular weight, identification, and quantity of the sample of interest.

The liquid chromatography phase, specifically the reverse phase chromatography consists of the mobile phase and the stationary phase (Halouzka *et al*., 2020). As such, the mobile phase is polar whereas the stationary phase is non-polar. Upon injection of the sample into the liquid chromatography, it is subsequently adsorbed in the stationary phase and are separated along the column based on their relative affinity. The compounds that have the highest affinity ate the last to separate as they pass through the column. The effluent coming from the liquid chromatography is ionized through electrospray ionization by a nebulizing gas. As such, the charged ions pass on to a series of mass spectrometer filters and ultimately to a detector. Within the liquid chromatography, samples are detected based on their retention time in the column whereas the mass spectrometer separation is according to the mass-to-charge ratio whereas detection serves to determine the quantity of each ion (Mutiga *et al*., 2021).

## 2.9 Principles of light microscopy

The light microscope is an instrument used for the visualization of fine details of objects. In the case of biological objects, the light microscope operates by the principle of enhancing the magnification of an image through a series of glass lenses (Dey, 2018). The lenses initially focus a beam of light through an object of interest and the convex objective lenses so as to enlarge the image. Viewing of the enlarge image is through the binocular eyepieces, which are secondary in regard to the magnification of the object of interest. Such microscopes are referred to as compound microscopes, whereby the total magnification is arrived at by the sum of the objective magnification and the eyepiece magnification (Dey, 2018).

Visibility of the objects is possible owing to differences in light absorption by different components of the object being examined. The reasons for these differences in absorption is as a result of tissue processing steps such as fixation and staining. There are different staining methods that adopt various colored dyes that have specific affinities to cellular structures. The object of interest is on a permanent slide and uses visible light to illuminate it from beneath. Some microscopes have a third lens referred to as a condenser situated between the object and the light source, which focuses light on the object of interest. The resolving power of the best compound light microscope is 0.2 µm (Ryan *et al*., 2017).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Experimental site

Germination assays and histology work were conducted at the Plant Transformation Laboratory (PTL), Kenyatta University, Nairobi, Kenya. Liquid chromatography tandem mass spectrometry (LC-MS/MS) of the legume root exudates was conducted at Biosciences east and central Africa hub, ILRI, Nairobi, Kenya.

## 3.2 Plant Material

Four types of legumes obtained from Simlaw Seeds, Nairobi were used to conduct the study. The selected legumes used were two cowpea genotypes KEN-KUNDE-1 and M66, a common bean genotype (Nyayo), a garden pea genotype, and pigeon pea genotype. *Striga hermonthica* seeds used in germination bioassays were obtained from Kibos in Western Region of Kenya (0.0699° S, 34.8169° E). *Striga gesneroides* seeds were provided by Prof. Julie Scholes from Sheffield University.

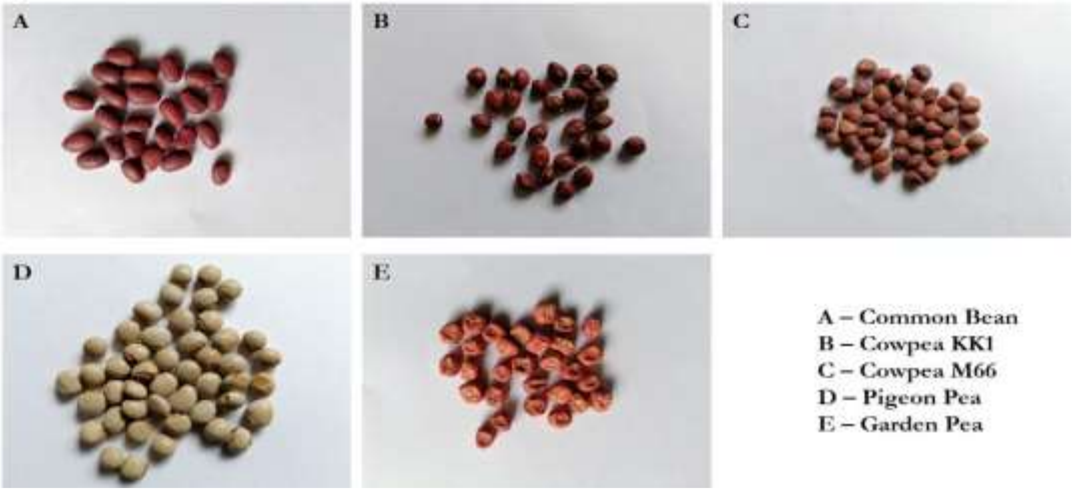
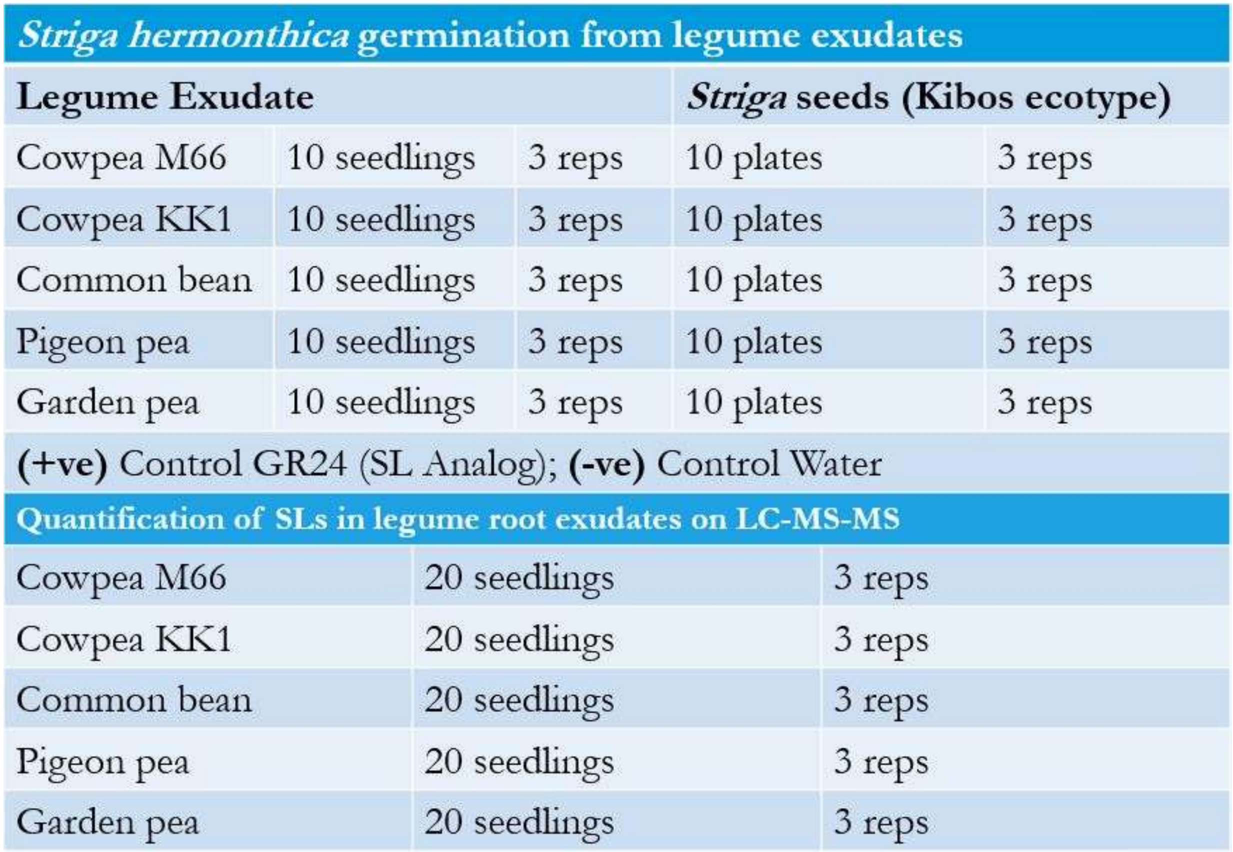


**Figure 3.1: Legumes used in the study**

### 3.3 Experimental Design

The study design adopted was a completely randomized controlled design.

**Table 3.1: Experimental design for germination assays and strigolactone profiling**



| ***Striga hermonthica*** **germination from legume exudates** | | | | |
|---|---|---|---|---|
| **Legume Exudate** | | | ***Striga*** **seeds (Kibos ecotype)** | |
| Cowpea M66 | 10 seedlings | 3 reps | 10 plates | 3 reps |
| Cowpea KK1 | 10 seedlings | 3 reps | 10 plates | 3 reps |
| Common bean | 10 seedlings | 3 reps | 10 plates | 3 reps |
| Pigeon pea | 10 seedlings | 3 reps | 10 plates | 3 reps |
| Garden pea | 10 seedlings | 3 reps | 10 plates | 3 reps |
| **(+ve)** Control GR24 (SL Analog); **(-ve)** Control Water | | | | |

| **Quantification of SLs in legume root exudates on LC-MS-MS** | | |
|---|---|---|
| Cowpea M66 | 20 seedlings | 3 reps |
| Cowpea KK1 | 20 seedlings | 3 reps |
| Common bean | 20 seedlings | 3 reps |
| Pigeon pea | 20 seedlings | 3 reps |
| Garden pea | 20 seedlings | 3 reps |

### 3.4 Preparation of legume root exudates

Seeds of the selected legumes were germinated in vermiculite at 28˚C for 7 days. The seedlings were then transferred to 50 ml boiling tubes containing 40% Long Ashton nutrient solution whose preparation procedure is described in Hudson (1967). Cotton wool plugs were used as support material for the seedlings with the root system immersed in the nutrient solution. The seedlings were maintained upright in a hydroponic nutrient media for 1 week in the glass house at 28˚C. The boiling tubes were covered with aluminum foil to exclude light. Seedlings were then maintained in 20 ml test tubes containing distilled water for a period of 48 hours (Mallu *et al*., 2021). The test tubes were also covered with aluminum foil to exclude light. After removal of the plants from the test tubes, crude root exudates were transferred to universal bottles and stored at -20˚C.

