16th World Congress on Parasitic Plants

3rd to 8th July 2022
Nairobi, Kenya

Abstracts

I. Host resistance
II. Parasitic Plant Biology and Ecology
III. Genes and Genomes
IV. Molecules and Biochemistry
V. Control and Management
VI. Parasitic plants-Hosts-Microbes interactions
VII. Phylogeny and evolution of parasitic plants
VIII. Posters

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3rd to 8th July 2022

Hybrid format: On Zoom, or Tulipa Hall, Eka Hotel,
Nairobi, Kenya

Preamble

The World Congress on Parasitic Plants (WCPP) is a series of biennial meetings organized by the International Parasitic Plant Society (IPPS: https://www.parasiticplants.org/). The tradition started in Malta in 1973 and provides a platform for experts on parasitic plants from all over the world to exchange ideas and discuss recent progress made in the field of plant parasitism. This year, Kenya (International Crops Research Institute for the Semi-Arid Tropics and Kenyatta University) will host the 16th WCPP between 3rd and 8th July 2022.

Program

SUNDAY, JULY 3	
16.00 - 20.00	Registration open
18.00 - 21.00	Welcome cocktail by the Bar Deck (courtesy of Plants People Planet) *All delegates are welcome*
MONDAY, JULY 4	
	Opening ceremony **Steven Runo, Kenyatta University, Kenya**
09.00 - 09.10	Harro Bouwmeester (President IPPS), Damaris Odeny (ICRISAT)
09.10 - 09.20	Rebbie Harawa (ICRISAT Regional Director - ESA)
09.20 - 09.30	Kenyatta University Vice Chancellor
09.30 – 09.45	Chris Thorogood (Editor, Plants People Planet)
09.45 – 10.15	**Keynote:** Harro Bouwmeester (University of Amsterdam, Netherlands) *Changes in the composition of the strigolactone blend exuded by the host reveals their biological significance*
10.15 – 10.45	**Coffee break and group photo**
	Session I: Host Resistance I **Jim Westwood, Virginia Tech, USA**
10.45 – 11.20	Keynote: Patrick Ongom (IITA, Nigeria) *Discovery and deployment of molecular markers for resistance to Striga gesnerioides in cowpea*
11.20 – 11.55	Yonli Djibril (CNRST/INERA, Burkina Faso) *Gamma ray-induced resistance to Striga hermonthica into Burkina's Sorghum landraces*
11.55 – 12.20	Armel Rouamba (University of Kwa Zulu Natal, South Africa) *Genetic analysis of Striga hermonthica resistance in pearl millet through generation mean analysis*

12.20 – 12.45	Mathews Dida (Maseno University, Kenya) *Breeding of maize in Kenya with resistance to Striga hermonthica L.: Challenges and Opportunities*
12.45 – 14.00	**Lunch**
	Session II: Host Resistance II **Salim Al-Babili (KAUST, Saudi Arabia)**
14.00 – 14.25	Danna Sisou (The Hebrew University of Jerusalem, Israel) *Genomic characterization of sunflower resistance to sunflower broomrape* (*Orobanche cumana*)
14.25 – 14.50	Min-Yao Jhu (University of Cambridge, UK) *Heinz-resistant tomato cultivars exhibit a lignin-based resistance to field dodder (Cuscuta campestris) parasitism*
14.50 – 15.15	Immaculate Mwangangi (University of Greenwich, UK) *Combining post-attachment Striga resistance with improved host nutrition*
15.15 – 15.40	Livia Stavolone (IITA, Nigeria) *African maize landraces reveal a novel mechanism of resistance to Striga hermonthica*
15.40 – 16.10	**Coffee break**
	Session III: Parasitic Plants Biology and Ecology **Susann Wicke (Humboldt University, Germany)**
16.10 – 16.35	Keynote 1: Peter Tóth (Slovak University of Agriculture in Nitra, Slovakia) *How and why broomrapes lie and what they say*
16.35 – 17.00	Paul Mugisha (Makerere University, Kampala, Uganda) *PUSH-PULL APP: A case study of using ICT to enhance the adoption of Striga control interventions among small scale farmers in Africa*
17.00 – 17.25	Gregorio Ceccantini (University of São Paulo, Brazil) *Hydraulics and functional aspects of loranthacean woods*
17.25 – 17.50	Guy Atsmon (Newe Ya'ar Research Center, Israel) *Hyperspectral imaging facilitates early recognition of Orobanche cumana below-ground parasitism on sunflower under field conditions*
17.50 – 18.25	Chris Thorogood (Oxford Botanic Garden and Arboretum, UK) *Desert hyacinths: An obscure solution to a global problem?*
18.25 - 19.30	**Poster viewing with drinks**
19.30 - 21.00	**Dinner**
21.00 –	**Bar conference centre open**
TUESDAY, JULY 5	
	Session IV: Genes and genomes **Damaris A. Odeny (ICRISAT, Kenya)**
09.00 – 09.35	Keynote speaker 1: Jim Westwood (Virginia Tech, USA) *A Report on the Analysis of the Orobanche cumana Genome*
09.35 – 10.00	Anne Greifenhagen (University of Hohenheim, Germany) *CLE peptides in the Interaction of Parasitic and Host Plants*
10.00 – 10.25	Sylvia Mutinda (Kenyatta University, Kenya) *Weighted gene co-expression networks explain Striga resistance in sorghum*

10.25 – 10.55	**Coffee break**
10.55 – 11.30	Keynote 2: Satoko Yoshida (NIST, Japan) *Structural and signaling diversity of haustoria in Orobanchaceae parasitic plants*
11.30 – 11.55	Imen Tanniche (Virginia Tech, USA) *Minimum Synthetic Inducible Promoters for Host Response to Parasites*
11.55 – 12.20	Josephine Sarah Kunguni (ICRISAT, Kenya) *Gene expression in finger millet within six days of Striga hermonthica infection*
12.20 – 12.45	Julia K Leman (Humboldt University, Germany) *A role for lateral haustoria in obligate parasites*
12.45 – 13.10	Songkui Cui (NIST, Japan) *Lignification occurs after perceiving haustorium inducing factors to facilitate prehaustorium induction in Striga hermonthica*
13.10 – 14.10	**Lunch**
	Session V: Molecules and Biochemistry **Harro Bouwmeester (University of Amsterdam, The Netherlands)**
14.10 – 14.45	Keynote 1: Salim Al-Babili (KAUST, Saudi Arabia) *New Insights in the role of canonical strigolactones and abscisic acid in Striga seed germination*
14.45 – 15.10	Jean-Bernard Pouvreau (Nantes Université, France) *Cytokinins are new major compounds involved in seed germination of parasitic weeds*
15.10 – 15.35	Shelley Lumba (University of Toronto) *How to become a successful plant parasite: Insights into Strigolactone Signalling in Striga*
15.35 – 16.05	**Coffee break**
16.05 – 16.30	Lyuben Zagorchev (Sofia University, Bulgaria) *Photosynthetic activity of Cuscuta campestris, influenced by gall formation by Smicronyx spp.*
16.30 – 16.55	Atsushi Okazawa (Kobe University, Japan) *Structure-Activity Relationship of Aromatic Carbonyl Thioureas for Inhibition of Orobanche minor Radicle Elongation*
16.55 – 17.20	Emeline Dossa (University of Kwa Zulu Natal, South Africa) *A meta-analysis of the effects of Striga control methods on maize, sorghum, and millet production*
17.20. – 18.30	**Poster viewing with drinks** *Virtual poster presentations from Natsumi Aoki, Mengqi Cui, Lei Xiang, Fiston Masudi, Christine Namala, Melaku Gedil and Sukhmanpreet Kaur – 5 minutes each, in that order.*
18.30 – 19.30	**IPPS general assembly**
19.30 – 21.00	**Dinner**
21.00 –	**Bar conference centre open**

WEDNESDAY, JULY 6	
All day	Conference tour + Conference dinner
07.30 - 14.30	Conference tour and lunch in Embu (Led by **Ann Wakaire** and **Sylvia Mutinda**)
14.30 – 17.00	Departure back to Nairobi
19.00 – 23.00	Conference dinner at the Carnivore restaurant
THURSDAY JULY 7	
	Session VI: Control and Management **Jonne Rodenburg (University of Greenwich, UK)**
09.00 – 09.35	Keynote 1: Jonathan Gressel (Weizmann Institute of Science, Israel) *Lessons learnt from the history of developing herbicide resistant crops for control of parasitic Orobanchaceae*
09.35 – 10.10	Keynote 2: Frank Chidawanyika ICIPE, Kenya *Striga control and management using push-pull technology*
10.10 – 10.35	Peter Lueth (Toothpick Company Ltd., Kenya) *The Toothpick Project – Seed Treatment to Control Striga hermonthica*
10.35 – 11.05	**Coffee break**
11.05 – 11.30	Amit Wallach (Newe Ya'ar Research Center, Israel) *Management of Phelipanche aegyptiaca in cabbage (Brassica oleracea var. capitate)*
11.30 – 11.55	Muhammad Jamil (KAUST, Saudi Arabia) *Advances in suicidal germination technology for effective Striga control*
11.55 – 12.20	Admire I. T. Shayanowako (University of KwaZulu-Natal, South Africa) *Combining ability and gene action controlling yield, yield components and Striga resistance among FOS compatible and Striga resistant maize genotypes*
12.20 – 12.45	Esra Cignitas (Batı Akdeniz Agricultural Research Institute, Antalya, Turkey) *Comparison of the effect of tomato root extracts obtained by two different extraction methods on the germination of broomrape at four different temperatures*
12.45 – 14.00	**Lunch**
	Session VII: Parasitic Plants-Host-Microbe Interactions **Steven Runo (Kenyatta University, Kenya)**
14.00 – 14.25	Benjamin Thiombiano (University of Amsterdam, Netherlands) *The struggle for food: metabolomics uncovers chemical signals in sorghum that can help to combat the parasitic weed scorch, Striga*
14.25 – 14.50	Lucie Poulin (UFR Science and Technology, France) *Branched broomrape – rapeseed interaction mediated by microbial activity*
14.50 – 15.15	Lisa Martinez (Nantes Université, France) *Soil suppressive microbiota in plant-parasitic interaction between Phelipanche ramosa and Brassica napus in western France*
15.15 – 15.40	Yongge Yuan (Taizhou University, China) *Dodder parasitism limited the effect of arbuscular mycorrhizal fungi on litter decomposition*
15.40 – 16.15	Henry S. Nzioki (KALRO, Kenya) *Preliminary studies on response of Kichawi Kill™ to yields and incidences of striga-resistant and striga-tolerant maize varieties in Kenya*

16.15 – 16.45	**Coffee break**
	Session VIII: Phylogeny and Evolution of Parasitic Plants **Stefan Wanke (Technische Universität Dresden, Germany)**
16.45 – 17.10	Keynote speaker 1: Susann Wicke (Humboldt University of Berlin, Germany) *Deep phylogenetic discordance hampers reconstructing the eco-evolutionary paths of the Broomrape family (Orobanchaceae)*
17.10 – 17.35	Keynote speaker 2: Emily Bellis (Arkansas State University, USA) *Host-specific selection across the Striga hermonthica genome*
17.35 – 18.00	Matthias Jost (Technische Universität Dresden, Germany) *Phylogeny and and comparative plastome evolution of Hydnoraceae*
18.00 – 18.25	Joel Masanga (Kenyatta University, Kenya) *The Exigent threat of Dodders in Eastern Africa*
18.25 – 19.00	**Keynote: Lytton Musselman (Old Dominion University, USA)** *IPPS and conferences history*
19.00 – 19.30	Poster awards and honoring of IPPS members
19.30 – 21.00	**Dinner**
21.00 –	Bar conference centre open
FRIDAY JULY 8	
05.30 – 12.30	Nairobi National Park
13.20 – 14.20	Lunch /departure

ABSTRACTS

I. Host resistance

Keynote 1

Changes in the composition of the strigolactone blend exuded by the host reveals their biological significance

Harro Bouwmeester, Changsheng Li, Yanting Wang, Lemeng Dong

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The Orobanchaceae are obligate root parasitic plants that are completely dependent on a host for the completion of their lifecycle. To minimise the risks of this dependency, these parasitic plants use chemical cues secreted from the roots of their host to synchronise their development with their host, such as germination stimulants and haustorium inducing factors. Strigolactones are the major germination stimulants for many of the orobanchaceous root parasitic plants, but have also been demonstrated to be a plant hormone – inhibiting shoot branching - and a rhizosphere signal for symbiotic arbuscular mycorrhizal (AM) fungi. Intriguingly their role as branching inhibiting hormone works as a visible marker for the amount of strigolactones produced: genetic variation in branching (or tillering) correlates with strigolactone exudation and in both pea and rice highly branched genotypes are less infected by root parasites. The multiple roles that the strigolactones have with positive as well as negative effects for the host may well be the evolutionary driving force behind the large structural diversity in the strigolactones, with by now over 30 structures identified across the plant kingdom and many more of which the structure still needs to be elucidated. Several studies have shown that different strigolactones have different activity in different biological processes, such as germination of parasitic plant seeds. However, the *in vivo* biological relevance of the structural diversity in the strigolactone blends exuded by plants is largely unknown. To further unravel the evolution and importance of this structural diversity, knowledge of their biosynthesis and perception by the parasitic plants is required. We use several approaches to elucidate strigolactone biosynthesis, such as RNAseq and coexpression analysis, and heterologous reconstitution of entire strigolactone biosynthetic pathways in *Nicotiana benthamiana*. Using this approach, we identified a number of new strigolactone biosynthetic genes in tomato, rice and maize. In the latter we show that differences in expression of one of these new genes result in changes in the strigolactone profile and, consequently, a strong decrease in Striga germination and infection. With these biosynthetic genes we can engineer plants with altered strigolactone profiles in which we can study the biological consequences of these changes and its potential to create resistant crop genotypes. The progress in our understanding of the strigolactone biosynthetic pathway and the biological importance of the structural diversity in the strigolactones will be discussed.