### 3.4.1 Preconditioning of *Striga hermonthica* seeds and Incubation

*Striga hermonthica* seeds weighing 0.08 g were surface sterilized in 10% (v/v) commercial bleach for 10 minutes with vigorous agitation. For this, 5ml of commercial bleach was added to a 50ml falcon tube and topped up with distilled water. The solution was divided in two falcon tubes equally (25 ml) and the 0.08 g of *S. hermonthica* seeds added. Shaking was done manually but vigorously for 10 minutes (Mwakha *et al*., 2020). The seeds were then rinsed three times through a sieve cloth folded in a funnel placed on a conical flask with distilled water to remove the bleach. The seeds were then spread on Whatman (GFA) filter papers that were placed on 90mm petri dishes. A 1 ml pipette was used to add 5 ml of distilled water to the *Striga hermonthica* seeds then sealed with parafilm and wrapped with aluminum foil. The seeds were then incubated at 30˚C for 14 days to break seed dormancy (Mbuvi *et al*., 2017).

### 3.4.2 Germination of *Striga hermonthica* using legume root exudates

The root exudates stored at -20˚C were thawed and then used to induce germination of pre-conditioned *Striga hermonthica* seeds in the petri dishes. A pipette was used to add 5 ml of crude root exudates to the pre-conditioned *S. hermonthica* seeds. Additionally, 5 ml of 0.1 ppm GR24, a synthetic germination stimulant was added to pre-conditioned *Striga* *hermonthica* seeds as positive control whereas distilled water was used as negative control. The petri plates were then sealed with parafilm and wrapped with aluminum foil and incubated at 30 ˚C for 24 hours. *Striga hermonthica* seeds were observed for germination under a Leica MZ10F stereomicroscope fitted with a DFC320FX camera and pictures taken. The percentage that had developed radicles was scored. Seeds were considered to have germinated if the radicles protruded from their seed coats. The germination frequency of *Striga hermonthica* seeds was determined by the function $[(x/y\times100) + (x/y\times100) + (x/y\times100)]/3$, where x is the number of germinated *Striga hermonthica* seeds and y is the total number of *Striga hermonthica* seeds in a particular field of view (Mallu *et al.*, 2021). Image J software was used to count the germinated *Striga hermonthica* seeds as well as the total number of seeds in a particular field of view (Mwakha *et al*., 2020).

### 3.5 Analysis of strigolactones from legume root exudates

For strigolactone profiling, each legume type was germinated in three different pots to get three replicates whereby each pot had approximately over 20 seedlings. Exactly 20 germinated seedlings at 10-days old were transferred in bottles containing distilled water wrapped in aluminum foil for collection of crude root exudates after 48 hours. After removal of the seedlings, 30 ml of crude exudate from the respective legumes was put in 50 ml falcon tubes to conduct the extraction process.

#### 3.5.1 Extraction and concentration of strigolactones from crude legume root exudates

The extraction was done according to Sato *et al*. (2005), whereby exactly 15 ml of ethyl acetate was added to the crude exudates in the falcon tubes, shaken vigorously, and placed on the vortex momentarily. The falcon tubes were allowed to sit to achieve phase separation. Upon phase separation, the supernatant was collected with the aid of a 1 ml pipette and transferred to a new falcon tube. The extraction step was repeated on the same root exudates to top up the supernatant collected initially. Exactly, 10 ml of 0.2 M $KH_2PO_4$ (potassium di-hydrogen phosphate) was then added to the collected supernatant, shaken vigorously, and placed momentarily on the vortex. The falcon tubes were allowed to sit to achieve phase separation. The formed supernatant was then pipetted into new falcon tubes through anhydrous magnesium sulfate supported with cotton wool placed in a funnel (Sato *et al*., 2005). The falcons containing the collected samples were then placed on a Nitrogen Evaporator (N-EVAP$^{TM}$ 112) for concentration. Nitrogen gas was used to evaporate the liquid sample until a white residue formed at the base of the falcon tubes. Exactly, 2 ml of ethyl acetate was added to the falcon tubes to dissolve the residue with the aid of a vortex. The solution was then transferred to 2 ml glass vials for storage and subsequent use. A volume of 100 µl of the sample was put into new glass vials and topped up with 1900 µl of 55% methanol.

### 3.5.2 LC-MS/MS analysis of strigolactones in the legume crude exudates

A total of 15 glass vials containing the samples were put into the LC-MS/MS machine for analysis. Strigolactone identification and quantification was done by comparing retention time and mass transitions with those of available strigolactone standards (Mutiga *et al.*, 2021). The standards available for this experiment included strigol, orobanchol, 2-epi-orobanchol, 5-deoxystrigol, and 2-epi-5-deoxystrigol. Selection of the aforementioned standards was based on availability as well as ensuring the standards consisted of both orobanchol-type and strigol type strigolactones. For each strigolactone standard, 100 µl was prepared in 1900 µl of acetonitrile as described by Mutiga *et al*. (2021). The identification and quantification of strigolactones was performed using the Shimadzu Nexera liquid chromatography coupled to the LC-MS/MS 8050 triple quadrupole mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). Infusion of neat standards was performed in the mass spectrometer to identify strigolactones as per their corresponding retention times. The data was called using LabSolutions software (Mutiga *et al*., 2021).

## 3.6 Determination of non-host/parasite interaction

The legumes were germinated in pots of vermiculite for 1 week. Seedlings of the five different legumes were transferred to a root observation chamber (rhizotron) – 25 ×25 ×5 cm Perspex plate – packed with vermiculite (Dayou *et al*., 2021). The rhizotrons were subsequently wrapped with aluminum foil and supplied with Long Ashton nutrient solution twice a day. The plants were maintained in a glasshouse whereby day and night temperatures were set at 28˚C and 24˚C respectively, and relative humidity set at 60% (Mbuvi *et al*., 2017). After 1 week, the seedlings that had well-developed roots were infected with GR24-pre-germinated *Striga hermonthica* seedlings as described by Mbuvi *et al*. (2017) by aligning them on the legume roots using a soft paintbrush. Controls for the incompatibility studies included Maize line Panar, infected with *Striga hermonthica* and *Striga gesneroides*, and Cowpea genotype M66 that is a host for *Striga gesneroides*. The plants were then returned to the glasshouse for 9 days before carrying out microscopic screening (Dayou *et al*., 2021).

### 3.6.1 Microscopic screening for *Striga hermonthica* resistance

To determine the extent of parasite infection in the roots of cowpea, pigeon pea, garden pea, and common bean (non-hosts), as well as maize, small sections of *Striga hermonthica*-Cowpea (KEN-K-1), *Striga hermonthica*-Cowpea (M66), *Striga hermonthica*-Pigeon pea, *Striga hermonthica*-Garden pea, *Striga hermonthica*-Common bean, *Striga hermonthica*-Maize (CML 144), *Striga gesneroides*-Cowpea (M66), and *Striga gesneroides*-Maize (CML 144) were dissected from the roots on the 9th day after infection under the Leica MZ10F stereomicroscope fitted with DFC 310FX camera. The tissues were fixed using Carnoy's fixative (4:1, ethanol: acetic acid). The tissues were then pre-infiltrated in Technovit 1 solution (Haraeus Kulzer GmbH, Germany) and absolute ethanol in 1:1 ratio for 1-2 hours. The tissues were subsequently infiltrated in 100% Technovit 1 solution for 15 minutes. The tissues were then replaced into fresh Technovit 1 solution for 3 days (Mbuvi *et al*., 2017).

The embedding process of the tissues involved placing tissues into Eppendorf (1.5 ml) tube lids containing Hardener 2 and Technovit 1 solution in a 1:15 ratio (Mwakha *et al*., 2020). Moulds were left in open air to dry before wrapping them in aluminum foil for incubation at 37°C to accelerate the drying process. Eppendorf lids containing the dried resin were then mounted on histoblocs using the Technovit 3040 mounting media kit. Sectioning of the tissues was done by cutting 5µm sections using a Leica RM 2155 microtome (Leica instruments GmbH) before transferring the tissues onto microscope slides (Dayou *et al*., 2021). The tissue sections were then stained using 0.1% toluidine blue O (Sigma, USA) in

100 mM phosphate buffer at pH 7 for 2 minutes, then rinsed with distilled water and dried at 65°C for 30 minutes on a hot plate. The sections were then mounted onto glass slides with DePex (BDH, Poole, UK), observed and photographed using a Leica DM100 microscope fitted with a Leica MC190 HD camera (Dayou *et al*., 2021).

### 3.7 Data Analysis

To determine the ability and efficiency of legume-derived exudates, to induce *Striga hermonthica* germination, protruding radicles from pre-germinated *Striga hermonthica* seeds were counted using Image J software version 1.48 and expressed as a percentage of all seeds in the petri dish. A total of 10 petri dishes replicated 3 times was used. Data was subjected to a one-way analysis of variance (ANOVA), implemented in SAS version 9.2, for generation of means and standard deviations of the mean. A Tukey's post-hoc test at $p \leq 0.05$ was performed for mean separations. Data was presented in graphs using GraphPad Prism version 6 software. For strigolactone profiling, a data visualization package, ggplot implemented in R software, was used to generate stacked bar plots of strigolactone proportions (in percentages) detected following analysis of crude root exudates using LC-MS/MS.

# CHAPTER FOUR

# RESULTS

## 4.1 Efficiencies of stimulation of germination of *Striga hermonthica* seeds

Seeds of the selected legumes germinated successfully in vermiculate as well as in the Long Ashton nutrient solution as shown in figure 4.1a and 4.1b. Following the induction of germination, *Striga hermonthica* seeds that germinated following treatment with root exudates had a radicle emanating from the seed coat as shown in figure 4.2 (a-f).

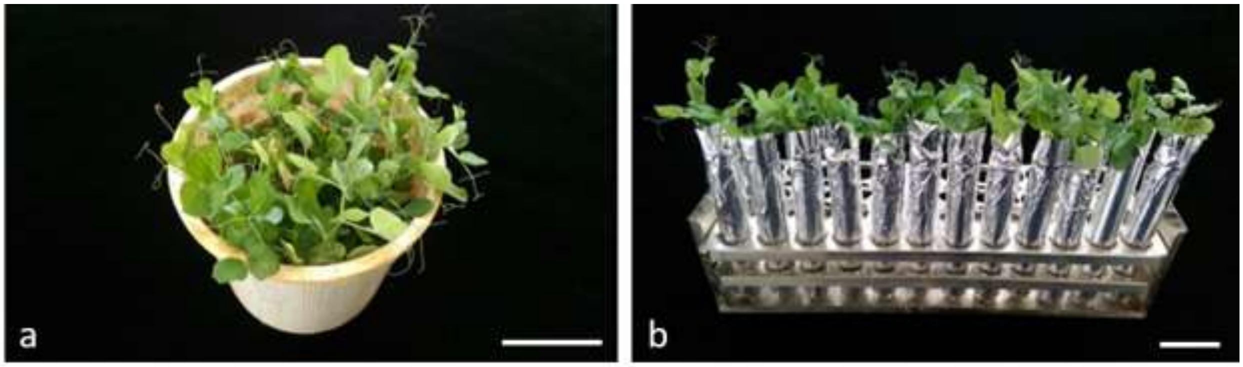


**Figure 4.1: (a) Germinated Garden Pea seedlings in vermiculite after 10 days (Bar = 5.5 cm; (b) Garden pea seedlings in Long Ashton nutrient media in boiling tubes. (Bar = 4 cm)**

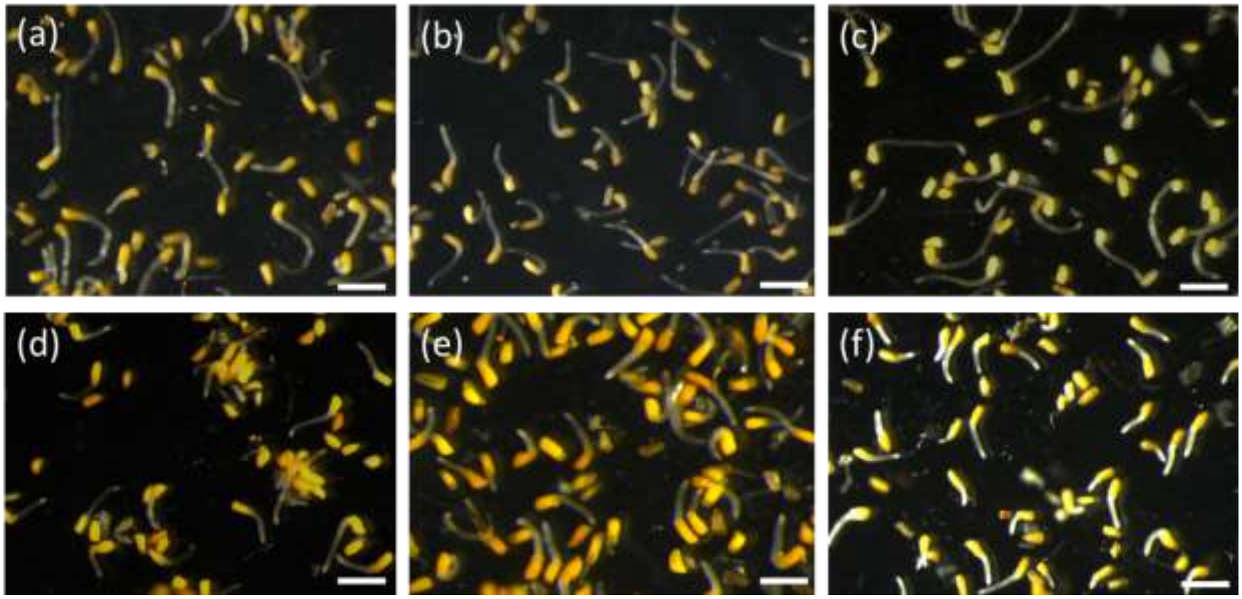


**Figure 4.2:** ***In vitro*** **germination of** ***Striga hermonthica*** **seeds using root exudates of (a) Common Bean, (b) Garden Pea, (c) Pigeon Pea, (d) Cowpea KK1, (e) Cowpea M66, and (f) artificial germination stimulant GR24 showing a protruding radicle. Scale bar = 1 mm**

To assess the ability of crude exudates to induce germination, pre-conditioned *Striga hermonthica* seeds were treated with exudates from the selected legumes. These results are presented in Figure 4.3. Crude exudates collected from the selected legumes over a period of 48 hours induced germination of *Striga hermonthica*. GR24 was used as a positive control in the assay and subsequently induced the highest germination percentage of 63.93%, on the other hand, germination was not observed from the seeds that were treated with distilled water, which was used as a negative control. The mean of germination frequency of common bean was 49.1% ±14.78, Cowpea KK1 45.9% ±8.4, Cowpea M66 43.8% ±16.52, Garden Pea 46% ±18.73, Pigeon Pea 38.6% ±18.45, GR24 63.9% ±10.54, and Water 0.0% ±0.0. ANOVA revealed that there were significant differences in the ability to induce germination of *Striga hermonthica* seeds from the selected legumes, GR24 (positive control), and water (negative control). However, there was no statistical significance among the five legume exudates in triggering germination, thus showing similarity in potency of germination cues.

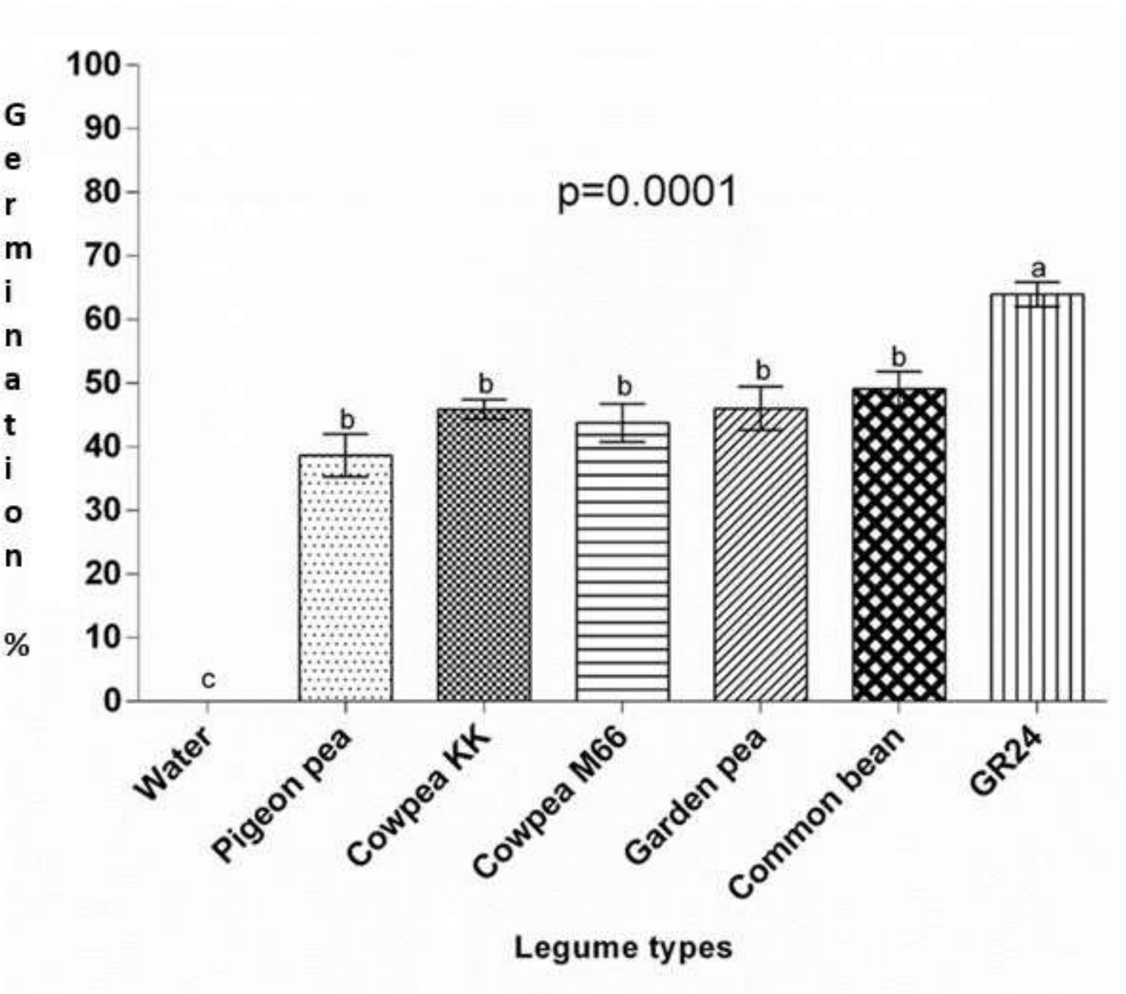


**Figure 4.3: Bar graph showing induction of *Striga hermonthica* germination by root exudates from selected legumes. Letter indicate significant differences among the groups according to Tukeys HSD test. (p≤0.05)**

### 4.2 LC-MS/MS Identification and quantification of strigolactones

Collection of root exudates was done is bottles covered in aluminum foil as shown in figure 4.4. Following LC-MS/MS analysis, the major strigolactones that were detected in the root exudates of the selected legumes following the LC-MS/MS analysis were orobanchol, strigol, 2-epi-orobanchol, and 2-epi-5-deoxystrigol. Orobanchol was detected at a retention time of 4.4 minutes; strigol was detected at a retention time of 5.0 minutes; 2-epi-orobanchol was detected at a retention time of 4.9 minutes; and 2-epi-5-deoxystrigol was detected at a retention time of 18.2 minutes.