Keywords: strigolactones, structural diversity, Striga, germination, resistance

Keynote 2

Discovery and deployment of molecular markers for resistance to *Striga gesnerioides* in cowpea

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The parasitic weed [*Striga gesnerioides* (Willd.) Vatke] is a principal pest of cowpea [*Vigna unguiculata* (L.) Walp.] that severely hinders its production in West and Central Africa. Multiple races of *S. gesnerioides* exist across the cowpea growing areas of the sub-region. Screening and breeding efforts have resulted in identification of a few resistant sources and some race specific genes underpinning resistance have been detected, yet deployment of associated markers in breeding is still limited. Here, we utilized a 52K cowpea iSelect single nucleotide polymorphism (SNPs) to decipher genomic regions underneath striga resistance and explore marker conversion for easy deployment. A minicore panel consisting of 365 cowpea accessions was phenotyped for reaction to *S. gesnerioides* across two locations in Northern Nigeria. The accessions displayed clear genotypic differences in response to *S. gesnerioides* attack, allowing detection of resistant lines. A genome-wide scan uncovered a major gene signal on chromosome Vu11 and three minor regions on chromosomes Vu02, Vu07 and Vu10 and, represented by the peak SNPs 2_32324*, 2_27836, 2_32524* and *2_42259* respectively. The candidate genes proximal to these regions included *Vigun11g014000.1*, *Vigun02g188800.1*, *Vigun07g249100.1, and Vigun10g001200.* The associated SNPs have been successfully converted into Kompetitive Allele Specific PCR (KASP) assays for validation and disposition for routine striga resistance breeding in cowpea.

Keywords: striga, cowpea, SNPs, genome-wide scan, candidate genes

Gamma ray-induced resistance to *Striga hermonthica* into Burkina's Sorghum landraces

Yonli Djibril[1*], Dao Karim[2], Nikiema M. Philippe[1], Sawadogo Assetou[3], Ouédraogo Margueritte[1,2], Traoré Hamidou[1], Sawadogo Mahamoudou[2], Jankuloski Ljupcho[4], Hingane J. Anupama[4], Mukhtar Ali Ghanim Abdelbagi[4]

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Sorghum bicolor (L.) Moench. is the staple crop in terms of cereal production and per capita consumption in Burkina Faso. Sorghum production is highly affected by *Striga hermonthica* attacks leading to yield losses

ranging 35 - 40% in 1.3 million ha of infested fields. The methods most commonly used by farmers to control *Striga* are cultural practices that seldom lead to long-term reductions in *Striga* populations. This study aims at inducing *Striga*-resistance into preferred Sorghum landraces that are *Striga*-sensitive with low yielding. Dry seeds of three Sorghum landraces Kb, RSOE15 and S29 were irradiated with 300, 350 and 400 Gy. The irradiated seeds and controls were sown. M1 panicles were harvested, and planted as M2 panicle-to-one progeny. M2 plants were selected and advanced to M3 using pedigree selection method using plant vigor, tiller number, early maturity and grain and panicle types as selection criteria. M3-M5 populations were screened to *Striga* in rain-fed cropping conditions. Sorghum hills were artificially infested with 10^3 viable *Striga* seeds hill^{-1} at the planting. Screenhouse test was conducted to confirm Sorghum phenotype observed in field while Agar gel and rhizotron bioassays were carried out on the putative resistant lines to highlight distinctive *Striga*-resistance mechanisms in Sorghum mutants. Field screenings revealed induced genetic variability among mutant lines and 52 lines significantly reduced the number of or exhibited good grain yield performance despite the infection by *S. hermonthica* ecotype from Burkina Faso. For verification purpose, all 52 M6 mutant lines including 16, 28 and 8 lines respectively generated from Kb, RSOE15 and S29 Sorghum landraces, were pot-screened to *Striga* in screenhouse conditions. Screenhouse experiments confirmed *Striga*-resistance in four mutant Sorghum lines leading to characterize four forms of resistance mechanisms. Further studies will enable to pyramid these resistance mechanisms in one genotype for more sustainable resistance.

Keywords: Sorghum, induced mutation, *Striga*-resistance, resistance mechanisms

Genetic analysis of *Striga hermonthica* resistance in pearl millet through generation mean analysis

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Striga (*Striga hermonthica* [Del.] Benth.) is a noxious parasitic weed causing substantial yield loss or crop failure in pearl millet and major cereal production systems of Sub-Saharan Africa (SSA). Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a nutritionally rich under-utilized crop species, and *Striga* species severely curtail its potential yield. Host plant resistance is one of the most economical and sustainable integrated control strategies for low-input pearl millet farming systems in Burkina Faso. Knowledge of the genetic basis of *Striga* resistance is fundamental for parental selection and trait integration in resistance breeding programs. The objective of this study was to determine the gene action and inheritance of *S. hermonthica* (*Sh*) resistance in newly developed pearl millet populations to guide selection and genetic advancement. Bi-parental crosses were derived from contrasting pairs of pearl millet lines 108IP-10579 (*Striga* resistant)/67IP-8129 (*Striga* susceptible), and 2IP-3098 (*Striga* resistant)/81IP-8786 (*Striga* susceptible). The two sets of parental lines, and their subsequent F_1, F_2, and backcross generations were evaluated under greenhouse and *Sh* infested field conditions using a randomised complete block design with three replications. Data on the number of *Striga* counted at 60 (SN60) and 80 (SN80) days after planting were collected and subjected to the generation mean analysis (GMA) procedure and the area under the *Striga* number progress curve (ASNPC) was computed. The analysis of variance showed significant ($P < 0.001$) differences among the breeding populations across the two sets for the assessed *Striga* parameters. *Striga* resistance and ASNPC in the pearl

millet populations are conditioned by multiple genes. Duplicate gene action controlled the inheritance of the number of emerged *Striga*. Unique F_2 individuals with *Striga* resistance were selected from the two sets for genetic advancement through recurrent selection methods for pearl millet variety development by integrating desirable agronomic and farmer-preferred traits.

Keywords: area under *Striga* number progress curve, gene action, generation mean analysis, pearl millet, *Striga hermonthica*, trait inheritance.

Breeding of maize in Kenya with resistance to *Striga hermonthica* L.: Challenges and Opportunities

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Striga hermonthica (Striga) is a widespread parasitic weed that causes considerable cereal crop losses in eastern Africa. Yield losses can reach 70 to 100% if susceptible cultivars are planted under severe infestation. In Western Kenya, *Striga* weed resistant maize landraces can be traced back to two populations, Kitale Synthetics of *Kenya Flat Whites* and *Ecuador 574* pioneered by earlier Kitale breeders. Additional mid-altitude landraces and open pollinated varieties (OPVs) such as *Ababario* and *Jowi* have also been developed from the two genotypes through diversifying selection. Inbred lines developed from these OPVs have good potential as *Striga* resistant hybrid parents, especially because they tend to segregate or occasionally mutate into two complementary groups that could be exploited for hybrid production. However, there are currently impediments to deploy these genotypes for sustained genetic gain owing to the narrow genetic diversity and lack of consistent and clear way of grouping the tropical lines into heterotic groups. We propose a novel method of classifying Kenyan mid-altitude flint maize into a complementary heterotic group. This method is based on the ability (or lack thereof) of the test line to dilute the purple coloration of the tester line in F_1generation. These inbred tester lines are then used to assign genotypes into complementary heterotic groups, thereby assigning related lines in opposite or same heterotic groups. Our breeding efforts resulted in the release of *Striga* resistant hybrid variety, Maseno EH 14, which is a three-way cross variety with yield potential of 8 to 9 tons/ha. The proposed method of hybrid development will enable maize breeders to assemble diverse lines into two complementary groups for more efficient selection of single cross hybrid parents. This method also promises to facilitate the development of new *Striga* weed resistant maize varieties with yield potential that can almost double the current yields.

Genomic characterization of sunflower resistance to sunflower broomrape (*Orobanche cumana*)

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The sunflower broomrape (*Orobanche cumana* Wallr.) is considered a major threat for sunflower cultivation across Europe, the Mediterranean region, and Asia, leading to sever yield losses. To control broomrape infestation, several herbicides and crop management approaches are commonly practiced. However, on the long term, breeding for resistance to broomrape remains the most efficient and environmentally-friendly approach. Many resistant sunflower cultivars were developed over the years, yet the broomrape has a remarkable ability to evolve and overcome the resistance mechanisms. Thus, new genetic resources of resistance are necessary. Here I will focus on the genomic basis of sunflower resistance to broomrape. To address this, the sunflower association mapping (SAM) population comprised of 287 accessions was screened for broomrape resistance in a high-throughput system based on image processing procedure allowing to quantify and characterize the mode of resistance. The results obtained in the high-throughput system were evaluated under field conditions with a representative panel of 77 accessions which were characterized as susceptible, post haustorial-resistant, and pre-haustorial resistant were selected in accordance with the results obtained in the high-throughput system. The 77 genotypes were grown in randomized block design with two treatments: broomrape infested, and non-infested plots. Phenotypic traits were recorded along the growing season and indicated that the high-throughput system well predict the response of sunflower to infestation under field conditions. Thus, a Genome-wide association analysis was conducted to identify QTLs and candidate genes that underlie broomrape resistance based on the high-throughput screening. Significant associations between genomic regions and the response to broomrape infestation were detected on chromosomes 1, 4, 5, 7, 9, 11, 16 and 17 which support previous findings and provide new candidate QTLs for broomrape resistance in sunflower.

Heinz-resistant tomato cultivars exhibit a lignin-based resistance to field dodder (*Cuscuta campestris*) parasitism

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Cuscuta species (dodders) are agriculturally destructive parasitic angiosperms. These parasitic plants use haustoria as physiological bridges to extract nutrients and water from hosts. *Cuscuta campestris* has a broad host range and wide geographical distribution. While some wild tomato relatives are resistant, cultivated tomatoes are generally susceptible to *C. campestris* infestations. However, some specific Heinz tomato hybrid cultivars exhibit resistance to dodders in the field, but their defense mechanism was unknown. Here, we discovered that the stem cortex in these resistant lines responds with local lignification upon *C. campestris* attachment, preventing parasite entry into the host. *LIF1* (*Lignin Induction Factor 1*, an *AP2*-like transcription factor), *SlMYB55*, and *CuRLR1* (*Cuscuta R-gene for Lignin-based Resistance 1*, a *CC-NBS-LRR*) are identified as factors conferring host resistance by regulating lignification. *SlWRKY16* is upregulated upon

C. campestris infestation and acts as a negative regulator of *LIF1* function. Intriguingly, *CuRLR1* may play a role in signaling or function as a receptor for receiving *Cuscuta* signals or effectors to regulate lignification-based resistance. In summary, these four regulators control the lignin-based resistance response in specific Heinz tomato cultivars, preventing *C. campestris* from parasitizing these resistant tomatoes. This discovery provides a foundation for investigating multilayer resistance against *Cuscuta* species and has potential for application in other essential crops attacked by parasitic plants.

Keywords: *Cuscuta campestris*, *Solanum lycopersicum*, resistance, lignin, dodder, tomato

Combining post-germination striga resistance with improved host nutrition

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Parasitic weed *Striga hermonthica* siphons nutrients, metabolites and water from its host plant, causing severe yield losses in sub-Saharan Africa. The use of striga-resistant cultivars is considered to be a crucial component in integrated striga management. Studies also show that fertilisers play a key role in the reduction of striga-infection levels and crop performance. The combination of striga-resistant germplasm and targeted host-plant nutrition is therefore proposed to be a feasible and effective integrated striga management. Most research has focussed on the role (macro-) nutrients play in pre-germination striga resistance. Our understanding of the effect of host-plant nutrition on post-germination striga resistance is much more limited. In this study, we used rhizotron assays to determine the interaction between post-germination striga resistance and host-plant nutrition. Three sorghum genotypes were selected based on their mechanism of post-germination resistance (N13: mechanical barriers, Framida: hypersensitive response, IS9830: incompatibility reaction) and these were compared to a susceptible check (Ochuti). These genotypes were subjected to four different fertiliser treatments: 1. a lower-benchmark level (25% of recommended macro- and micro-nutrients), 2. an upper-benchmark level (100% of both types of nutrients) and two intermediates, i.e. 3. 100% macro + 25% micro, and 4. 25% macro + 100% micro-nutrients. Recommended levels of macro-nutrients, alone or in combination with micro-nutrients, generally reduced striga infection levels whereas with application of only micro-nutrients at a recommended level, the infection levels remained the same as with the lower-benchmark treatment. Across genotypes, fertiliser-induced reductions in infection levels were more pronounced on susceptible Ochuti, than on resistant genotypes. Histological analysis of striga attachments on the host roots showed impaired penetration of the striga haustorium on the host roots endodermis at the recommended levels of macro-nutrients, alone or in combination with micro-nutrients across the genotypes.

Keywords: sorghum, genotypes, post-attachment resistance, nutrients, rhizotron

African maize landraces reveal a novel mechanism of resistance to *Striga hermonthica*

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The parasitic weed *Striga* (spp.) is a major biotic constraint and a severe threat to subsistence cereal grain production (sorghum, maize, millet, etc.) in tropical and subtropical regions of Africa and Asia. Considerable efforts have been invested in breeding for *Striga* resistance in cereals, however, the level of protection achieved to date is incomplete and the resistance is mainly determined by the coexistence of several mechanisms controlled by multigenic and quantitative systems. Sources of *Striga* resistance in maize have been limited, albeit a maize inbred line (ZD05), derived from the wild maize progenitor *Zea diploperennis*, has been previously characterized for its significant tolerance to *Striga hermonthica* (striga) infestation. To gain insights into the molecular mechanisms underlying such resistance, we performed RNAseq profiling of the tolerant ZD05 and a susceptible (5057) maize genotype both under striga-infested and non-infested conditions, and at three different time points from attachment to the development of striga shoots. The transcriptome analysis revealed differential expression of genes involved in cell wall rearrangement and in secondary metabolism, and, unexpectedly, differential regulation of benzoxazinoid (BX) genes involved in the hydroxamic acids pathway (DIMBOA). Quantitative biological test of root exudates from susceptible and tolerant maize inbred lines was used to assess the effects of hydroxamic acids against striga. The resulting level of parasitization correlate with the DIMBOA content in roots with the resistant line showing higher concentration of this compound compared to the susceptible line. Furthermore, we proved that expression levels of BX-genes and hydroxamic acids concentration were inversely correlated to the number of striga sprouts per host plant, both in sister lines of a mapping population (ZD05x5057) and in genetically unrelated maize lines showing different levels of tolerance to striga. Finally, we confirmed the involvement of three BX-genes (bx1, bx2, bx6) of the DIMBOA pathway in striga tolerance through a loss of function experiment, by showing that BX-deficient mutant maize lines are more susceptible to striga infestation. These results indicate that ZD05 uses a polygenic mechanism of resistance based on multiple biochemical and physiological pathways to resist against striga infestation. The role of DIMBOA in the mechanism of resistance to striga in maize reported for the first time opens new opportunities for developing alternative control strategies including development of biotechnologically improved lines and rapid and accurate tools for selection of resistant maize lines in breeding programs.

Keywords: striga, maize, *DIMBOA, gene expression, allelopathy*

II. Parasitic Plant Biology and Ecology

Keynote

How and why broomrape lie and what they say

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Plants interact with surrounding organisms through ubiquitous chemical communication. Volatile organic compounds (VOCs), as secondary metabolites are the main channel for these talks. The aim of this study was to investigate the VOCs of a holoparasitic plant – Butterbur broomrape (*Orobanche flava*). Floral VOCs were collected using dynamic headspace analyses at 10 different localities in the Slovak Republic and subsequently analyzed using gas chromatography and mass spectrometry. At the same time, pollinator communities visiting the individual broomrapes were observed. We found a clear difference in the floral phenotype and in the composition of pollinators between the lower and higher mountain valleys. New rarely described VOCs and almost unknown pollinators of *O. flava*, which occurs in the natural ecosystems of the Slovak Republic will be presented. We discuss significant variations in VOCs and the intraspecific variability of floral scents. Clear differences in the floral scent chemistry and the corresponding grouping of pollinators between broomrapes in different regions will be discussed. How and why broomrape is telling the truth and what it says will be discussed.

Keywords: parasitic plants, Orobanchaceae, *Orobanche flava*, pollinators, flower volatiles

PUSH-PULL APP: A case study of using ICT to enhance the adoption of Striga control interventions among small scale farmers in Africa

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Africa's agricultural sector has currently been characterized by strong demand for Internet~~;~~ Communication, and Technology (ICT). This demand, when fully harnessed, has the potential of enabling farmers to overcome major agricultural production challenges such as Striga infestation; in Africa. Using Internet of Things (IoT) and Hypertext preprocessor (PHP) software approaches, a mobile application was created to enhance the adoption of Push-Pull technology, a Striga-control farming innovation that was developed by ICIPE Kenya and

its partners. The app was designed with the ability to operate offline and with visual and audio properties. It was also programmed with simplified English grammar, and 2D illustrations to enhance usability and other user-friendly features. The high app impressions, downloading, and user bouncing rate as well as positive reviews of this mobile application after being availed to farmers indicated that ICT is one of the effective strategies in accelerating the adoption of farming innovations.

Keywords: Push-pull technology, Push-pull App, Striga, IPM, Internet of Things, Offline

Hydraulics and functional aspects of Neotropical Loranthaceae woods

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Loranthaceae is one of the largest parasitic plant lineages, gathering the largest number of genera in the order Santalales and comprising both root and stem parasites. Although both obtain nutrients directly from their host's vascular system, there are some differences in conditions in which the nutrients are found depending on the parasitized organ. In order to the flux of water from the roots towards the leaves to happen, plants establish a negative water potential gradient in their xylem. Therefore, Leaf water potential is lower than stem's (more negative) which is lower than root's water potentials This means stem parasites have more difficulty to obtain water and nutrients from their hosts, needing to operate under more negative water potentials to do so, having greater risk of developing hydraulic failures such as embolism. Because of that, we expect to find differences in both wood anatomy and hydraulic conductivity between root parasites and stem parasites in this family. We selected 13 South American genera of Loranthaceae for wood anatomy and theoretical hydraulic comparison, including the early divergent genus *Gaiadendron* (root parasite tree) and many different genera of mistletoes including different types of haustoria and plant sizes. All samples were sectioned using classical anatomy techniques and analyzed with light microscopy. Quantitative anatomy measurements were taken from the sections and used as basis for the calculations of the hydraulic properties. Mistletoes showed some classically xeromorphic-associated features such as thicker vessel and fibre walls, more organized axial parenchyma with very lignified cell walls and wider rays. We also found that *Gaiandendron* had greater average vessel diameter and hydraulic weighted vessel diameter (HWD) than most mistletoe species, but lower vessel density. According to Hagen- Poiseuille's law of laminar flow in capillary tubes, the water transport efficiency scales to the fourth power of the conduit's width, thus we expected to find the specific conductivity of *Gaiadendron* to be much higher than the conductivity found for mistletoes. As expected, *Gaiadendron* had in general higher conductivity (Kps) than most mistletoes, although there was considerable variance between species. Surprisingly, *Tripodanthus* the greatest values of conductivity al all species, surpassing by far all other mistletoe species and contradicting the original prediction. Furthermore, mistletoes generally had larger pit chambers and pit fractions than *Gaiadendron*, what could indicate more permeable and efficient intervascular water transport. This, at least in theory, should lower the overall resistivity of the main bottleneck in the system. Our results support the hypothesis that the change of habit from root-parasitism to stem-parasitism was accompanied by a drastic change in wood organization to endure the harsher conditions (such strong water deficit and low water potential), acquiring narrower and less conductive vessels, classically associated with water deficient environments.

Keywords: mistletoe hydraulic architecture, xylem hydraulics, functional wood anatomy, Santalales

Hyperspectral imaging facilitates early recognition of *Orobanche cumana* below-ground parasitism on sunflower under field conditions

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Sunflower broomrape (*Orobanche cumana*) is a root parasitic weed that limits sunflower yield in large areas of Europe and Asia. Early detection of the parasite can facilitate site-specific control of this weed. However, most of its life-cycle takes place in the soil sub-surface and by the time that *O. cumana* shoots emerge, the damage to the crop is irreversible. The main aim of our study is to evaluate the potential use of hyperspectral imaging for the early detection of parasitism by monitoring changes in spectra of the host plants. A field experiment was conducted on infested and non-infested sunflower plants, imaged by a ground-based hyperspectral camera in two early parasitism stages that are relevant for herbicide application. A logistic regression model was used to classify infected and non-infected plants, at early sub-surface parasitism stages, 31 and 38 days after sunflower planting, with 76 and 89% accuracy, respectively. A partial dataset, containing only 10 spectral bands of the hyperspectral dataset gave 73 to 82% accuracy, suggesting the potential of multi-spectral sensors for the detection task. Sampling pixels from specific sunflower leaf segments improved the classification compared to non-specific sampling. This study thus contributes to establishing a basis for future development of site-specific weed management of *O. cumana* as well as of other broomrape species.

Keywords: broomrape, site specific weed management, classification

Desert hyacinths: An obscure solution to a global problem?

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'Desert hyacinths' (Cistanche) are a remarkable genus of parasitic plants, some of which are traded widely for herbal medicine or have historical local importance as food. Despite their importance, little is known about the biology of most species and their taxonomy remains confused, hindering identification. In the context of a global desertification crisis, there is potential to expand cultivation of Cistanche beyond China, as an ancillary crop alongside vegetation planted to halt land degradation. To realise this potential and to monitor trade to control any possible unsustainable harvesting of threatened wild populations, robust taxonomy informed by both morphological and molecular data is needed.

III. Genes and Genomes

Keynote 1

A Report on the Analysis of the *Orobanche cumana* Genome

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Orobanche cumana is an interesting species due to its specific affinity for sunflower hosts and economic impact. The relatively recent history of *O. cumana* and sunflower sympatry, along with the evolution of virulence races of the parasite suggests that it is capable of rapid evolution. The genome sequence of *O. cumana* is revealing insights into the parasite and its interactions with its host. By combining first-generation PacBio sequencing and optical mapping, the assembly is of high quality, with 763 scaffolds and an N50 of 67 Mb, 85% of the estimated 1.5 Gb genome is assembled into 19 chromosome-scale scaffolds. Highlights of the analyses include a substantially reduced capacity for metabolism, consistent with enhanced dependence on the host. Additionally, sampling and resequencing of populations from Europe to China indicates the existence of five major groups of *O. cumana*. The assembly also provides a context for understanding the evolution of key parasitism genes, for example the *KAI2d* (*KARRIKIN INSENSITIVE 2*) genes that mediate perception of germination stimulants prove to be mostly clustered within 3 Mb of each other. Their arrangement in tandem duplications suggests opportunities for the evolution of sunflower-specific germination stimulant receptors. Insights into mechanisms of evolution, horizontal gene transfer, and potential for *O. cumana* miRNAs to act as virulence factors during parasitism will be discussed.

Keywords: *Orobanche cumana*, sunflower, genome evolution, populations, KAI2d.

LE peptides in the Interaction of Parasitic and Host Plants

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To infect host plants, all parasitic plants develop a multicellular feeding organ, the haustorium. Haustoria develop from proto-haustoria to parasitically active mature haustoria. The transition from proto- to mature haustoria is not well-understood. Previous studies in the root parasite *Phtheirospermum japonicum* (Pj) and its host *Arabidopsis thaliana* (At) revealed that the maturation of haustoria coincides with the transcriptional up-regulation of *Subtilisin-like proteases* (*SBTs*) and requires SBT activity in nascent haustoria. We discovered that SBT substrates CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) pre-propeptide encoding genes are co-expressed with *SBT*s in Pj, *Striga hermonthica* and *Striga asiatica*. We show with promoter-reporter constructs that *PjSBT1.2.3* and *PjCLE2.1* are co-expressed during haustorium development in different domains of the Pj haustorium. Further, PjSBT1.2.3 cleaved proPjCLE2.1 to release the bioactive PjCLE2.1 peptide in vitro. By initiating different receptor-mediated signal transduction cascades, highly mobile CLEs regulate many different processes in plants. Application of the mature PjCLE2.1 peptide impaired host root growth in At. In Pj, PjCLE2.1 induced proto-haustorium formation. Consistently, CRISPR/Cas9 mediated knock-outs of *PjCLE2.1* formed relatively fewer proto-haustoria than controls. In conclusion, PjCLE2 represents a new class of endogenous Haustorium-Inducing-Factors (HIFs) that we aim to further elucidate on a molecular level in ongoing experiments.

Keywords: Phtheirospermum, CLE, SBT, haustorium, parasite-host crosstalk, Haustorium-Inducing-Factors

Weighted gene co-expression networks explain Striga resistance in sorghum

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Striga resistance in sorghum is believed to be due to diverse mechanisms. However, molecular mechanisms of Striga resistance remain unclear. We performed a comparative transcriptome on five genotypes of sorghum exhibiting diverse Striga resistance mechanisms. using weighted gene co-expression network analysis (WGCNA). We found that out of a total of 43 significant modules, 7 modules were enriched for pathways involving typical Striga/pathogen resistance. General categories of Striga resistance pathways were i) pathogen triggered immunity, ii) cell wall enhancement synthesis of secondary metabolites, and iii) hypersensitive response. Importantly, some modules were enriched for specific cell wall enhancements. For example, cellulose, pectin, and flavonoid biosynthesis. Additionally, some modules were specifically enriched in some genotypes allowing WGCNA to clearly delineate mechanisms of Striga resistance in the sorghum genotypes studied. Based on these results, Striga resistance mechanisms in the sorghum genotypes studied can be summarized as follows: IS1814 has primarily cell wall enhancement mechanisms including cellulose, pectin, lignin, and flavonoid biosynthesis. The main defense mechanisms in IS9830 is pathogen triggered immunity that leads to cell wall enhancement though cellulose, pectin and flavonoid biosynthesis. In IS14963, the primary defense is lignin, flavonoid, and a string HR. N13 appear to have multiple defenses based on the cell wall but not to the high extent of the other genotypes. IS41724 has multiple layers of cell wall defense (cellulose, pectin, and lignin) as well as – albeit to a less extent, HR.

Keywords: Striga resistance mechanisms, Sorghum

Keynote 1

Structural and signaling diversity of haustoria in Orobanchaceae parasitic plants

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Parasitic plants in Orobanchaceae cause severe agricultural problems in worldwide, but the molecular basis for parasitism remains poorly understood. The haustorium, an organ for host invasion and connection, has been termed as “the essence of parasitism”. Orobanchaceae parasitic plants form haustoria on its own roots and invade host roots. The haustorium structures can be categorized to terminal and lateral; the former is formed by deformation of radicle tips and observed in obligate parasitic plants, such as *Striga* spp. and *Phelipanche*, or *Orobanche* spp. The latter is formed at lateral parts of roots without disturbing root tip meristematic activity, and mostly observed in facultative parasitic plants, including model species such as *Phtheirospermum japonicum* or *Triphysaria versicolor*. To understand molecular mechanisms for haustorium formation, we use two species in Orobanchaceae, *Striga hermonthica* and *Phtheirospermum japonicum*. Comparison of two species suggested the diversity of signaling and structures of haustoria. The initiation of haustoria is recognized with formation of prehaustoria, bump-like early haustorial structures with divided and expanded root cells. The prehaustoria are induced by haustorium-inducing factors (HIFs), low molecular weight compounds present in the rhizosphere. To date, host-derived phenolic acids and quinones have been known as HIFs for *S. hermonthica* and *P. japonicum*, and it has been reported that the plant hormone cytokinins can induce prehaustoria in the obligate holoparasite *Phelipanche*. We tested the prehaustorium-inducing activities of cytokinins and found that cytokinins induce prehaustoria in *Striga*, but not in the facultative parasite *P. japonicum*. To understand signaling pathway of HIFs, we screened a chemical library to isolate novel inhibitors of prehaustorium formation. Investigation with various inhibitors suggested that the induction of prehaustoria by cytokinins in *Striga* follows a signaling pathway that is partly different from that of quinones. These results suggest the complex and robust signaling pathways for haustorium initiation. Furthermore, we have reconstructed the three-dimensional structures of haustoria of *P. japonicum* and *S. hermonthica* by stacking thin-section images of the haustoria. The resulting 3D structures indicate different xylem connection structures of two species. *Striga* inserts oscula into the host xylem vessels to form direct xylem connections, while *P. japonicum* xylem cells do not form oscular structure, but surround the host xylems to establish pore-to-pore connection. Inside *Striga* haustoria, donut-shaped hyaline body cells were observed, while *P. japonicum* haustoria possess paratracheal parenchyma cells adjacent to a xylem bridge. These structural differences may reflect aggressiveness of parasitic species or evolutional diversity.