**Figure 4.4: Collection of legume roots exudates in distilled water for a period of 48 hours for LC-MS/MS analysis. (a) common bean, (b) cowpea (M66), (c) pigeon pea (d) garden pea, (e) cowpea KK1. Each bottle contains 20 seedlings. Bar = 7 cm**

The most abundant strigolactone detected from the selected legumes was 2-epi-5-deoxystrigol, which was detected in garden pea, cowpea (KK1), common bean, cowpea (M66), and pigeon pea whereas strigol and 2-epi-orobanchol were detected in small amounts. The quantity of strigolactone profiles in the legumes were expressed in picograms per ml per seedling in a period of 48 hours. Cowpea M66 had comparatively high amounts of 2-epi-orobanchol and strigol while strigol was absent in pigeon pea as shown in Table 4.1.

**Table 4.1: Concentration of strigolactones produced by selected legumes in pg/ml/48hours**

| Legume | 2-epi-5-deoxystrigol | Orobanchol | 2-epi-orobanchol | Strigol |
|---|---|---|---|---|
| Common Bean | 2204 ±199.19 pg | 128.2 ±71.9 pg | 8.22 ±0 pg | 10.41 ±0.26 pg |
| Cowpea KK1 | 3200 ±0 pg | 225.1 ±28.2 pg | 17.4 ±2.53 pg | 13.53 ±1.82 pg |
| Cowpea M66 | 4260.2 ±693.9 pg | 371.9 ±12.5 pg | 114.65 ±17.83 pg | 49.79±4.13 pg |
| Garden Pea | 2808 ±391.6 pg | 161.5 ±39.1 pg | 10.8 ±3.75 pg | 13.27 ±0.78 pg |
| Pigeon Pea | 5757.4 ±1297.8 pg | 199 ±67.1 pg | 15.79 ±0 pg | 0.0 |

Following tabulation of the quantity of detected strigolactones in picograms, the strigolactones in each legume type were expressed as percentages as shown in Table 4.2.

**Table 4.2: Percentages of strigolactone types in each of the selected legumes**

| Legume | 2-epi-5-deoxystrigol | Orobanchol | 2-epi-orobanchol | Strigol |
|---|---|---|---|---|
| Common Bean | 93.75% | 5.45% | 0.35% | 0.44% |
| Cowpea KK1 | 92.59% | 6.51% | 0.5% | 0.39% |
| Cowpea M66 | 88.82% | 7.75% | 2.39% | 1.04% |
| Garden Pea | 93.8% | 5.39% | 0.36% | 0.44% |
| Pigeon Pea | 96.4% | 3.33% | 0.26% | 0.0% |

Plotting of the percentages variations of the strigolactones types in each legume under study showed a clear distinction of the proportion as shown in Figure 4.5. 2-epi-5-deoxystigol was produced in relatively high amounts across all legumes compared to any other type of strigolactone. Cowpea M66 had comparatively high amounts of strigol and 2-epi-orobanchol.

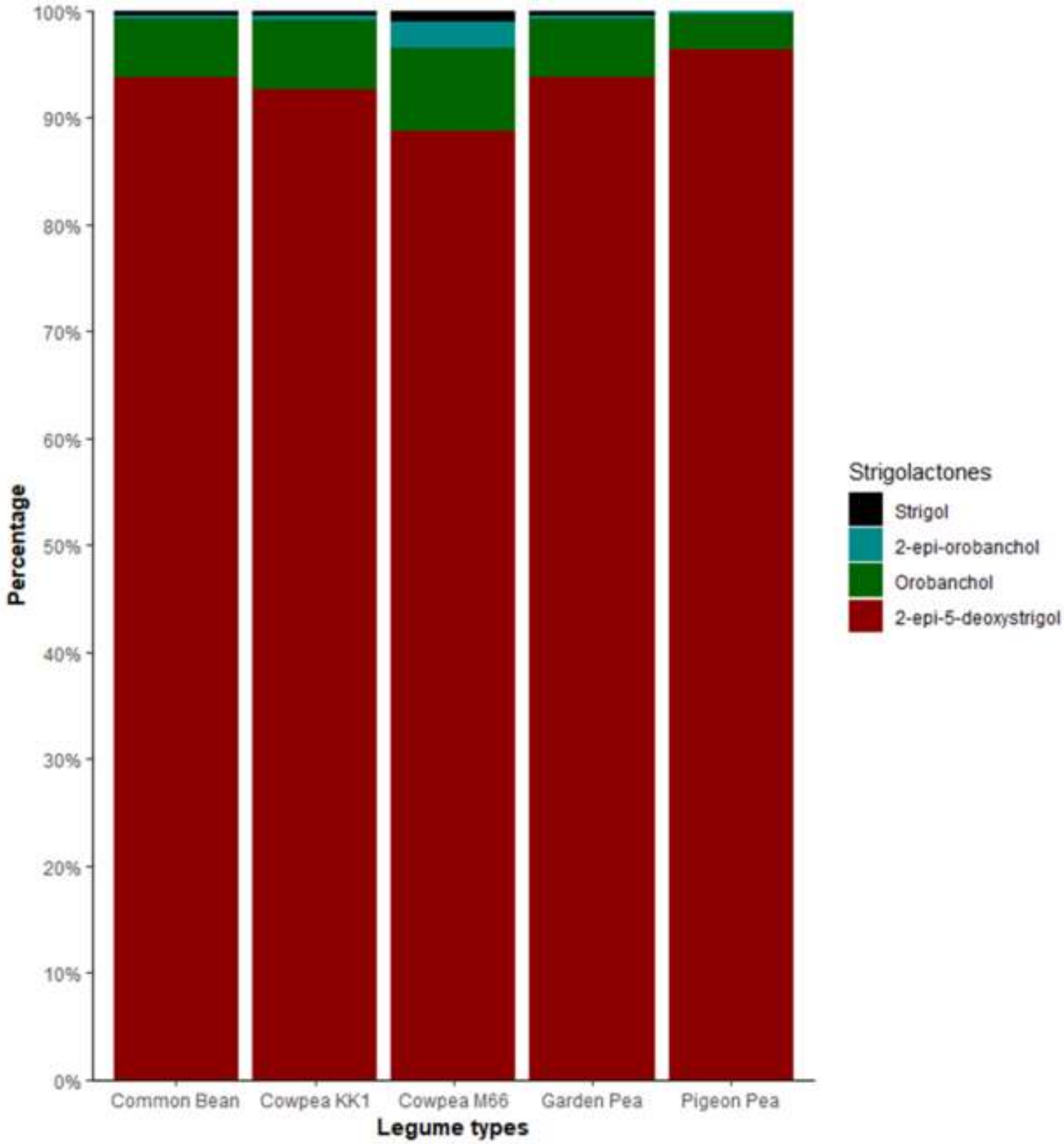


**Figure 4.5: Percent stacked bar graph showing variation in the proportions of the strigolactones strigol, 2-epi-orobanchol, orobanchol, and 2-epi5-deoxystrigol in the root exudates of selected legumes. 2-epi-5-deoxystrigol had the highest proportion; cowpea M66 had comparatively high amounts of strigol and 2-epi-orobanchol; strigol and 2-epi-orobanchol were in trace amounts; and pigeon pea did not detect any strigolactone. Color-coded key represents the respective strigolactones**

## 4.3 Microscopic Screening of *Striga hermonthica* Resistance

There were marked differences in growth and development observed in both compatible and incompatible response of *S. hermonthica* and *S. gesneroides* towards hosts and non-hosts. In the case of maize at 9 days after infection with *S. hermonthica*, which in this case was used as a control for compatibility, the parasite exhibited rapid shoot development with a characteristic three scale leaf pairs and a definite swelling at the point of attachment with the host root (Fig 4.6 ai). A transverse section of the of the swelling as well as the host root at the point of attachment indicated a complete parasitic invasion of the host root cortex by the haustorium and a subsequent establishment of a xylem-to-xylem connection (Fig 4.6 aii).

On cowpea (M66), at 9 days after infection with *S. gesneroides*, the parasite showcased a distinct swelling of the haustorium at the point of attachment (Fig 4.6 ci). A cross section of the point of attachment revealed that the haustorium had successfully penetrated the host root cortex and established a xylem-to-xylem connection. Additionally, there was an observation of phloem-to-phloem connection between the host and parasite (Fig 4.6 cii). On cowpea (M66), at 9 days after infection with *S. hermonthica*, the parasite exhibited shoot development but the seed coat was still intact. However, the haustorium of the parasite formed at the point of attachment did not have a huge swelling (Fig 4.6 bi). A transverse section at the point of attachment revealed that the haustorium penetrated the non-host root cortex and subsequently established a xylem-to-xylem connection (Fig 4.6 bii).

On maize, at 9 days after infection with *S. gesneroides*, which in this case was used as a control for incompatibility, the parasite formed a connection with the non-host but the haustorium was not well-formed with the characteristic swelling. However, the seed coat of the parasite was still intact (Fig 4.6 di). A transverse section of the point of attachment between the parasite and maize root revealed parasitic invasion of the root cortex and an establishment of xylem-to-xylem connection between the non-host and parasite. Additionally, tubercles of *S. gesneroides* were seen to establish connections with the non-host xylem (Fig 4.6 dii).