Keywords: haustorium, haustorium-inducing factor, Orobanchaceae, xylem bridge, hyaline body

Minimum Synthetic Inducible Promoters for Hot Response to Parasites

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One approach to generating parasitic plant resistant hosts is to engineer the host to express a gene that would stop parasite growth soon after haustorial penetration. While much work is being done to identify effective resistance genes, little has been done to identify promoter regions to drive expression of such genes. We previously studied gene expression in *Arabidopsis thaliana* parasitized by *Cuscuta*, and set out to develop a parasite-inducible promoter. As a starting point, we searched RNAseq data to find *A. thaliana* genes that were induced by the parasite, and then identified functional elements in the regulatory regions of these genes. Rather than clone a multi-kb long regulatory region, we aimed to construct a minimum synthetic inducible promoter (MSIP) comprising elements identified from the native inducible promoters. We designed seven MSIPs containing different combinations of transcription factor binding sites and fused them independently to the β-glucuronidase (GUS) reporter gene, and generated transgenic *A. thaliana* lines. GUS staining was performed on the *A. thaliana* lines with *Cuscuta* attachments. Most of the MSIPs were induced by *Cuscuta* but varied in their expression in non-parasitized tissues. The MSIP with the greatest parasite-specific expression contained stress-inducible transcription factor binding sites. Furthermore, roots of *A. thaliana* parasitized by *Phelipanche aegyptiaca* showed a similar MSIP induction pattern in roots, suggesting that parasitic plants trigger common plant transcriptional machinery. These MSIPs represent valuable tools for driving expression of parasite resistance genes.

Keywords: *Cuscuta*, parasitic plant, minimum synthetic inducible promoter, transcription factor binding sites, parasite specific-expression, Phelipanche

Gene expression in finger millet within six days of *Striga hermonthica* infection

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Finger millet is an important cereal crop in Africa and southern parts of India. One of the key biotic constraints to finger millet production in Africa is the parasitic weed, *Striga hermonthica*, which causes up to 100% crop loss. To understand the molecular mechanism involved in finger millet–*Striga* interactions, we performed transcriptome profiling of finger millet infected with *Striga* alongside controls at three different time point. The roots of two *Striga* resistant (LESK10 and OKHALE1) and one susceptible (GBK029646A) genotype were infected with pre-germinated *Striga* and fresh leaf tissues of inoculated finger millet collected at 1 (24 hours), 3 and 6 days for RNA sequencing. A chitinase was the most highly upregulated pathogenesis-related protein in all genotypes within 24 hours of inoculation. Major disease resistance proteins, including NBS-LRR

class family proteins, were induced within 24 h of inoculation in OKHALE1 and LESK10, most of these genes were not induced in the susceptible genotype within the same time period. Other classes of genes that showed differences in their induction between resistant and susceptible genotypes included cytochrome P450, transcription factors, detoxification and signaling genes. The top enriched GO terms were electron transporters, chlorophyll binding and proton exporting ATPase activity. KEGG pathways that were enriched in resistant varieties included plant hormone signal transduction, phenylpropanoid biosynthesis and MAPK signaling pathway. *MAX1*, a cytochrome P450 has been reported to catalyze strigolactone production, which is required for *Striga* germination and its establishment in the host. Most of the putative candidate genes identified in the current study are consistent with previous analysis in other crops and provides a novel opportunity for more studies in the crop. Further exploration and quantification of these putative *Striga* defense response genes will improve our understanding of finger millet - *S. hermonthica* interactions and facilitate the development of more resistant genotypes.

Keywords: Strigolactone, MAX1, cytochrome P450, NBS-LRR, *Eleusine coracana*

A role for lateral haustoria in obligate parasites

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Haustoria, multicellular organs connecting parasites to host vasculature, are an essential and unique feature of plant parasitism in flowering plants. Different degrees of parasitism exist, ranging from serendipitous exploitation of (facultative hemiparasites) to complete dependence on (obligate holoparasites) an available host plant, and examples of this variety can all be found within the Orobanchaceae family. Lateral haustoria develop predominantly from the root elongation zone, and can be numerous and/or short-lived. Terminal haustoria develop from the apical meristem of the radicle and are characteristic of most obligate parasites. In addition to their initial connection to a host root via a terminal haustorium, obligate parasites such *Striga hermonthica* (hemiparasitic) and *Phelipanche ramosa* (holoparasitic) also develop lateral haustoria at later developmental stages. The lateral haustoria of *S. hermonthica* have been shown to penetrate host vasculature and develop mature and differentiated tissue, indicating they may play an important role in addition to the terminal haustoria. However, lateral haustoria biology in obligate parasites is understudied, and the eco-evolutionary importance of lateral haustoria for obligate parasites is not known. To better understand the function and relevance of lateral haustoria within the Orobanchaceae, we have undertaken histological and molecular work of lateral haustoria from *S. hermonthica* and *P. ramosa* to specifically assess 1) the capacity of lateral haustoria to exploit host nutrients and 2) the structural and transcriptional differences between lateral and terminal haustoria within and between each species. Our study shows, among others, that late-stage lateral haustoria are essential for parasite fitness. By using a combination of ecological assays and omics-enabled molecular analysis, this work will bring novel information regarding the significance of secondary parasite-host attachments of obligate parasites.

Keywords: haustoria, secondary parasite-host attachments, *Striga*, histology, transcriptomics

Lignification occurs after perceiving haustorium inducing factors to facilitate prehaustorium induction in *Striga hermonthica*

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Notorious parasitic plants *Striga hermonthica* sense host-derived signaling molecules including quinones, monolignols, and flavonoids to induce penetrative organ, the haustorium. The molecular events triggered by these haustorium inducing factors (HIFs) in prehaustorium formation remain poorly understood. Here we show that HIFs induce tissue lignification at root tip for facilitating prehaustorium formation in *S. hermonthica*. To understand cell-type specific HIF response, we firstly used fluorescent monolignol probes as HIFs and found that monolignol probes, while inducing prehaustorium, rapidly incorporate into the outer-facing cell wall of the epidermis at the root tip, later covering the entire prehaustorial surface. Structure, activity and tissue localization relationships of monolignols reveal that while 3'-methoxyl group on the aromatic ring is required for prehaustorium induction it is dispensable for cell wall incorporation, indicating that recruitment and sensing of monolignols are regulated by different pathways. Each of three HIF categories, monolignols, quinones and flavonoids all induced lignification in *S. hermonthica* reflecting that lignification is an endogenous process converged at downstream in response to host signals. Genes involved in monolignol biosynthesis and lignin polymerization including RBOHs and peroxidases are indeed upregulated after exposure to HIFs and among these peroxidases play an important role in promoting prehaustorium induction. Our study uncovers the mechanism of parasitic plant organ development where cell wall modification acts as driving forces for integrating host plant signals.

Keywords: prehaustorium, monolignols, lignin, Striga hermonthica, haustorium inducing factors

IV. Molecules and Biochemistry

Keynote 1

New Insights in the role of canonical strigolactones and abscisic acid in Striga seed germination

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Striga hermonthica produces an exceptionally high number of seeds that remain viable in soil for more than a decade. Germination of theses seeds is induced by host-released germination stimulants, mainly the plant hormone strigolactone (SLs). There are two types, canonical and non-canonical, SLs, which are distinguished based on the presence of the ABC-ring in the former ones. However, the question whether the structural diversity is coupled with particular functions remains one of the most intriguing questions in SL biology. To address this question, we generated rice CRISPR-Cas9 rice mutants that lack canonical SLs, i.e. 4-deoxyorobanchol and orobanchol, characterized them with respect to morphological phenotypes related to SL-deficiency and investigated the potential of their exudates in inducing Striga seed germination. Our results indicate that canonical SLs are not important for rice tillering, but play a significant role in rhozospheric communication. In the second part of my talk, I will present our results on the role of abscisic acid in the conditioning and germination of Striga seeds, which indicate a role of this hormone as a further rhizospheric signal in Striga life cycle.

Keywords: Striga, Canonical Strigolactones, Seed Dormancy. Suicidal Germination

Cytokinins are new major compounds involved in seed germination of parasitic weeds

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Seeds of the root parasitic weeds broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) are fascinating integrators of rhizosphere signals and excellent models for studying hormonal crosstalks during seed germination. Indeed, seeds of these species must perceive allelochemicals to germinate, and they have adapted to sense up to picomolar ranges of those so-called 'germination stimulants'. Generic and ancestral compounds belong to the strigolactone family, although parasitic species can adapt to their preferred hosts by perceiving non-strigolactones compounds. Perception of germination stimulants triggers a decrease in abscisic acid (ABA). Because of the intimate link between strigolactones, ABA, and cytokinins (CK) in other developmental frameworks, such as during the regulation of shoot and root branching, we anticipate a major role for CK during the germination of parasitic weeds. Here, we demonstrate that natural and synthetic CK stimulate seed germination of the witchweed *Striga hermonthica*, while they inhibit that of the broomrapes *Phelipanche ramosa* and *Orobanche cumana*. A similar bioactivity is observed upon exogenous application of INCYDE, a plant growth regulator that inhibits the CK-degradation enzyme cytokinin oxidase/dehydrogenase (CKX). By combining pharmacology approaches with gene expression analyses, we demonstrate that ABA catabolism precedes CK signaling along seed germination in *P. ramosa*. Mass spectrometry analyses indicate that there are overall no significant changes in CK levels, at the exception of isopentenyladenine-9G (iP9G) that accumulates over germination time. Finally, structure-activity relationship analyses confirmed germination bioassays in that CK activity declines upon glycosylation. These results demonstrate that germination stimulants induce first the decrease in endogenous ABA levels then CK inactivation to fulfill germination in broomrapes. This work positions CK as promising natural compounds to fight against parasitic plants. Finally, the contrasted bioactivity of CK on broomrapes and witchweeds leads to question whether the acquisition of strigolactone dependency for germination is a unique event within the Orobanchaceae family.

Keywords: Orobanchaceae, seed, germination, cytokinin, strigolactone, antagonism

How to become a successful plant parasite: Insights into Strigolactone Signalling in Striga

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Infections caused by parasitic plants like Striga hermonthica (witchweed) dramatically reduce the yield of major food crops in Africa and pose a serious threat to food security on the continent. The first committed step of a successful infection is the germination of Striga seeds in response to a group of related small molecule hormones called strigolactones (SLs), which are emitted by host roots. Intriguingly, Striga seeds germinate underground, and seedlings develop without the need for light. I will present our recent advances into the mechanisms of SL signalling and the role of the SL pathway in Striga germination. Upon crystallizing an SL-bound ShHTL receptor, we detected a variety of conformational changes. Our structure-function analysis suggested a mechanism of how the products of SL hydrolysis are expelled. These results indicate the importance of hydrolysis in SL perception and subsequent signalling. In the second half of the presentation, I will discuss downstream components of the SL pathway and how this pathway can replace the light requirement for germination. We propose that the ability of SL to act as 'liquid light' could explain how Striga evolved its underground germination behaviour in response to a host signal.

Photosynthetic activity of *Cuscuta campestris*, influenced by gall formation by *Smicronyx* spp.

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Weevils of the genus *Smicronyx* (Coleoptera: Curculionidae) are gall-forming beetles, specialized as epiparasites on both root (*Striga hermonthica*) and stem (*Cuscuta* spp.) parasitic plants. Besides being regarded as a possible approach for biological control of the agriculturally important weed species, they also represent an intriguing study object because of their evolutionary adaptation to attack other parasites. During a period of five years (2017 – 2021) in Bulgaria, *Smicronyx* galls were found in over 10% of all studied populations of the introduced *Cuscuta campestris*, but not in any of the native *Cuscuta* species (*C. appoximata*, *C. epithymum* or *C. europaea*). They formed fusiform or irregular-shaped galls, approximately 1.5 cm long, with a clearly defined larval chamber, greenish inner cortex and yellowish outer cortex. Although abundant in all affected populations, no clear signs of any inhibition of *Cuscuta* growth were observed. Based on this, we aimed to characterize the apparent increase in photosynthetic activity in the galls, compared to non-photosynthetic stems. As expected, the gall and particularly the inner cortex showed significantly higher content of chlorophyll *b* and especially chlorophyll *a*. Various photosynthetic parameters, estimated by prompt chlorophyll *a* fluorescence (OJIP test) suggested photosynthetic activity, comparable, but lower than in a green plant (*Arabidopsis thaliana*), with a notable peak in the inner gall cortex and more efficient functioning of photosystem I (PSI). Blue-native/SDS PAGE separation of thylakoid complexes also showed higher abundancy of PSI and LHCII (light-harvesting complex II) components in gall cortices in comparison to the *Cuscuta* stem. Immunoblot detection of RuBisCO large subunit, however, did not confirm any significant increase in the galls. Furthermore, the increased activity of superoxide dismutase in the galls, but not other antioxidant enzymes, confirmed the increase of light-dependent photosynthetic reactions in these structures. Hydrolytic enzymes – α-amylases and especially proteases, also showed increased activity in the galls and particularly in the outer cortices. Based on these results we hypothesized that during the gall formation, the weevil larvae manipulate the otherwise non-photosynthetic plant to increase photosynthetic activity in the inner cortex, while the metabolically distinct outer cortex serves as a storage and mobilization structure for starch and proteins. In such a way, the galls are better fitted to ensure nutrients supply for the larvae, but these alterations are not necessarily detrimental to the parasitic plant itself.

Keywords: insect galls, *Cuscuta campestris*, photosynthetic activity, OJIP test

Structure-Activity Relationship of Aromatic Carbonyl Thioureas for Inhibition of *Orobanche minor* Radicle Elongation

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Trisaccharide planteose accumulated in the endosperm of *Orobanche minor* dry seed is hydrolyzed by an α-galactosidase, OmAGAL2, after perception of strigolactones (Okazawa *et al*., *J. Exp. Bot*., **73**, 1992–2004, 2022). The planteose metabolism is essential for promoting an early stage of germination (Wakabayashi *et al*., *J. Exp. Bot*., **66**, 3085–3097, 2015). Therefore, OmAGAL2 and its homologous α-galactosidases are possible targets for the control of root parasitic weeds. Several compounds were obtained as OmAGAL2 inhibitors by the screening of chemical libraries. It was revealed that several OmAGAL2 inhibitors retarded the radicle elongation in germinating *O. minor* seeds. Here, we focused on thiourea, **PI-28**, as a lead compound for the development of inhibitors with higher activities and conducted a structure-activity relationship study. Aromatic carbonyl thioureas were synthesized from phenols, 2-chloroacetamide, and aryl isothiocyanates in a two-step reaction. Twenty-six thioureas were synthesized and their effects on the radicle elongation

of *O. minor* germinating seeds were evaluated. As a result, thioureas derived from aryl isothiocyanates possessing Br or Cl atom at 4-position of the benzene ring exhibited potent inhibitory activities. The lengths of radicles of *O. minor* germinating seeds treated with some newly synthesized thioureas at 1 ppm (final concentration) were less than 20 % of those of non-treated germinating seeds, while **PI-28** at the same concentration reduced the radicle lengths to around 90 % of the control.

A meta-analysis of the effects of *Striga* control methods on maize, sorghum, and millet production

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Parasitic *Striga* weeds severely damage cereal crops in sub-Saharan Africa (SSA), leading to yield losses or crop failure in susceptible varieties. Several *Striga* control methods are recommended globally, including cultural practices, chemical herbicides, biological agents, and host resistance alone or combinations of these approaches (labeled as integrated *Striga* management (ISM)). A limited study compared the relative efficacy of the recommended *Striga* control methods or their combinations for ISM in cereal crop production in SSA. The objective of this paper was to undertake a meta-analysis and provide a detailed comparison of the *Striga* control methods in maize, sorghum, and millet production to guide *Striga* management. The study was conducted based on 66 research articles that reported on various control measures such as cultural practices, crop protection chemicals, biological agents, host resistance, and ISM. The following data were collected: grain yield (GY) response of the assessed crops, and *Striga* parameters such as damage rating scores (SDR) and emergence counts (SEC). Results showed that *Striga*-resistant maize varieties possessing major resistance genes displayed high mean yield values at 2053 kg ha^{-1} varying from 281 kg ha^{-1} to 6260 kg ha^{-1}, and reduced SDR at 4.7, varying from 2 to 7. Likewise, sorghum varieties with *Striga* resistance genes achieved greater GY with a mean yield response of 1738 kg ha^{-1}, varying from 850 kg ha^{-1} to 2162 kg ha^{-1}. A relatively low GY was achieved in maize and sorghum production when deploying ISM (e.g., cultural control + host resistance and host resistance + chemical herbicides), and chemical *Striga* control. Effective ISM and pre-and post-emergent herbicides have not yet been identified for effective *Striga* control and yield gains. *Striga* damage negatively affected GY in maize, as revealed by the significant correlation ($r=-0.36$, $P<0.001$) between GY and SDR. In maize, a relatively weak correlation was detected between GY and SEC ($r=0.003$, $P=0.96$). Sorghum GY was negatively correlated with SEC, although non-significantly ($r=-0.30$, $P=0.36$). Relatively few studies have evaluated *Striga* control methods in pearl millet and finger millet, limiting the opportunity for effective comparison. The study recommends SDR as the best selection criterion for improving GY performance in maize, while SEC and SDR are parameters of choice in sorghum selection programs for better GY under *Striga* infestation. Overall, the meta-analysis indicates that host resistance is the most effective method for controlling *Striga* infestation and boosting GY in maize and sorghum. There is a need to investigate the best combinations of the reported control methods to recommend an ISM package across target production environments of common cereals in Africa. This work was supported by the African Centre for Crop Improvement (ACCI) and the Organisation for Women in Science for the Developing World (OWSD).

Keywords: cereal crops, meta-analysis, Integrated *Striga* Management, ISM, *Striga* control methods, *Striga* parameters

V. Control and Management

Keynote 1

Lessons learnt from the history of developing herbicide resistant crops for control of parasitic Orobanchaceae

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My interest in parasitic weeds was piqued by a chance discussion with an eminent scientist who was looking for solutions. This eventually led to a solution: commercialization of imidazolinone-resistant maize in Kenya. *Lesson 1: When you have a seemingly intractable problem – discuss it with everyone, someone unexpected may have an answer.* As I dealt with herbicides, I asked whether these parasites made their own amino acids. I was crestfallen when he said that the leading text book claims they get their organic nitrogen from their hosts. Luckily, I found literature that countered the claim: tissue culture media of various Orobancaceae did not contain organic nitrogen and sub-lethal rates of glyphosate, a herbicide inhibiting aromatic amino acid biosynthesis, controlled a parasite on fava beans. *Lesson 2: text book claims without evidence can be wrong.* We hypothesized the resistant crop should have target site resistance to the herbicide, as metabolic resistance could at best provide short-lived control. *Lesson 3: as farmers do not want to continually reapply herbicide during a crop season, depending on metabolic herbicide resistance will rarely be a commercial success.* With hard pressure on three companies we were able to obtain 2 crops with 3 transgenic target-site resistances, and successfully showed the ease of *Phelipanche aegyptiaca* control. *Lesson 4: Despite the dire need, industry won't register transgenic crops for parasitic weed control, governments should pressure them to do this*. This lesson still holds; no one has tested South-African developed glyphosate resistant maize for *Striga* control. Luckily, I knew that mutant maize was being developed with resistance to acetolactate synthase inhibiting herbicides, and I personally visited the seed company doing the development, and the chemical company that wanted its release and requested samples, and was refused because Africa was not considered a potential market. *Lesson 5: Governments that need products for their farmers, should apply the needed pressure on industry.* When this maize was finally commercialized in the USA, we asked a colleague to purchase some and test if for the control if *Striga asiatica* in the USA, and to send seeds to Kenya for testing with *S. hermonthica*. The system worked well in both venues. The cost of herbicide from the primary manufacturer was such that we proposed seed treatment so that an order of magnitude herbicide could be used. Thus began a long collaboration with an international breeding organization. Together we modelled that *Striga* resistance would appear in five years, based on other weed resistances. This did not happen and we realized that because of the high concentration of herbicide in the rhizosphere, weeds would have to be homozygously resistant. *Lesson 6: With models it is easy to be wrong by a factor of million.* Despite using one generation of marker assisted breeding, it took nearly 15 years to backcross this single gene character into Africanized breeding lines. *Lesson 7. A local commercial breeding company, with a desire to market a product would have done this in less than half the time.* Despite the fact that no one had registered intellectual property rights for use of herbicide on the resistant maize, the developing group signed contracts with that organization which limited flexibility and raised costs to farmers. *Lesson 8: Why be holier than though to the detriment of the product?* When it became apparent that with insufficient early rains, there was some toxicity to the crop, and with heavy rains, the herbicide was washed away, we saw a slow release formulation might be better. Describing the problem to a colleague in the pharmaceutical industry came up with a solution – *see Lesson 1. The most important lesson comes from the satisfaction of helping growers conquer parasitic weeds.*

Keywords: target site herbicide resistance; parasitic weed control

Keynote 2

Striga control using the push-pull technology

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Push–pull technology is based on multi-cropping system developed by the International Centre of Insect Physiology and Ecology, Rothamsted Research (UK) and national partners for integrated pest, weed and soil management in cereal–livestock farming systems. Lepidopteran cereal pests are attracted to Brachiaria grasses trap plants (pull) and are repelled from the main cereal crop using a repellent legume intercrop (push), desmodium (Desmodium spp.). Desmodium root exudates effectively control the parasitic striga weeds [Striga hermonthica and Striga asiatica] by causing suicidal germination. Desmodium also improves soil fertility through nitrogen fixation, improved biomass and reduced soil erosion. These companion plants serve as fodder facilitating milk production. The current talk will discuss the development of PPT highlighting the mechanisms important for suppression of Striga and the promotion of soil health.

The Toothpick Project – Seed Treatment to Control *Striga hermonthica*

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Witchweed (*Striga hermonthica*) is a parasitic weed that causes high yield losses in maize on more than 200,000 ha in Kenya alone. Striga particularly affects maize, sorghum and millet on approximately 40 million farms across Africa. A new biological herbicide developed in Kenya called Kichawi Kill is able to control Striga effectively. The product was approved for use by the PCPB in Kenya in September 2021. It is self-produced in the villages using an inoculum provided by Toothpick Company Ltd. By using the product, yield increases of up to 100% could be achieved on Striga-infested areas. But the product has some disadvantages, which are a complicated production process, a very low storage stability and a high application rate. Additionally, the product has to be applied manually and therefore can only be used in maize production. For this reason, efforts have been made to formulate the antagonist, *Fusarium oxysporum* strain DSM 33471, as a powder and to use it as a seed treatment agent. The presentation deals with the production of the Fusarium spore powder, its properties, its application to the seed, and its herbicidal effect proven by two first field trials. The used *Fusarium oxysporum* strains has been isolated from a Striga plant in Kenya. The strain is producing the amino acids Leucine, Methionine and Tyrosine. These are responsible for a second mode of action apart from the disease-causing effect of the fungus. Whereas Leucine and Tyrosine have an herbicidal effect, Methionine is triggering the germination of the Striga seed in the soil. Additionally, the strain has been selected concerning its resistance toward the fungicide Captan, which is commonly used in Kenya to treat maize seed. Results of two field trials on the effect of seed treatment on *Striga hermonthica* are presented. According to these, by treating maize seed with *F. oxysporum* DSM 33471 on Striga-infested areas on 17 small-holder farms in the counties Bungoma, Kakamega, Siaya and Vihiga, yield increases of up to 89% have been observed. A second trial carried out by the Kenyan Agricultural and Livestock Research Organization (KALRO) showed a reduction of Striga plants of up to 93%.

Keywords: *Striga hermonthica*, *Fusarium oxysporum*, maize, biological herbicide

Management of Phelipanche aegyptiaca in cabbage (Brassica oleracea var. capitate)

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Phelipanche aegyptiaca, a member of the Orobanchaceae family, is a root parasitic plant that causes severe yield losses and is considered one of the main weeds in vegetable and field crops worldwide. After seed germination, and once established the vascular connections to the host plant roots, *P. aegyptiaca* becomes a major sink that draws nutrients, and water from the host resulting in crucial damage to the host plant. One of the most effective ways to manage P. aegyptiaca in the field is via herbicide application as previously was demonstrated for crops. The main objective of this research was to develop a protocol for the management of *P. aegyptiaca* in cabbage. In this work, two herbicides (glyphosate and ethametsulfuron-methyl) were examined via two application methods (foliage and the root rhizosphere) in laboratory and field conditions. The herbicides were tested for *P. aegyptiaca* control as well as for crop safety. in the laboratory *P. aegyptiaca* control was evaluated using polyethylene bags (PEB) system at pre- and post-attachments stages of parasitism. In the field, herbicide was applied in three sequential applications at 21, 35, and 49 days after transplanting with six different rates. Non-treated plots served as control. Our results show that under laboratory conditions, glyphosate and ethametsulfuron-methyl can control *P. aegyptiaca* when applied at both different stages of parasitism. Furthermore, the field experiments (seven different field trials), revealed that glyphosate foliage application was found to be effective for the control of *P. aegyptiaca* at a rate of 72 gr ae ha-1 with both applications methods. However, *P. aegyptiaca* control with ethametsulfuron-methyl was achieved at a rate of 18 gr ai ha-1 only when overhead irrigation was applied. Based on the results from this study, the protocol was developed to control *P. aegyptiaca* in cabbage under field conditions. The protocol suggests that P. aegyptiaca control can be achieved by three sequential herbicide applications. Applications of ethametsulfuron-methyl must be followed with overhead irrigation while glyphosate at a rate of 72 g ae ha-1 can be applied on cabbage foliage.

Keywords: broomrape, herbicide, field control

Suicidal Germination Technology to Alleviate Striga Infestation: Progress and Updates

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Striga hermonthica, commonly known as “Purple Witchweed”, is an obligate root parasite, infesting staple crops in sub-Saharan Africa, Middle East, and parts of Asia. This parasitic weed has become a major threat to food security causing up to 90% yield loss equating to 10 billion US$ annually that exacerbate hunger

and poverty in many African countries. The long-term management of *Striga* is hampered by huge number and longevity of seeds, vast genetic variability, complex life cycle, and subterranean nature of damage. The seeds can remain viable more than 15 years in soil and germinate only after exposure to hot and humid favorable conditions followed by perception of host-released stimulants, mainly strigolactones (SLs). After germination, *Striga* attaches to host roots to siphon off water and nutrient from the host. Although several extensive efforts have been deployed for *Striga* control, the success is still far away. Lowering *Striga* seed reservoir is crucial to minimize the underground damage by this parasite. The induction of *Striga* seeds' germination through synthetic SL analogs in the absence of the host leads to the death of germinating seedlings few days after germination. This scenario builds the basis for a promising strategy "suicidal germination", which can reduce the *Striga* seed bank in the infested soil. However, the success of suicidal germination technology depends not only on active and potent SL analogs, but also on simple, efficient, and handy formulation as well as a suitable application protocol. In this poster, we have highlighted recent progress on suicidal germination technology, carried out at King Abdullah University of Science and Technology (KAUST) in collaboration with our research partners. First, we have designed and synthesized more than 30 easy-to-synthesize SL analogs and discovered Methyl Phenlactonoates 3 (MP3) as one of the potent germination stimulants. Next, with the cooperation of industrial partner UPL India, we developed an effective and suitable formulations "Emulsifiable Concentrate (EC) or Granular" of MP3 and Nijmegen-1 to apply under field conditions. Finally, we have developed a rainfed-based application protocol that fit well to prevailing African field conditions. In conclusion, we have made tremendous efforts on suicidal germination technology during the past few years and developed several potent simple SL analogs in suitable formulations that can be applied by an established rainfed based protocol in *Striga* control program for African Agriculture. This study was supported by the Bill and Melinda Gates Foundation (grant number OPP1136424 to S.A.) and King Abdullah University of Science and Technology.

Combining ability and gene action controlling yield, yield components and *Striga* resistance among *FOS* compatible and *Striga* resistant maize genotypes

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Parasitic weeds of the genus *Striga* are increasingly threatening production and productivity of maize in sub-Saharan African countries. Effective and economic control of the weed can be achieved using *Striga* resistant maize genotypes that are compatible to *Fusarium oxysporum f.sp. strigae* (*FOS*), a biological control agent of *Striga* species. The objective of this study was to select *Striga* resistant and *FOS* compatible breeding populations of maize with good combining ability for grain yield, yield components, *Striga* resistance or tolerance. Four selected subtropical maize populations with partial *Striga* resistance and *FOS* compatibility were crossed to two broad-based *Striga* resistant testers, Z.Diplo.BC4C3-W-DTC1 and DTSTR-wSYN15, using a line by tester mating design. The testcrosses were evaluated under field and controlled environment conditions during the 2017/2018 growing season. The experiments were laid out using a randomized complete block design with four replications. Analysis of variance revealed that general combining ability (GCA) effects were significant ($p< 0.05$) for all assessed traits, whereas specific combining ability (SCA) effects were only significant for ear aspect score, kernel rows cob^{-1}, *Striga* damage rating score at 8 and 10 weeks after planting. The GCA effects were larger than SCA effects for ear aspect score, grain yield, cob diameter, kernel row^{-1}, *Striga* emergence counts at 8 and 10 weeks after planting. This suggests the prevalence of additive gene action in controlling the inheritance of these traits. SCA effects were larger than GCA estimates for kernel rows cob $^{-1}$, *Striga* damage rating score at 8 and 10 weeks after planting, which implied that nonadditive gene action was predominant in conditioning the three traits. Test crosses such as M.Pearl/DTSTR-wSYN15, M.Pearl/Z.Diplo.BC4C3-W-DTC1 and ZM1423/Z.Diplo.BC4C3-W-DTC1 were selected for further breeding based on high grain yield and significant negative SCA effects for *Striga* emergence counts and host damage rating scores. Both additive and nonadditive gene actions were present in controlling grain yield, *Striga* resistance and tolerance traits indicating the potential of recurrent selection methods in improving the selected populations.