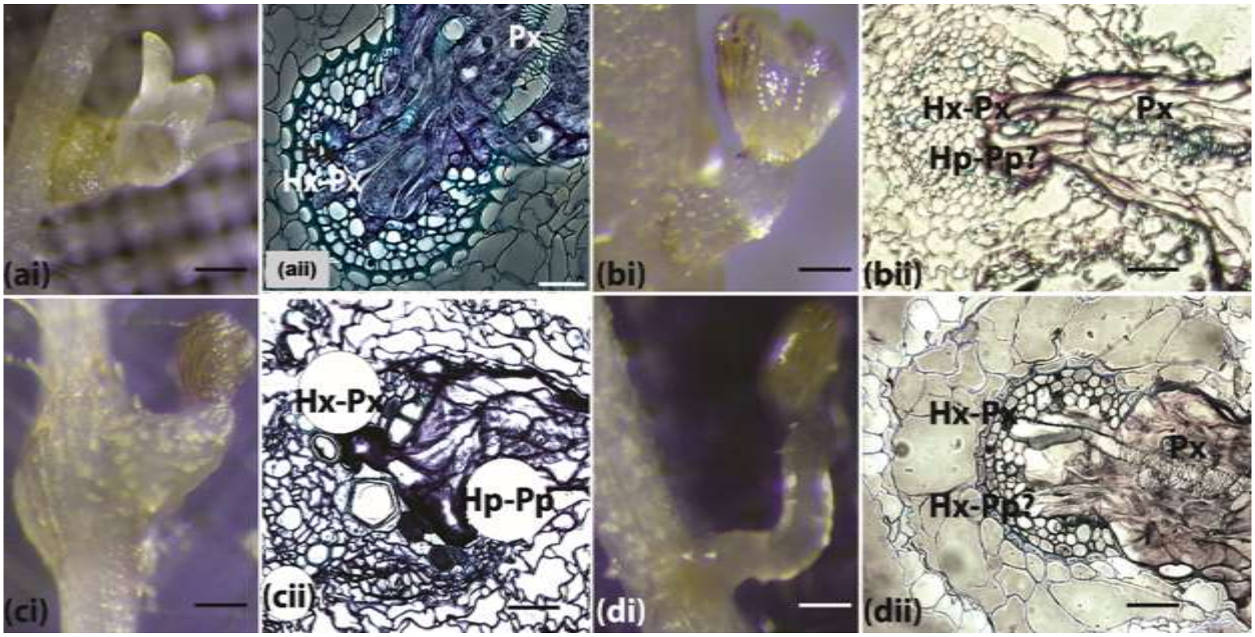


**Figure 4.6: Compatibility and incompatibility of host and non-host plants to *Striga hermonthica* at 9 DAI. (ai) Colonization of Panar maize line by *Striga hermonthica* showing a well-established haustorium (bar=1 mm). (aii) Transverse section of root tissue of Panar maize line 9DAI showing parasite penetration of cortex and endodermis as well as vascular connections (Hx-Px) (bar=0.1 mm). (bi) Colonization of Cowpea (M66) by *S. hermonthica* showing a well-established haustorium (bar=1 mm). (bii) Transverse section of a root tissue of Cowpea (M66) 9 DAI showing parasite penetration of cortex and endodermis as well as vascular connections (Hx-Px) (bar=0.1 mm). (ci) Colonization of Cowpea (M66) by *Striga gesneroides* showing a well-established haustorium (bar=1 mm). (cii) Transverse section of root tissue of Cowpea (M66) 9DAI**

**showing parasite penetration of cortex and endodermis as well as vascular connections (Hx-Px) (bar=0.1 mm). (di) Colonization of Panar maize line by *S. gesneroides* showing a well-established haustorium (bar=1 mm). (dii) Transverse section of a root tissue of Panar maize line 9 DAI showing parasite penetration of cortex and endodermis as well as connection between host and parasite xylem (Hx-Px). (Bar=0.1 mm)**

There were differences observed in the incompatibility responses to *Striga hermonthica* in the non-host legumes. In the case of garden pea, there was a well-established haustorium at the point of attachment at 9 days after infection with *S. hermonthica*. However, a black coloration was observed at the point of attachment. Additionally, the seed coat of the parasite was still intact (Fig 4.7 ai). A transverse section at the point of attachment with the garden pea root revealed that the parasite had penetrated the root cortex as well as the endodermis and established a host-parasite xylem-to-xylem connection (Fig 4.7 aii). On pigeon pea at 9 days after infection, the parasite had a well-established haustorium but there were signs of necrosis exhibited by dark coloration at the point of attachment. Additionally, the seed coat of the parasite was still intact (Fig 4.7 bi). A transverse section at the point of attachment revealed that the parasite had penetrated the root cortex but it was arrested at the endodermis, indicating that there was no xylem-to-xylem connection (Fig 4.7 bii). In the case of common bean at 9 days after infection with *S. hermonthica*, the parasite had a well-formed haustorium at the point of attachment with the common bean root. However, the seed coat of the *S. hermonthica* seed was still intact (Fig 4.7 ci). A transverse section of the point of attachment revealed the parasite's invasion of the root cortex but its development was arrested at the endodermis. The endodermis hindered further development and there was no host-parasite xylem-to-xylem connection (Fig 4.7 cii).

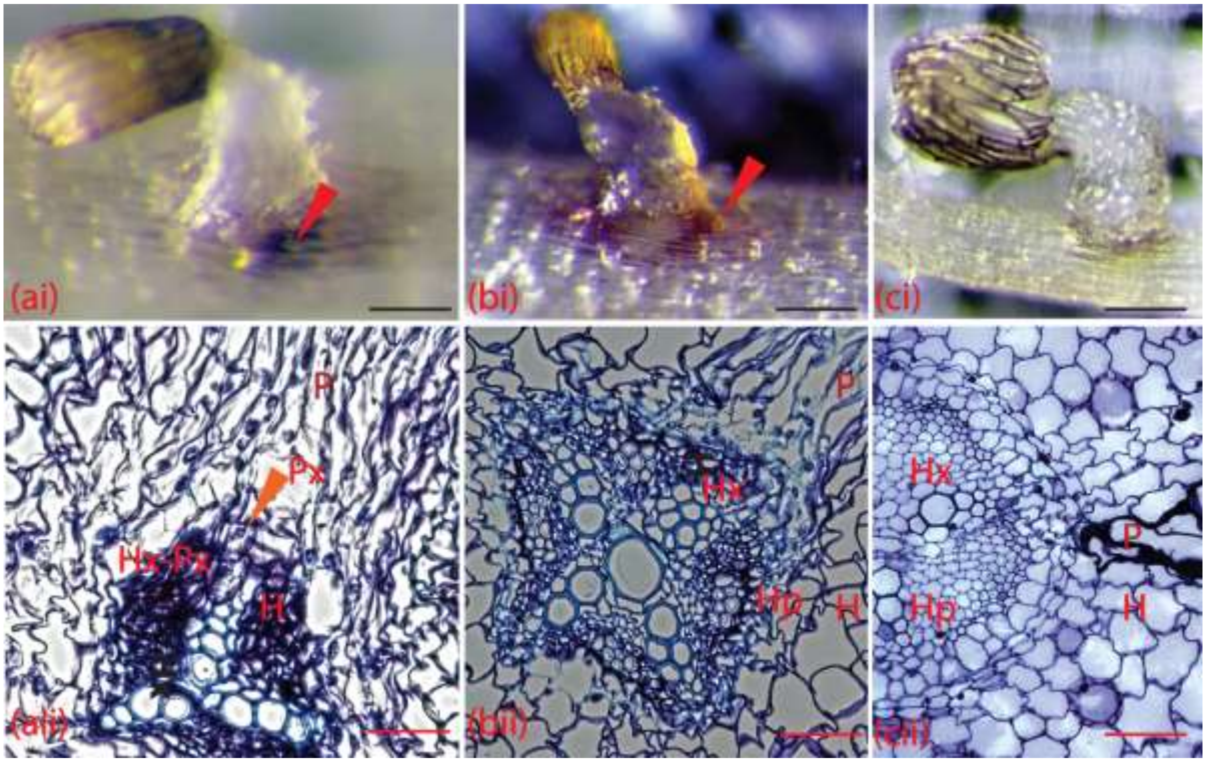


**Figure 4.7: Incompatibility of non-hosts to *Striga hermonthica* 9 DAI. (ai) Colonization of garden pea root by *S. hermonthica* showing a well-established haustorium but with some signs of a hypersensitive reaction (bar=1 mm). (aii) Transverse section of root tissue of garden pea showing penetration of the non-host root cortex and endodermis, as well as establishment of vascular connections (Hx-Px) (bar=0.1 mm). (bi) Colonization of pigeon pea root by *S. hermonthica* showing a well-established haustorium but with some signs of a hypersensitive reaction. (bar=1 mm). (bii) Transverse section of an embedded root tissue of pigeon pea showing the parasite xylem reaching the non-host phloem region but failing to establish xylem-xylem connection. (bar=0.1 mm). (ci) Colonization of common bean root by *S. hermonthica* showing a well-established haustorium (bar=1 mm). (cii) Transverse section of root tissue of common bean showing penetration of the root cortex but parasite development is arrested at the endodermis. (Bar=0.1 mm)**

The non-host legumes were left in the rhizotrons for 21 days after infection with *Striga hermonthica*. Expectedly, the non-hosts did not support parasite development at the 21-day stage whereby seedlings did not showcase any vegetative development. Contrarily, sorghum, a host for *S. hermonthica* showed vegetative development of the parasite as shown in figure 4.8

**Figure 4.8: Comparison of; A). Cowpea (non-host), 21 days after infection. B). Sorghum, (host), 21 days after infection. Red arrows show parasite arrest and death in cowpea whereas in sorghum they show vegetative development of the parasite. Bar = 2 cm**

# CHAPTER FIVE

# DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

Having knowledge of the strigolactone profile of both host and non-host plants is essential in understanding parasitism of *Striga hermonthica* with the aim of coming up with effective management strategies of the parasite. As such, this study employed selected legumes to determine their ability to induce *S. hermonthica* germination using crude root exudates *in vitro*, profile the quantity and type of strigolactone produced in each legume root exudate, and conduct incompatibility studies between legume trap crops and *Striga hermonthica* through histological analysis.

Based on the findings of assay for stimulation of germination of *Striga* seeds, all the selected legumes showed satisfactory germination efficiencies. There was no significant difference in stimulation activity of the root exudates obtained from the selected legumes, which is an indication that the legumes used in the study are effective in *Striga* germination activity. There was no significant difference across all the selected legumes. A study by Namutembi *et al*. (2020) on the use of trap crops to assess emergence of *Striga* as well as maize yield showed that the root exudates produced by these trap crops induce germination of *Striga* seeds. Trap crops induce suicidal germination of the *S. hermonthica* seeds in soil, thus reducing the parasite's seedbank (Namutembi *et al*., 2020). *In vitro Striga hermonthica* germination assays by non-host legume trap crops showcase the potential of suicidal germination as a control strategy against *Striga hermonthica*. The idea of the pre-germination assay of *S. hermonthica* seeds was to stimulate the germination of the parasite seeds in the absence of its host (Mallu *et al.*, 2021). Traore *et al.* (2011) showed that there is a variation in the ability of root exudates to induce abortive germination of *Striga hermonthica* among the three trap crops namely cotton, cowpea, and groundnuts. The *Striga* seed germination observed in the root exudates of the selected legumes indicate that they are well-suited trap crops in the control of *Striga* in farmers' fields. This work, albeit being conducted in artificial laboratory conditions, showed the practicality of suicidal germination as a measure for controlling the *Striga hermonthica* infestation on farmers' fields. Induction of germination of roots of parasitic weeds such as *Striga* in the absence of an appropriate host has been a subject of a number of research studies (Samejima *et al*., 2016). This is to demonstrate the importance of employing management strategies such as trap crops, intercropping, and crop rotation. Conversely, mono-cropping of host plants of *Striga hermonthica* such as maize and sorghum leads to an accumulation of large seedbanks of the parasite in soil (Hailu *et al*., 2018). Given that *S. hermonthica* is an obligate parasite, its germination in absence of its host is a suicidal (Mwakha *et al*., 2020). The production of naturally occurring strigolactones is complex and not cost-effective. Therefore, the alternative option is to produce strigolactone analogs such as the GR series to overcome the aforementioned challenges (Kountche *et al*., 2019).

In this study, GR24, an artificial germination stimulant, induced the highest germination percentage of 63.9% of *S. hermonthica* seeds. This is in line with what Kim *et al*. (2010) who reported that GR24, which is a synthetic analog elicits over 60% germination of *Striga* seeds. However, the GR compounds have instability in alkaline soil conditions (Babiker, 1988). This is owing to sensitivity to hydrolysis within the enol ether unit, thus making their practicality for use in field conditions challenging. In addition, this becomes challenging especially under natural conditions in *Striga*-infested rain-fed African fields. Kountche *et al*. (2019) reported that the application of strogolactone analogs in the absence of host plants has the potential to cleanse heavily *Striga*-infested fields. In this regard, the use of non-host plants or trap crops of *Striga hermonthica* is a more promising control strategy of *Striga* infestation (Samejima *et al*., 2016; Mwakha *et al*., 2020).

Following the assay of germination frequencies of the aforementioned legumes, it was essential to profile root exudates for type and quantity of strigolactone produced so as to ascertain the specific stimulants responsible for germination. The collection of root exudates hydroponically from the legume roots required a set-up with many plants (20 plants) because the production of strigolactones is low (Sato *et al*., 2005). Yoneyama *et al*. (2012) showed that strigolactone production is enhanced under P and N deficiencies. The results showed that each of the legumes used in the study produced more than one type of strigolactone. The strigolactone that was produced abundantly was 2-epi-5-deoxystrigol in all the legume types whereas the least produced was 2-epi-orobanchol and strigol. Orobanchol was produced in significantly moderate amounts across all legumes. This result is in line with the work of Ueno *et al*. (2011), who reported that leguminous crops such as cowpea mainly produces orobanchol type of strigolactones. Consequently, natural canonical SLs can be divided into two types: orobanchol-type with α-oriented C ring and strigol-type with β-oriented C ring (Xie, 2016; Yoneyama, 2019)). Roots produce a wide array of secondary metabolites into the rhizosphere and strigolactones are part of these metabolites (Nasreldin, 2018). The results indicate that there is a variation in the type of strigolactones detected in the crude exudates, which is in line with the study by Nasreldin (2018), who detected orobanchol, sorgomol, and 5-deoxystrigol in sorghum genotypes.

The production of a mixture of strigolactones in the selected legumes’ root exudes is in line with previous works on sorghum whereby there was the production of different strigolactone profiles from the root exudates (Awad *et al*., 2006: Yoneyama *et al*., 2010). The different types of strigolactones detected from the crude exudates of the selected legumes have a role to play in the germination-inducing activity of *S. hermonthica* seeds. This is because even the strigolactones produced in minute amounts in the picogram scale have a significant effect on germination stimulatory activity. Uraguchi *et al*. (2019) reported that sphynolactone-7, a hybrid molecule, has a femtomolar-range potency for inducing germination of *Striga* seeds, which indicates that albeit being produced in picomolar concentrations, germination of *Striga* seeds can be high. In this regard, the two strigolactones namely 2-epi-orobanchol and strigol, which were produced in small amounts have significant impact on germination. Yoneyama *et al*. (2009) reported that different types of strigolactones exhibit different germination stimulatory activity, which can be over 100-fold different.

Having conducted germination assays as well as strigolactone profiling of the selected legumes, it was essential to conduct histological analysis so as to determine the extent of parasitism. As such, this study examined the interaction between *Striga hermonthica* and *Striga gesneroides* with some of their subsequent host and non-host plants. According to Yoshida and Shirasu (2009), there are four different types of incompatible interactions observed between *Striga* and its host as well as non-host plants namely; layer I after vascular connection; layer II at the endodermal cell; layer III at the cortex cell; and layer IV before the attachment of *Striga* to the host or non-host. The aforementioned layers represent the levels of resistance that can be expressed by the host or non-host plants towards parasitism (Mutuku *et al*., 2020). This study set out to determine non-host resistance towards *Striga hermonthica* by selected legumes. Based on the histological analysis, there was a variation in the levels of parasitism by *S. hermonthica.*

The selected legumes used in this study are non-hosts for *S. hermonthica* and, therefore, are unable to complete their life cycle. The essence of this was to demonstrate the utility of trap crops and how they contribute to suicidal germination of *Striga* seeds when incorporated in farmers' fields. According to Spallek *et al*. (2013), *Striga* takes 3 to 7 days to find a host otherwise the seedling will die due to depletion of the endosperm reservoir. As such, 9-days post-artificial infection with preconditioned *S. hermonthica* seedlings was an appropriate time-point for assaying suicidal germination induced by germination cues originating from the legume root exudates.

This study revealed compatibility and incompatibility interactions towards *Striga* parasitism. Conventionally, *Striga hermonthica* is deemed compatible with monocots whereas *Striga gesneroides* is considered compatible with dicots (Jamil *et al*., 2021). In the case of *Striga hermonthica* on maize, successful parasitism was evident by the formation of a primary and secondary haustoria. In addition to the well-defined haustoria, there was also rapid shoot development at 9 days post-infection. According to Okonkwo and Nwoke

(1978), primary haustoria is formed when parasite's radicle comes into contact with the host root.  Additionally, the root parasites often form haustoria at the point of contact with the host root. Further, the secondary haustoria is usually formed when there is contact between the host root and the parasitic secondary roots (Yoshida *et al*., 2016). The formation of host-parasite xylem-to-xylem connection is an indication of successful parasitism and an onset of the process of obtaining water and nutrients at the expense of the host (Goyet *et al*., 2019). The aforementioned observed features are an indication of compatibility between *S. hermonthica* and maize host.

Another case of compatibility was observed between cowpea and *S. gesneroides*. There was a bulge of the haustoria at the point of contact between the parasite and host. According to Gurney *et al*. (2003), this is a sign of differentiation of the haustoria, which is important for growth and development of the parasite. Histological analysis at the point of contact indicated that there was continuous vasculature connection of the host and parasite, which is essential for constant supply of water and minerals (Yoshida *et al*., 2016). Dorr *et al*. (1997) reported that the host and parasite xylem-to-xylem connections are made in *Striga* whereas phloem-to-phloem connections have not been widely reported. However, this study was able to observe phloem-to-phloem connections between cowpea and *Striga gesneroides*, which is important for the transfer of photosynthates from the host to *Striga*.

Experiments were also run to determine incompatibility of *Striga* parasitism. In the first instance, *S. hermonthica* formed a haustorium with cowpea, which is in line with what Okonkwo and Nwoke (1978) reported that a primary haustoria forms when the parasite's radicle comes in contact with the root. Although there was formation of a xylem-to-xylem connection, cowpea was not able to support growth and development of *S. hermonthica* past 21 days after infection, which is a case of suicidal germination owing to the absence of an appropriate host (Kountche *et al*., 2019). The other case of incompatibility was between *S. gesneroides* and maize whereby there was a formation of xylem-to-xylem connection. However, there was no growth development of the parasite due to the unsuitability of the non-host.

Other legumes namely common bean, pigeon pea, and garden pea were used to show incompatibility. For instance, there was evident haustorium formation in common bean at the point of attachment but the parasite's development was arrested at the endodermis level. This observation is in line with what Gurney *et al*. (2006) reported in rice towards *S. hermonthica* where parasite growth was arrested at the endodermis level. It was observed that the endodermis provided a substantial barrier to vascular penetration by *Striga*. In the case of pigeon pea, there was formation of a haustorium but with some evidence of necrosis at the point of attachment. Necrosis is a type of hypersensitive reaction put up by a host against invasion by a parasite (Kavuluko *et al*., 2020). Consequently, this hypersensitive reaction is responsible for the failure of formation of xylem-to-xylem connection between the host and parasite. Hypersensitivity is common in resistant hosts or non-host plants so as to halt parasitism (Dayou *et al*., 2021). A similar hypersensitivity was also observed in garden pea but *S. hermonthica* was able to establish xylem-to-xylem connection. Albeit the establishment of the vascular connection, the parasite cannot be sustained for long and dies off due to non-host incompatibility (Mutuku *et al*., 2020).