Keywords: combining ability, compatibility, *Fusarium oxysporum f.sp.strigae*, *Zea mays* (L.), resistance breeding, *Striga* resistance

Comparison of the Effect of Tomato Root Extracts Obtained by Two Different Extraction Methods on The Germination of Broomrape at Four Different Temperature

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Broomrapes (*Phelipanche* and *Orobanche* spp.) are obligate root parasitic weeds belong to the Orobanchaceae family. These parasites attack many economically important crops including Solanaceae, Fabaceae and Asteraceae, causing severe losses in yield and quality. Strigolactones are the plant hormons which control the interactions between parasitic plants, host plant and arbuscular mycorrhizal fungi (AMF), and plant development as well. They are chemical signals of broomrape as a host-derived germination stimulant. To find the relationship between broomrape seeds and complex strigolactones secreted from the rhizosphere provides new possibilities for the control of the broomrape. The host plant root exudates are largely used for broomrape seeds germination bioassays. Different methods are used to obtain root extracts for in-vitro bioassays. Aqueous root extracts obtained by soaking plant roots in water or extractions using organic solvents by grinding the roots, are used. The aim of this study was to determine the effects of two different extraction methods and four temperature conditions on the germination of broomrape. Analytical method extraction and methanol extraction are used. Germination tests were carried out at 18, 20, 22 and 24 degrees. While tomato plant was used as host plant to obtain root extracts, *Phelipanche aegyptiaca* species was used as parasite plants. Tomato plants were grown under controlled conditions hydroponically until they reach the 6-leaf stage. The experiment was executed in in-vitro trial with 1 x 2 x4 factorial design 5 replications and repeated twice. After one-week preconditioning period of the broomrape seeds, root extracts were added and germinated seeds were counted one week later under the microscope. Germination rates were calculated as a percentage (%). While germination rates in analytical method extraction were 52.6% as average, in methanol extraction was 3.2%. The analysis of the collected data has shown significant differences between temperature and extraction methods on germination rate. Interaction between extraction method and germination temperature was statistically significant. The results obtained that analytical method extraction can be used for broomrape germination bioassay for tomato plant in in-vitro bioassay.

Keywords: broomrape, root extract, strigolactone, germination

VI. Parasitic plants-Hosts-Microbes interactions

The struggle for food: metabolomics uncovers chemical signals in sorghum that can help to combat the parasitic weed scorch, Striga

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Millions of hectares of African arable fields are infected with the seeds of the parasitic weed Striga hermonthica. Striga uses chemical cues exuded by the roots of its hosts to guide its infection process. A better understanding of these cues could provide new targets for Striga resistance breeding programs. To identify new signaling molecules driving the infection of sorghum by Striga we used a metabolomics approach. To achieve this, the metabolite profiles of roots and root exudates and the Striga infection process of 59 genetically diverse sorghum genotypes were analyzed using unbiased LC-QTOF mass spectrometry and a series of bioassays, respectively. Both datasets were then integrated using advanced multivariate models to pinpoint metabolic features playing a role in the Striga infection process. These models confirmed 5-deoxystrigol as the top predictor for germination, while orobanchol was negatively associated; we also identified another negative regulator of germination that has not been reported before but was conformed in a germination bioassay. In addition, we identified a number of known and unknown metabolic features that associated with haustorium formation and confirmed these using bioassays. Our work shows that the use of multivariate models that combine metabolic profiles with phenotypic data can be used to identify chemical features that play a crucial role in biological processes such as the host plant-parasitic plant interaction. Our findings shed more light on the belowground chemical communication between Striga and sorghum, which will help define the most important targets for Striga resistance breeding in sorghum.

Keywords: Parasitic plant-host interaction, chemical cues, haustorium formation, metabolomics

Branched broomrape – rapeseed interaction mediated by microbial activity

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Branched broomrape or *Phelipanche ramosa* is a Mediterranean parasitic weed that can infest a wide range of either wild or cultivated host plants. It generally causes extensive yield losses on tomato, tobacco and hemp in Europe. In recent decades, a new genotype has emerged in western France, which appears to be particularly specialised in rapeseed cropping systems[1]. It has since caused significant damage, sometimes leading farmers to abandon this crop. Among the many factors proposed to explain such a large but localised infestation are both the specific molecular interaction between rapeseed and *P. ramosa*, and the intermediary involvement of soil microbes. Firstly, following on from previous results[2], we confirmed using *Arabidopsis thaliana* as a *Brassicaceae* model plant, that the most important signaling pathway for the parasitic interaction in the *Brassicaceae* rhizosphere was the glucosinolate pathway. Indeed, *Brassicaceae* secondary metabolites known as glucosinolates can be hydrolysed by myrosinase enzymes into isothiocyanates which are the main stimulants of germination. Secondly, we showed, using co-culture experiments and in vitro assays, that microbial myrosinase activity in heavily parasitized soils had an impact on the levels of isothiocyanates in the rhizosphere and thus on germination and early cycle of parasitic plants. As previously proposed for parasitic seed microbiota[3], soil microbiota was shown to be largely involved in the germination of parasitic seeds. A number of fungal and bacterial candidates were additionally isolated and characterized further to validate the model.

Soil suppressive microbiota in plant-parasitic interaction between *Phelipanche ramosa* and *Brassica napus* in western France

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The root obligate holoparasite *Phelipanche ramosa* of the *Orobanchaceae* family has become a great biotic constraint for winter oilseed rape (WOSR) farmers in western France. Yet, still no efficient nor sustainable control methods are available due to intricate interactions with environmental and biological factors[1,2]. However, recent observations of parasitic plant decline in historically heavily infested fields might fill some of these gaps. It is strongly suspected that this so-called disease suppression mainly results from the effects of individual or specific groups of microorganisms[3,4]. This hypothesis was investigated using two adjacent WOSR-cultivated soils with similar physicochemical properties but contrasted levels of parasitism, characterized as suppressive and conducive soils. The differential reduction of root infestation in the suppressive vs conducive soils could be reproduced in co-culture experiments as there was a delay of parasitism development, as well as more necrotic parasitic tubercles. Further assays using coculture plant exudates, revealed that the suppressive soil had no effect on germination stimulants of *P. ramosa*, and should be investigated on the subsequent stages. Analysis of initial soil microbial composition and rhizosphere community dynamics during parasitism provided information on the nature and mechanisms of the suppressive soil, especially for fungal communities which were more contrasted between the soils. Fungal strains were isolated from the suppressive soil and are being tested *in vitro* for parasitic plant inhibition, and should provide more detailed microbial mechanisms, as well as identification of possible *P. ramosa* - suppressive agents.

Dodder parasitism limited the effect of arbuscular mycorrhizal fungi on litter decomposition

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Effects from parasitism and parasitic litter input can affect the decomposition of litter, but the mechanism was unclear. We hypothesized that the decomposition of litter produced by the holoparasite *Cuscuta australis* was promoted more strongly by arbuscular mycorrhizal fungi (AMF) than the litter of its host *Bidens pilosa*, and parasitism by *C. australis* can indirectly regulate the decomposition of litter by AMF. We conducted two container experiments to compare the effect of AMF on the decomposition of ^{15}N-labelled *C. australis* and *B. pilosa* litter filled in hyphae-in-growth bags and test the effect of *C. australis* parasitism on the decomposition of *B. pilosa* litter by AMF. We found that the amount of ^{15}N that remained in bags with *C. australis* litter was significantly higher than that in the bags with *B. pilosa* litter. AMF significantly decreased the remaining ^{15}N and changed the bacterial community in bags with *B. pilosa* litter but did not significantly affect these factors in the bags with *C. australis* litter. The bacterial diversity and AMF hyphal density in bags with *C. australis* litter were significantly lower than those in bags with host litter. Parasitism significantly reduced the hyphal density of AMF and increased the ^{15}N that remained in litter bags. Our results indicated that AMF play a key role in the parasitism and litter effects of parasitic plants on soil nutrient cycling.

Preliminary studies on response of Kichawi Kill™ to yields and incidences of striga- resistant and striga-tolerant maize varieties in Kenya

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Kichawi Kill™ formerly referred to as Foxy T14 is a pre-emergent and post-emergent bioherbicide which was recently registered in Kenya for management of striga in maize. It contains virulence-enhanced *Fusarium oxysporum* f. sp. *strigae* as the active ingredient. The bioherbicide is formulated in boiled rice. The recommended application rate is 2g of inoculated boiled per every maize planting hole during planting. Kichawi Kill was developed based on striga susceptible maize varieties. However, its effect on striga populations and grain yields of striga resistant and striga tolerant maize varieties is not known. Preliminary studies were conducted on farmer's striga infested field in Western Kenya to investigate the effect of Kichawi kill on resistant and tolerant maize varieties. The treatments consisted of paired plots of Kichawi Kill treated and untreated plots. Data were collected on striga and agronomic maize parameters until the crop was harvested. Although the two varieties were responsive to Kichawi Kill, our preliminary results showed that the striga resistant maize variety was more compatible to Kichawi Kill, with a yield increment and emerged striga reduction of more than 50% in treated plots compared to control plots. The results suggest that varieties respond differently to Kichawi Kill. Future studies should focus on testing commercial maize varieties for compatibility to Kichawi Kill™ and, recommending varieties compatible with Kichawi Kill for cultivation in striga endemic areas of Kenya.

Keywords: Kichawi Kill, Striga-resistant maize, Striga-tolerant maize, Western Kenya

VII. Phylogeny and evolution of parasitic plants

Keynote 1

Deep phylogenetic discordance hampers reconstructing the eco-evolutionary paths of the Broomrape family (Orobanchaceae)

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Orobanchaceae are an exceptional family of flowering plants. Its members cover the entire trophic range from free-living, autotrophic plants to nonphotosynthetic parasites. The transition to parasitism coincides with massive genetic reconfigurations and shifts of ecological preferences. Here, we revisit the phylogenetic relationships in the most important model family of parasitic plants. To this end, we i) established a successful protocol to maximize the yield of a universal target capture kit for parasites at different stages of parasitic specialization, ii) assessed its performance compared with transcriptome- and genome-informed expectations, and iii) used the obtained hundreds of nuclear, plastid, and mitochondrial markers to reconstruct and test hypotheses of the evolutionary history of Orobanchaceae. We sampled 80 species from all major clades of Orobanchaceae, representing all trophic specializations and transitions into holoparasitism. We conducted Maximum Likelihood and Bayesian inferences using various data partitioning strategies, from which we reconciled the most likely species tree(s). Our study highlights a deep phylogenetic conflict in the Orobanchaceae family, perhaps due to rapid diversification after the emergence of the first parasites. Another deliverable of this work is the critical evaluation of employing universal target enrichment kits for phylogenetic studies of hemi- and holoparasitic plants. Although we focus only on Orobanchaceae, its independent transitions to a nonphotosynthetic lifestyle can be considered a proxy for the different stages of parasitism-related genetic divergences. Therefore, we anticipate that this study's results will be of interest beyond Orobanchaceae systematics. Additionally, the consolidation of phylogenetic hypotheses for improved phylogenetic resolution within this family will further our understanding of the evolutionary trends associated with plant parasitism. We will discuss potential biases that might skew phylogenetic affinities and the consequences that unresolved phylogenetic uncertainties bear for interpreting functional-genetic progression and eco-evolutionary paths into parasitism.

Keywords: Orobanchaceae, phylogenetics, evolution, marker bias

Keynote 2

Host-specific selection across the *Striga hermonthica* genome

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Many host-parasite systems involve a complex multi-step infection process including many stages of interaction between host and symbiont derived molecules. Theoretical studies suggest that as parasite populations adapt to their local host environment, loci involved in initial recognition stages are more likely to be targets of selection than those involved in later stages. We will present recent work investigating this hypothesis in the parasitic plant *Striga hermonthica* (purple witchweed), which attacks a range of cereal crop hosts in Africa and exhibits substantial variation in performance on different host species due in part to local adaptation. Whole genome sequencing of *S. hermonthica* populations from diverse hosts in western Kenya suggests that only a small portion of the genome is strongly differentiated by host species. Contrasting with theory emphasizing the role of early recognition loci for host specificity and results from other model systems, our findings support host-specific selection on later interaction stages, including genes with a potential role in haustorium development. Notably, even the strongest signals of host-specific selection detected in our study did not reveal any loci exhibiting complete or near complete differentiation between parasites on different hosts, indicating that there may be relatively few genetic barriers to parasitism of different cereal host species in the studied region.

Keywords: host-parasite adaptation

Phylogeny and and comparative plastome evolution of Hydnoraceae

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Phylogenetic relationships within the magnoliid order Piperales have been studied extensively, yet the relationships of holoparasitic Hydnoraceae to the remainder of the order remain a matter of debate. Since the first confident molecular phylogenetic placement of Hydnoraceae among Piperales, different studies have recovered various contradictory topologies. Here we present phylogenomic tree reconstruction based on 137 loci from all three subcellular genomes for all genera of Piperales. In addition, plastome comparative genomics, using seven out of eight known species of the genus Hydnora and three species of Prosopanche, reveal a high degree of structural similarity and shared gene content; contrasted by striking dissimilarities with respect to larger repeats (inverted and direct repeats).

Keywords: Hydnora, Prosopanche, repeats, Piperales, Lactoridaceae

The exigent threat of Dodders in Eastern Africa

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Invasive holoparasitic plants of the genus Cuscuta (dodder) threaten African ecosystems due to their rapid spread and attack on various host plant species. Most Cuscuta species cannot photosynthesize and hence rely on host plants for nourishment. After attachment through a peg-like organ called a haustorium, the parasites deprive hosts of water and nutrients, which negatively affects host growth and development. Despite their rapid spread in Africa, dodders have attracted limited research attention, although data on their taxonomy, host range, and epidemiology are critical for their management. Here, we combine taxonomy and phylogenetics to reveal the presence of field dodder (Cuscuta campestris) and C. kilimanjari (both either naturalized or endemic to East Africa), in addition to the introduction of the giant dodder (C. reflexa), a south Asian species, in continental Africa. These parasites have a wide host range, parasitizing species across 13 angiosperm orders. We evaluated the possibility of C. reflexa to expand this host range to tea (Camelia sinensis), coffee (Coffea arabica), and mango (Mangifera indica), crops of economic importance to Africa, for which haustorial formation and vascular-bundle connections in all three crops revealed successful parasitism. However, only mango mounted a successful postattachment resistance response. Furthermore, species distribution models predicted high habitat suitability for Cuscuta spp. across major tea- and coffee-growing regions of Eastern Africa, suggesting an imminent risk to these crops. Our findings provide relevant insights into a poorly understood threat to biodiversity and economic wellbeing in Eastern Africa, and provide critical information to guide development of management strategies to avert Cuscuta spp. spread.