The findings in this study showed that all the selected legumes are appropriate for use as trap crops since they induce high *S. hermonthica* germination as well as not supporting parasite development.

## 5.2 Conclusions

1. There was efficient stimulation of germination of *S. hermonthica* seeds by the 5 legume root exudates *in vitro*.
2. Identification and quantification of different strigolactones from the selected legumes was feasible, whereby 2-epi-5-deoxystrigol was the most abundant strigolactone followed by orobanchol with trace amounts of 2-epi-orobanchol and strigol.
3. Incompatibility mechanism between legume non-host and *S. hermonthica* was successfully determined through histological analysis. Cowpea and garden pea established a xylem-to-xylem connection with *S. hermonthica*, but the parasite did not persist past 21 days.

## 5.3 Recommendations

### 5.3.1 Recommendations from the study

1. Range of germination frequencies of the selected legumes (38.6%-49.1%) is adequate to induce suicidal germination of *Striga* seeds.
2. The endodermis should be considered as a significant barrier to parasite development in incompatibility studies.

iii. The legume trap crops should be incorporated in *S. hermonthica* management practices.

### 5.3.2 Recommendations for future research

i. Field studies should be conducted by intercropping the legume trap crops with host cereals to assess emergence of *S. hermonthica* plants compared to those planted as monocrops.

ii. There is need to further profile strigolactones found in legume trap crops using more strigolactone standards since more strigolactones are being characterized to date.

iii. Confounding effects (if any) on successful penetration of *S. hermonthica* in non-hosts will require further investigation.

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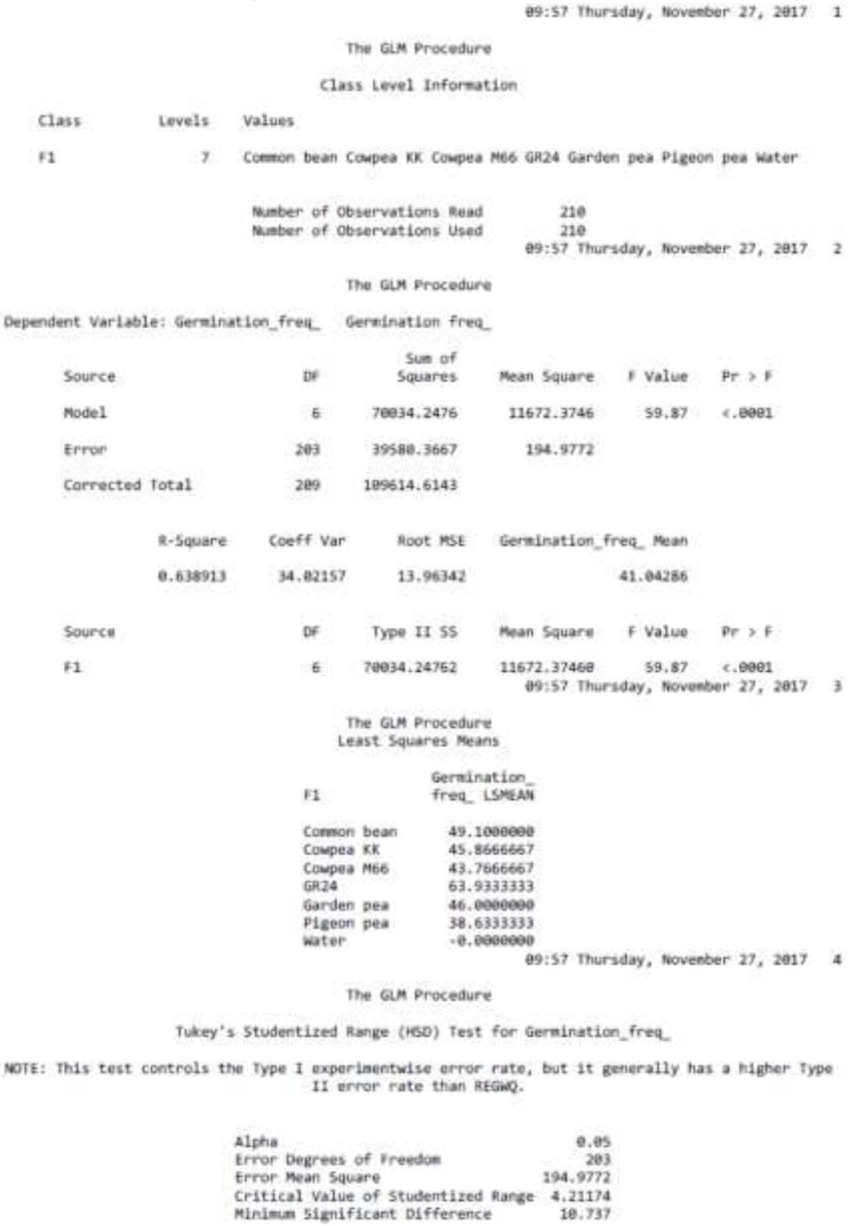
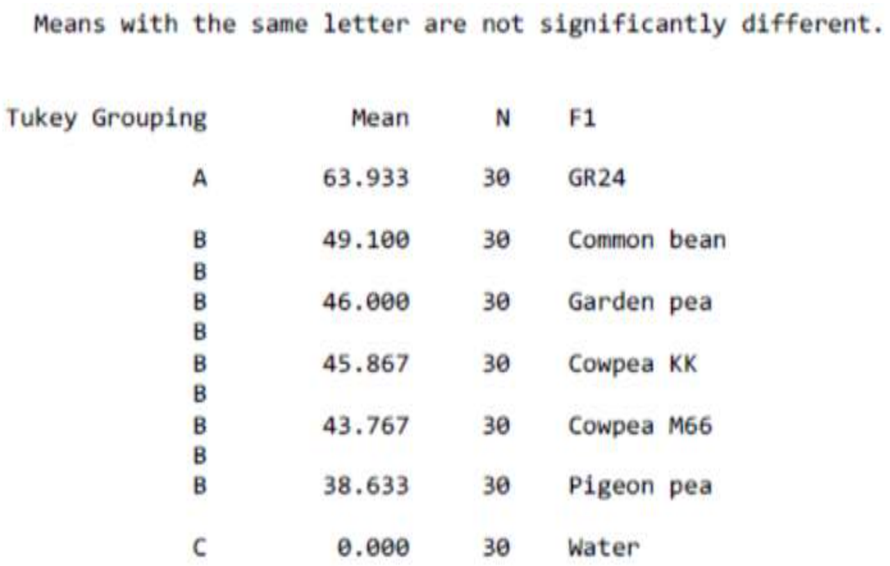
# APPENDICES

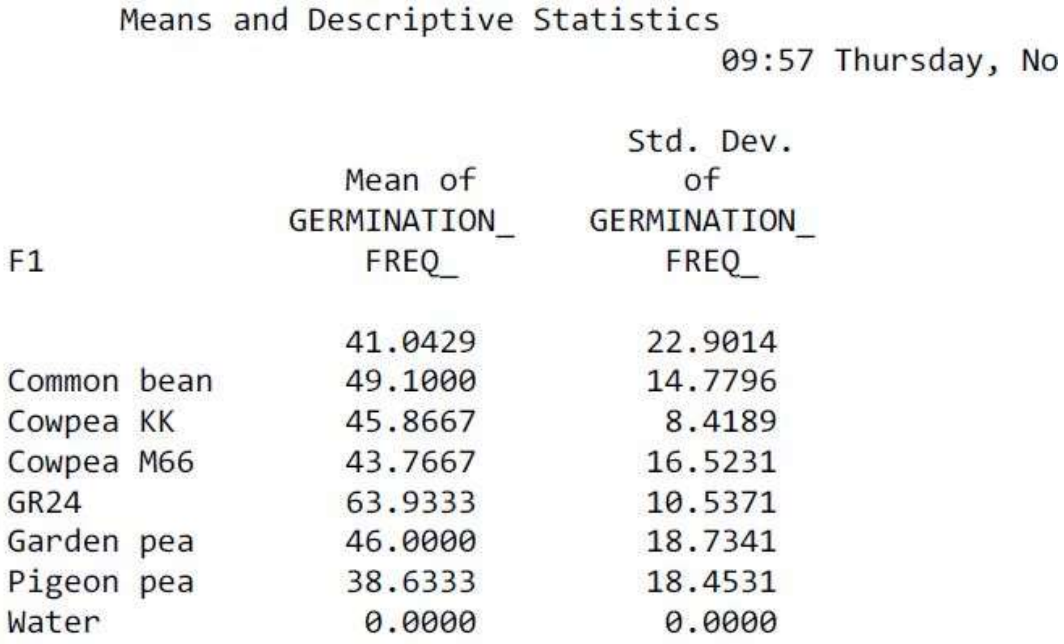
## Appendix 1: ANOVA for germination frequency of the selected legumes



```
                                                    09:57 Thursday, November 27, 2017   1

                                 The GLM Procedure

                              Class Level Information

      Class          Levels    Values

      F1                  7    Common bean Cowpea KK Cowpea M66 GR24 Garden pea Pigeon pea Water


                           Number of Observations Read         210
                           Number of Observations Used         210
                                                    09:57 Thursday, November 27, 2017   2

                                 The GLM Procedure

Dependent Variable: Germination_freq_   Germination freq_

                                        Sum of
        Source                  DF         Squares     Mean Square    F Value    Pr > F

        Model                    6      70034.2476      11672.3746      59.87    <.0001

        Error                  203      39580.3667        194.9772

        Corrected Total        209     109614.6143


                     R-Square     Coeff Var      Root MSE    Germination_freq_ Mean

                     0.638913      34.02157      13.96342                  41.04286


        Source                  DF     Type II SS     Mean Square    F Value    Pr > F

        F1                       6    70034.24762     11672.37460      59.87    <.0001
                                                    09:57 Thursday, November 27, 2017   3

                                 The GLM Procedure
                                Least Squares Means

                                             Germination_
                              F1             freq_ LSMEAN

                              Common bean      49.1000000
                              Cowpea KK        45.8666667
                              Cowpea M66       43.7666667
                              GR24             63.9333333
                              Garden pea       46.0000000
                              Pigeon pea       38.6333333
                              Water            -0.0000000
                                                    09:57 Thursday, November 27, 2017   4

                                 The GLM Procedure

                  Tukey's Studentized Range (HSD) Test for Germination_freq_

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type
                              II error rate than REGWQ.


                        Alpha                                   0.05
                        Error Degrees of Freedom                 203
                        Error Mean Square                   194.9772
                        Critical Value of Studentized Range  4.21174
                        Minimum Significant Difference        10.737
```