Keywords: Dodder, parasitic plants ecology and biology, highly invasive weeds

VIII. Posters

1. Potential Host-induced gene silencing in the parasitic plant *Striga hermonthica*

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Striga hermonthica, an obligate hemi-parasiticic plant, infects roots of targeted host plants including rice and maize. Controlling *Striga* infestations can be difficult due the nature of its small, hardy seeds that can lay dormant for extended periods of time before germination is triggered by host signaling. One method to combat *Striga* infestation the use of Host-induced Gene Silencing (HIGS) to silence *Striga*-specific transcripts that could contribute to parasite fitness and its ability to obtain nutrients from the host. Small interfering RNAs (siRNAs) have a broad range of function within plants including translational repression and post-transcriptional silencing.[1] One class of siRNAs known as phased small interfering RNAs (phasiRNAs) include secondary siRNAs which act in *trans* to further silence target transcripts.[2,3] The result is an amplified gene silencing effect. Successful HIGS experiments in *Cuscuta campestris* suggests that if successful, secondary siRNAs accumulation within *Striga hermonthica* derived from host-induced miRNAs may be a viable method of controlling *Striga* growth.[4,5] This study looks to test HIGS of endogenous transcripts of *Striga hermonthica* grown on *Orzya sativa* var. kitaake.

Keywords: siRNA, HIGS, Striga, gene silencing, host resistance

2. Virulence and Cross-host Interactions of *Striga hermonthica*

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Cereal crop production continues to record high yield gaps as a result of parasitism by *Striga hermonthica* irrespective of several studies conducted and different control measures applied. This results to aversion of farming and food insecurity. Even though the use of improved seed offers an alternative to bridge the gap, the availability of stable durable resistant seed is hindered by insufficient knowledge of the parasite diversity and host specificity that results in breakdown of resistance and invading new hosts. Using a completely randomized design, we used root exudates from 3 maize genotypes (H531, KSTP94 and WH403) to germinate six ecotypes of *Striga hermonthica* seeds sampled from maize, sorghum and finger millet fields in western Kenya. GR24 was used as a positive control and water, a negative control. Significant differences ($P<0.05$) were observed between maize genotypes in their ability to germinate different *Striga* ecotypes, as well as in comparison to the positive control. All exudates induced *Striga* germination but at varying proportions. The lowest *Striga* germination (23.7%) was recorded when root exudates from maize genotype H531 was used to germinate finger millet *Striga* ecotypes from Alomodoi. The highest germination percentage was recorded when KSTP94 root exudates were used to germinate Striga harvested from sorghum fields in Alomodoi (78%). High mean germination percentages were observed in ecotypes from sorghum fields (74.9% and 62.6%) compared to maize ecotypes (35.9% and 46%). In conclusion, H531 has a higher degree of pre-attachment resistance similar to the susceptible and resistant checks, WH403 and KSTP94 respectively. Ecotypes from sorghum *Striga* fields are more virulent as their germination percentage was higher.

Keywords: *Striga hermonthica,* Pre-attachment resistance, Parasite diversity, Cross-host interaction, Maize

3. Comparative transcriptomes of compatible and incompatible *Striga* interactions

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Resistance to Striga is based on a series of incompatible host/non-host interactions. Rice and pearl millet cultivars, Nipponbare and 29AW respectively have been reported to possess post-attachment resistance. Knowledge on the mechanisms underlying the interactions between *Brachiaria mulato II* and *Striga hermonthica*, specifically, is limited. This study aims to understand the molecular factors underpinning host and non-host interactions. Here we unearthed the mechanism of resistance in *Brachiaria mulato II*. Resistance was by mechanical barrier that barred the parasite invasion. Despite some studies on host – parasite transcriptome profiles, little is known about genetic factors underpinning non-host (*Brachiaria mulato II*) Striga interaction. We present a comparative analysis of different hosts (rice vs. pearl millet) and non –host (*Brachiaria mulato II*). We examined gene expression variation at 3DAI and 9DAI in Nippon bare, IAC 165, 29AW, SOSAT C-88 and Brachiaria to understand the genes and pathways involved in infectivity. Of particular note, was the up-regulation of defense genes, genes in the phenylpropanoid pathway and WRKY transcription factors. Transcriptome analysis disclosed a significant difference in number of DE transcripts between hosts (rice and pearl millet) in comparison with and non-hosts (*Brachiaria mulato II*). DEGs involved in various metabolic pathways such as serine threonine-protein kinase, o-methyltransferase, and leucine-rich repeat receptor-like kinase protein and ubiquitin-protein ligase were up regulated in Brachiaria mulato II Our study shows how taking advantage of host genetic variability to can upsurge resistance levels to Striga and provides the potential of understanding a host/non-host-resistant system in grasses.

Keywords: Host/non-host interactions, differentially expressed transcripts, Resistance mechanisms

Themes: Host Resistance/Genes and Genomes

4. A transcriptome atlas of Striga seed germination

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For Striga, germination is a "life and death" decision because of the limited seed reserves. Germination in the presence of an appropriate host would mean successful parasitism and an opportunity to complete its lifecycle, but germination without an appropriate host means that the seed will die after a few days. Studying how Striga makes this decision will help expand our mechanistic understanding of genetic underpinnings of parasite seed germination. Under natural conditions, this is a more complex process because Striga responds to different hosts based on the diverse chemical stimulants exuded by its different hosts. It is known that Striga germinates more effectively in response to 5-deoxy strigol exuded by most sorghum

genotypes relative to orobanchol emitted by a few genotypes with the *low germination stimulant 1* (*lgs1*) loss of function mutation. To add to this complexity, the root exudate is a blend of biomolecules, some of which would enhance or inhibit germination. For example, the phytohormones such as ABA, GA, JA interact in a cross talk that impinges on Striga germination. To shed light on the Striga germination process, we used the striga-sorghum patho-system in a comparative transcriptome approach that compared RNA profiles high inducers of germination, low inducers of germination (*lgs1*-like) and low inducers of germination (non-*lgs*-like) against the synthetic strigolactone (GR24) positive control. We sought to answer the following research questions: (i) what are the genetic factors that underpin Striga germination after conditioning? (ii) what are the genetic underpinnings of Striga germination in response to low germination inducers relative to high inducers? and (iii) what are the agonistic/ antagonistic interactions during Striga germination? We present the results in an atlas of color-coded atlas of models and genetic pathways them in the context of Striga management.

Keywords: Striga resistance mechanisms

5. Reduced exudation of strigolactones as a resistance mechanism of wild carrots against *Phelipanche aegyptiaca*

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Phelipanche aegyptiaca is an obligate root parasitic plant with a wide host range, which includes many crops. Carrot is one of the host crops of *P. aegyptiaca*, which causes a reduction in taproot biomass and sugar content leading to a decline in carrot quality and quantity. One way to control parasitic plants is to breed traits from resistant crop cultivars or wild accessions into susceptible genotypes. A screening of a wild carrot collection in Israel identified an accession (PI-21793) of *Daucus glaber* to be resistant to *P. aegyptiaca.* The main objective of this study was to identify mechanisms associated with the resistance of this accession. We employed a Polyethylene (PE) bag cultivation system to observe parasite resistance of *D. glaber* in comparison to a related wild carrot species, *D. littoralis*, and a cultivated carrot (*D. carota*). The results showed that both wild carrot accessions caused lower germination of *P. aegyptiaca* seeds. However, treatment with the synthetic germination stimulant GR24 increased the germination of *P. aegyptiaca* seeds, indicating reduced exudation of strigolactones as a resistance mechanism in *D. glaber* and *D. littoralis*. Furthermore, we observed post-attachment resistance in the *D. glaber* accession based on unsuccessful establishment of *P. aegyptiaca* tubercles with *D. glaber roots*. By contrast, *D. littoralis* supported healthy tubercle establishment despite similar *P. aegyptiaca* germination rates as with *D. glaber*. Hence, in *D. glaber*, both pre-and post-attachment mechanisms may be involved to successfully fend off *P. aegyptiaca*. Future research will focus on a more thorough quantitative and qualitative analysis of strigolactones from *D. glaber* and on determining mechanisms of post-attachment against *P. aegyptiaca*. Overall, this work indicates that wild carrots may be a good source of parasite resistance traits for cultivated carrot breeding programs.

Keywords: *Phelipanche aegyptiaca, Daucus carota, Daucus glaber, Daucus littoralis,* resistance, strigolactones

6. Gibberellin action-based strategy to control root parasitic weeds

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Striga hermonthica (*Striga*), a root parasitic weed, causes severe damage to crop production worldwide due to the presence of its seeds in the soil. Gibberellins (GAs), one of the plant hormones, are involved in seed germination, stem growth, and flowering in flowering plants, and their control techniques are widely used in agriculture. GAs also reduce SL production and secretion from roots by inhibiting SL biosynthesis. Application of this function could lead to using GAs as parasitic control agents of root parasitic weeds. We previously reported that an N-substituted phthalimide, 67D-I mimics GA activity in *Arabidopsis thaliana*. In this study, we tested the inhibitory activity of 67D-I on SL production in host rice and its ability to inhibit *Striga* parasitism on the host.

We measured the level of 4-deoxyorobanchol (4DO), a major endogenous SL in rice, in root and root exudates using the LC-MS/MS. 67D-I showed 4DO inhibitory activity in a dose-dependent manner. We then checked the *Striga* germination rate of the root exudates from 67D-I-treated rice. In accordance with the results of the 4DO level in root exudates, the culture media of 67D-I-treated rice showed less germination stimulating activity than those of mock-treated. Finally, we grew rice in pots and applied 67D-I to evaluate the effect of 67D-I on *Striga* infection. Weekly treatment with 67D-I reduced the emergence of *Striga* and protected the host from *Striga*-induced growth inhibition. Intriguingly, 67D-I did not even promote stem elongation in the host. These results indicate that 67D-I inhibits SL production and increases crop yield by controlling *Striga* damage.

Since shoot elongation of host plants can cause yield loss due to lodging, root parasitic weed control agents that do not have above-ground elongation activity may be helpful in agriculture. This study suggested that using GA regulators is one of the options to control root parasitic weeds.

Keywords: Gibberellin, plant growth regulator, plant hormone, *Striga hermonthica*, strigolactone

7. A SL biosynthesis inhibitor TIS108 suppresses the emergence of *Striga hermonthica* in rice pots and *Orobanche minor* in tomato pots by targeting cytochrome P450s

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TIS108 represses the endogenous levels of 4DO in rice (1). However, it has not uncovered the target protein(s) of TIS108. The 1H-1,2,4-triazole moiety can bind to heme iron in P450s and inhibit the function of various P450s. Therefore, it is reasonable to consider that the target sites of TIS108 could be P450s. We expressed the recombinant Os900, Os1400, Os5100 and Os1900 in yeast microsomes to estimate the inhibitory activities of TIS108. TIS108 efficiently inhibited the conversion of CL to CLA and CLA to 4DO by Os900, with IC_{50} values of both conversions of 0.15 µM. TIS108 also inhibited the conversion of 4DO to orobanchol by Os1400 with a IC_{50} value of 0.02 µM. Codon-optimized Os5100 clearly converted CL to CLA, which was inhibited by treatment of TIS108 in a dose-dependent manner. Conversely, the codon-optimized Os1900 did not convert CL to CLA, suggesting that Os1900 was not well expressed in yeast cells or the substrate of Os1900 is not CL. These results indicated that TIS108 targets CYP711As in rice. The inhibition of SL biosynthesis could be a new strategy for parasitic weed management. We performed tests to evaluate the effects of TIS108 on *Striga* infection and found that TIS108 suppresses Striga emergence without adverse effects on rice growing (2).

We have also confirmed that TIS108 reduced SL production in tomato plants without affecting tillering and shoot branching, respectively. TIS108 effectively reduced *O. minor* parasitism on tomato in a greenhouse pot test with no adverse effects on tomato.Similar results were obtained with S. hermonthica parasitism on rice plants (3). The target site(s) of TIS108 in tomato will be discussed.

Keywords: Striga, biosynthesis inhibitor, strigolactone

8. Signaling pathways for prehaustorium induction by quinones, phenolics and cytokinins in *Striga hermonthica*

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Parasitic plants derive water and nutrients from their hosts, and therefore the parasitic species having their host preference on crop species cause severe agricultural problems. Parasitic plant in Orobanchaceae parasitizes host roots through the organ called a haustorium that functions in nutrient and water acquisition. Prehaustorium, a primitive haustorial structure, is formed by sensing host-derived haustorium-inducing factors (HIFs). Quinones and phenolics, such as 2,6-dimethoxy-p-benzoquinone (DMBQ), acetosyringone and syringic acid, are well known HIFs for many Orobanchaceae parasitic species, including *Striga hermonthica and Phtheirospermum japonicum.* However, these HIFs are not so effective on non-photosynthetic holoparasitesvvv in *Phelipanche ramosa*. Little is known about the effects of cytokinins on photosynthetic hemiparasite species. Moreover, the relationships of signaling pathways for prehaustorium induction by quinones, phenolics and cytokinins are not well understood. Here we show that cytokinins act as HIFs in *Striga hermonthica* but not in *Phtheirospermum japonicum*. Using chemical inhibitors for each type of HIF, we demonstrated that cytokinins activate prehaustorium formation through a signaling pathway that overlaps with the quinone and phenolic HIF pathways in *S. hermonthica*. Furthermore, we analyzed gene expression during prehaustorium induction by quinone, phenolics and cytokinins as well as host root exudates. The results suggest that host root exudates contain a mixture of these types of HIFs. Our study reveals the importance of cytokinins for prehaustorium formation in obligate parasitic plants and redundancy and complexity of HIF signaling pathways may contribute to establish robust response against host roots-derived HIFs.