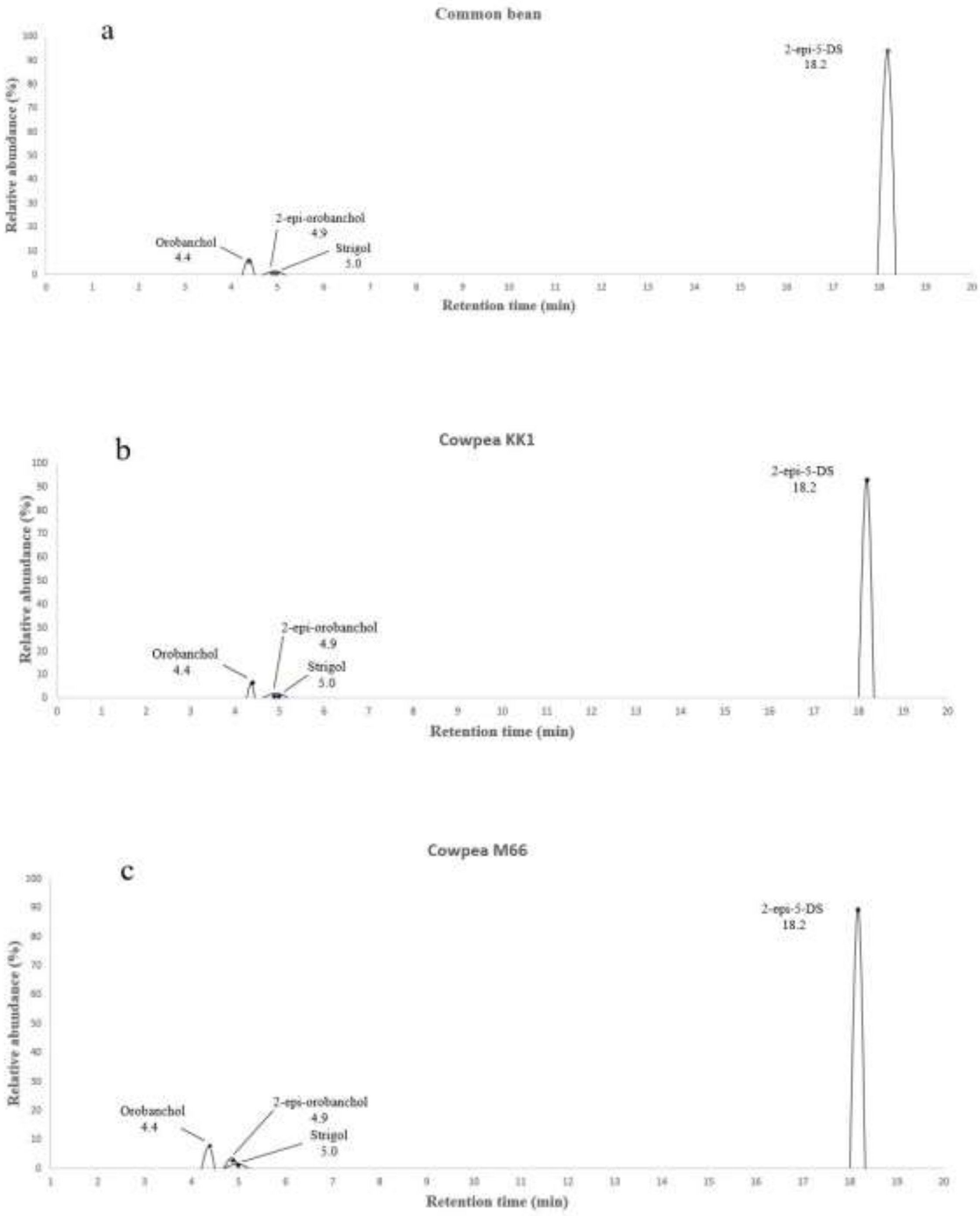


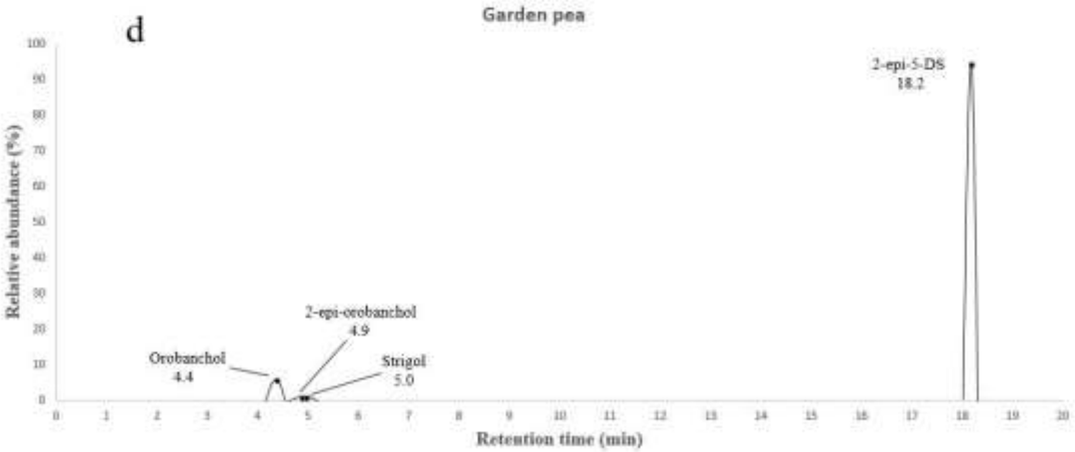
```
Means with the same letter are not significantly different.

Tukey Grouping          Mean      N    F1

             A        63.933     30    GR24

             B        49.100     30    Common bean
             B
             B        46.000     30    Garden pea
             B
             B        45.867     30    Cowpea KK
             B
             B        43.767     30    Cowpea M66
             B
             B        38.633     30    Pigeon pea

             C         0.000     30    Water
```

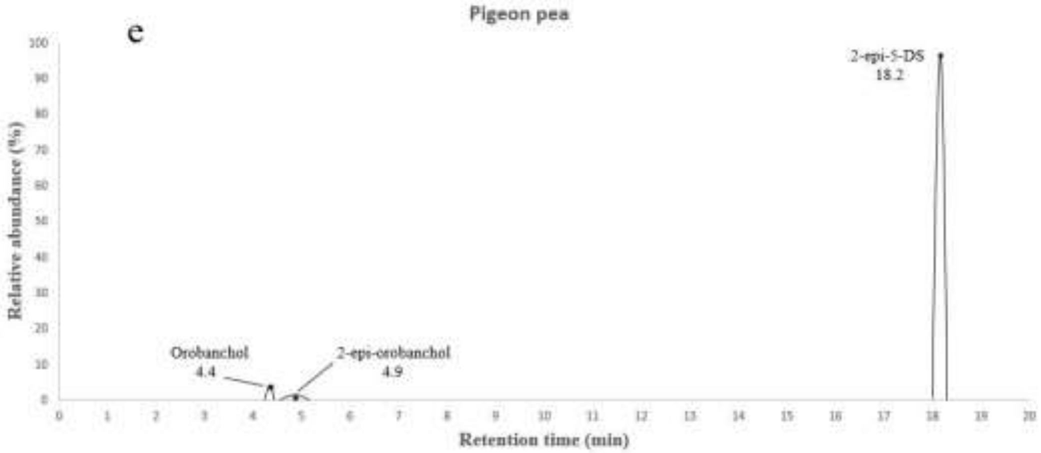


```
        Means and Descriptive Statistics
                                      09:57 Thursday, No

                                    Std. Dev.
                    Mean of            of
                  GERMINATION_    GERMINATION_
F1                    FREQ_           FREQ_

                    41.0429         22.9014
Common bean         49.1000         14.7796
Cowpea KK           45.8667          8.4189
Cowpea M66          43.7667         16.5231
GR24                63.9333         10.5371
Garden pea          46.0000         18.7341
Pigeon pea          38.6333         18.4531
Water                0.0000          0.0000
```

**Appendix 2: Chromatogram showing peak areas of strigolactones' relative abundance and respective retention times. Chromatograms a-e of the selected legumes.**




d
Garden pea
Relative abundance (%)
0
10
20
30
40
50
60
70
80
90
100
Orobanchol
4.4
2-epi-orobanchol
4.9
Strigol
5.0
2-epi-5-DS
18.2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Retention time (min)


e
Pigeon pea
Relative abundance (%)
0
10
20
30
40
50
60
70
80
90
100
Orobanchol
4.4
2-epi-orobanchol
4.9
2-epi-5-DS
18.2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Retention time (min)