Keywords: Haustorium induction, haustorium-inducing factor, *Striga hermonthica*, quinone, phenolic, cytokinin

9. Identification of Novel Strigolactones in a *Striga*-Susceptible Pearl Millet Line and Determining the Effect of Zaxinone on Their Release

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Pearl millet (*Pennisetum glaucum*) is a major staple food and the third most important cereal in sub-Saharan Africa. However, the production of pearl millet in Africa is severely impacted by *Striga hermonthica*, a root parasitic weed infesting cereals and causing yield losses up to complete crop failure. The germination of *Striga* seeds largely depends on perceiving germination signals, mainly strigolactones (SLs), released by the hosts. Therefore, determining the SL profile in *Striga* resistant and susceptible lines would pave the way for generating or breeding *Striga* resistant commercial lines. Here, we investigated two pearl millet lines (29Aw and SOSAT-C88-P10), which derive from a wild accession or the commonly planted SOSAT variety and exhibit contrasting phenotypes with respect to shoot architecture as well as *Striga* susceptibility. LC-MS analysis revealed different SL compositions in root exudates of the two lines; importantly, we identified four novel pearl millet SLs that are only present in the susceptible SOSAT-C88-P10 line. *Striga* bioassay further confirmed the seed germinating activity of these SLs. Recently, we have reported on the apocarotenoid metabolite as a growth-promoting compound and negative regulator of SL biosynthesis and release in rice. We also developed zaxinone mimics (MiZax) for basic research and application in agriculture. To test if we can use zaxinone and MiZax to reduce the seed germinating activity of pearl millet exudates, we applied these compounds to SOSAT-C88-P10 plants. Subsequent analysis, unraveled a reduction of root-released SLs and a decrease in *Striga* emergence. Moreover, zaxinone and MiZax significantly increased root length and biomass of hydroponically grown pearl millet seedlings. In conclusion, our results reveal novel SLs that contribute to *Striga* susceptibility in pearl millet and indicate the potential of exogenous application of the growth regulators zaxinone and MiZax in promoting the growth of this cereal and alleviating its susceptibility to *Striga*.

Keywords: Strigolactones, *Striga*, Pear Millet (*Pennisetum glaucum*), Zaxinone, Zaxinone Mimics (MiZax)

10. LATERAL ORGAN BOUNDARIES DOMAIN 25 functions as a key regulator of haustorium development in field dodders

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Parasitic plants reduce crop yield worldwide. Field dodder (*Cuscuta campestris*), one of the most widespread and destructive parasitic plants, severely reduces tomato yield. *C. campestris* is a stem parasite that attaches to its host, using haustoria to extract nutrients and water. We analyzed the transcriptome of six *C.*

campestris tissues and identified a key gene, *LATERAL ORGAN BOUNDARIES DOMAIN 25* (*CcLBD25*), as highly expressed in prehaustoria and haustoria. Gene coexpression networks from different tissue types and laser-capture microdissection RNA-sequencing data indicated that *CcLBD25* could be essential for regulating cell wall loosening and organogenesis. We employed host-induced gene silencing by generating transgenic tomato hosts that express hairpin RNAs to target and down-regulate *CcLBD25* in the parasite. Our results showed that *C. campestris* growing on *CcLBD25* RNAi transgenic tomatoes transited to the flowering stage earlier and had reduced biomass compared with *C. campestris* growing on wild-type (WT) hosts, suggesting that parasites growing on transgenic plants were stressed due to insufficient nutrient acquisition. We developed an *in vitro* haustorium system to assay the number of prehaustoria produced on strands from *C. campestris*. *Cuscuta campestris* grown on *CcLBD25* RNAi tomatoes produced fewer prehaustoria than those grown on WT tomatoes, indicating that down-regulating *CcLBD25* may affect haustorium initiation. *Cuscuta campestris* haustoria growing on *CcLBD25* RNAi tomatoes exhibited reduced pectin digestion and lacked searching hyphae, which interfered with haustorium penetration and formation of vascular connections. The results of this study elucidate the role of *CcLBD25* in haustorium development and might contribute to developing parasite-resistant crops.

Keywords: *Cuscuta campestris*, dodder, *Solanum lycopersicum*, haustorium, development, *LBD25*

11. A Novel Protocol of Genetic Transformation of *Cuscuta campestris*

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Cuscuta spp. (dodder) are holo-parasitic plants that depend on a host plant for all their life cycles. As a weed, it causes severe damages on major crops (such as tomato and legumes) in the world. Recently, *Cuscuta* has been studied as a model plant for understanding plant-plant interactions because mobile molecules (DNAs, RNAs, and proteins) are exchanged between *Cuscuta* and host plants through a unique organ called haustoria. Although *Cuscuta* genome sequences and multiple RNA-seq studies have been reported, molecular mechanisms of *Cuscuta* parasitism or host-parasite interactions have been poorly understood due to the lack of a stable genetic transformation system. In this study, we investigated *Agrobacterium*-mediated transformation of *Cuscuta campestris* to develop a novel protocol. We used two reporter genes (GFP and GUS) to track the transformation efficiency and transformation stability. *Cuscuta* seedlings were inoculated by two different *Agrobacterium* species, *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens*, to compare the susceptibility. At the same time, different factors; explant type, culture media with different plant growth regulators, and co-cultivation conditions have been tested to optimize the protocol. Taken together, we found some *Cuscuta* explants and callus showing strong GFP or GUS activities as results of transient and stable transformation. Under different regeneration media, some transformed calli even showed regeneration initiation, suggesting the possibility of stably transformed *Cuscuta* explants. This transformation method will accelerate functional characterization studies of *Cuscuta*.

12. Isolation of protoplasts from *Cuscuta campestris*

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Research on *Cuscuta* is currently limited by the lack of a protocol for transformation and regeneration of stably transformed plants. *Agrobacterium*-mediated attempts at transformation including biolistics, vacuum infiltration, and co-cultivation have resulted in transient transformation of a few, localized cells, but to date no selection or regeneration of transformed tissues has been achieved. While regeneration of whole plants from protoplasts is inherently challenging, the direct delivery of sequences of interest into plant protoplasts, combined with fluorescent cell sorting, allows for in depth expression analysis, and transcription factor/promoter interactions in plant protoplasts where *Agrobacterium* mediated studies are lacking. Protoplasts have been successfully isolated from rapidly dividing *in vitro Cuscuta campestris* tissue at concentrations of 10^{x104} protoplasts/mL. Improved yield of protoplasts will increase the potential efficiency of PEG-mediated transformation. Resulting transformed cells will be directly useful for studying genes of interest and may provide and alternative approach to the generation of transgenic plants.

13. Dispersal of *Phoradendron quadrangulare* (Santalaceae) by birds in urban areas of São Paulo, Brazil: the importance of a mistletoe for the conservation of an endangered bird species

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Parasitic plants have a noticeably bad reputation. Possibly because of our suspicions regarding human parasites, many consider these plants to be destructive pests that must be eradicated. However, as a group, they can play important roles as biodiversity attractors or food resources, especially in urban areas, where their presence may result in positive consequences at the community level. This project aimed to investigate the dispersal of the mistletoe *Phoradendron quadrangulare* (Santalaceae) parasitizing the exotic tree *Melia azedarach* (Meliaceae), as well as the diversity of bird visitors. We selected *M. azedarach* because it is the only urban *P. quadrangulare* host in São Paulo bearing juicy fruits. We compared two areas in Sao Paulo: *i*) city streets that presented almost no trees; *ii*) areas presenting a greater number of trees and shrubs and that could be considered an "urban park"— the Butantã campus of the University of São Paulo. To perform this comparison, focal observations were conducted, a method which consists in directly observing a plant or a set of plants and recording any events of interest. Just more than 80 hours of observation were carried out during 2019 and 2020, during the months of fructification (from May to September, approximately Fall to Spring), recording the birds that either visited or consumed fruits of the host and/or parasite pair. A total of 21 species of birds were identified. The most observed visiting birds of the host tree were: Sayaca Tanager (*Tangara sayaca*), Rufous-bellied Thrush (*Turdus rufiventris*), Great Kiskadee (*Pitangus sulphuratus*), and the Picazuro Pigeon (*Patagioenas picazuro*). From the abovementioned species, the main consumers of host fruits were the Rufous-bellied Thrush (*T. rufiventris*), Sayaca Tanager (*T. sayaca*), and the Great Kiskadee (*P. sulphuratus*). As for *P. quadrangulare*, only one species stood out as both visitor and consumer of fruits: the Golden-Rumped Euphonia (*Euphonia cyanocephala*). This rare and threatened bird was observed during the entire period, except for the month of May. The Sayaca Tanager (*T. sayaca*) was the second most frequent visitor of *P. quadrangulare*, although consumption of fruits was never observed. Our observation of *E. cyanocephala* as the most important consumer of *P. quadrangulare* fruits makes this species a strong candidate for parasite dirpersor. The fact that our observations point to this beautiful bird, the Golden-Rumped Euphonia (*E. cyanocephala*), as the *quasi*-exclusive consumer of *P. quadrangulare* fruits highlights the fact that the mistletoes may be key resources for some species. This might be especially significant in megacities like São Paulo which lack high densely forested areas. Regarding the conservation of this bird species, the mistletoe could be considered an important food resource, given the low diversity and quantity of urban trees – that represents fraction of the original diversity of the Atlantic Rain Forest biome. In the last decades São Paulo's landscaping design focused on the usage of exotic deciduous trees that bear dry fruits, such as *Tipuana tipu*, *Jacaranda mimosifolia,* or, similar native trees such as *Cenostigma pluviosum* and *Libidibia ferrea*. Therefore, *P. quadrangulare* may provide a necessary food source for the Golden-Rumped Euphonia, especially because its fructification period coincides with dry season months, when fewer small juicy fruits are available. Given the evidence of P. *quadrangulare* being an important food resource to the endangered Golden-Rumped Euphonia, we propose that the presence of this parasitic species in São Paulo might help further conservation efforts concerning *E. cyanocephala* and that, due to its importance, this mistletoe species should no longer be removed or exterminated.

Keywords: frugivory, animal-plant interaction, ornithochory, parasitic plants, Santalaceae, Santalales

14. Overexpression of ZAXINONE SYNTHASE1 in susceptible rice variety IAC165 reduces seed germination and emergence of *Striga hermontica*

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The rice variety IAC165 is susceptible to *Striga hermontica* parasitism and known as a high producer of strigolactones (SLs; Jamil et al., 2012), which are perceived by *Striga* seeds as a stimulant required for their germination. *ZAXINONE SYNTHASE1* (*ZAS1*) has been reported to produces zaxinone, a growth regulator that down-regulates rice SL biosynthesis and release. An increase in *ZAS1* expression should increase zaxinone levels, which would reduce SL production, thereby lowering the germination of *Striga* seeds in the soil. We have generated four independent overexpression lines of *OsZAS1* by *Agrobacterium* mediated transformation of IAC165 grain derived callus. Root exudate collected from the ZAS1OE lines showed lower Striga seed-germinating activity, compared to those of the WT. The reduction varies by dilution level of root exudates, which is a proxy for distance from the rice root, from no significant reduction at 3x dilution to over 50% reduction at 81x dilution. We also assessed the effect of the overexpression on *Striga* infestation under greenhouse conditions. Although there was a high variation between the different lines, the strong overexpressors were significantly less infested by *Striga*. The reduction in Striga emergence was more pronounced at 9 weeks and became less pronounced at 11 and 13 weeks progressively, indicating a delaying effect in *Striga* emergence. However, the ZAS1OE lines showed reduced plant height and delayed flowering compared to the wild type IAC165 in both greenhouse and field experiments. Even a mild improvement in *Striga* resistance comes at a cost of impaired growth for the IAC165 ZAS1OE rice. A more beneficial ratio between resistance gain and developmental detriment may be achieved by more specifically targeting strigolactones that are most prolific in promoting *Stiga* germination, while leaving other strigolactones with a more hormonal function unaffected.

Keywords: *Striga*, strigolactones, zaxinone, rice

15. Transcriptome landscape in haustoria for host-regulated vascular connection

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Parasitic plants form a multicellular organ, the haustorium, on their shoots or roots to parasitize host plants for acquisition of water and nutrients. The haustorium penetrates the host tissues, reaches host's vascular bundle, and establishes vascular connections between the host and the parasites called xylem bridges. Inside a mature haustorium, various cell types are differentiated, including xylem cells and procambium-like cells. However, little is known about how these cell differentiation are regulated and what are the identities of these cells. We use the model Orobanchaceae parasitic plant, *Phtheirospermum japonicum*, to solve these questions. *P. japonicum* form lateral haustoria on its roots and can parasitize the model autotrophic plant *Arabidopsis thaliana*. First, we have surveyed Arabidopsis mutants that show defects in their vascular development in *P. japonicum* infection assay to find out whether host vascular development affects on the xylem bridge formation. We found that the Arabidopsis *wodden leg* (*wol*) mutant, which have a mutation in a cytokinin receptor gene, can not induce normal xylem bridge formation in wild type *P. japonicum* haustoria, suggesting that host vascular development affects xylem cell differentiation in a haustorium. To understand molecular basis for host-regulated xylem bridge formation, we performed RNA sequencing of haustoria infecting *wol* mutant or wild type Arabidopsis, and found auxin-related genes and vascular development genes are differentially expressed. However, because RNA-seq samples were obtained from a mixture of cells, the cell-to-cell differences were not detected in this analysis. To further explore cell differentiation in highly heterogeneous cell populations in haustoria, we tried single nuclei RNA sequencing (snRNA-seq). Our results identified various cell types with specific transcriptome in a haustoria. Our study reveals heterogenous cell structures in a haustorium and provides a gene expression map of a haustorium at single-cell resolution.

Keywords: haustorium, Phtheirospermum japonicum, RNA sequencing, snRNA-seq

16. Identification of a Prehaustoria Suppressor in the Root Parasitic Plant *Phtheirospermum japonicum*

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Parasitic plants develop the specialized organ called haustoria to acquire water and nutrients from host plants. Formation of the prehaustoria, the preliminary structure of haustoria, requires the induction of host-derived Haustorium Inducing Factors (HIFs). Here we present the isolation of a mutant, named *spontaneous prehaustoria 1* (*spa1)*, in root parasitic species *Phtheirospermum japonicum*. Wild type does not produce prehaustorium in absence of host signal or HIFs, while *spa1* produces spontaneous prehaustorium even when host and HIFs are absent. Sucrose, but not other sugars, significantly enhances *spa1* phenotype. Moreover, *spa1* is hypersensitive to low concentration of HIFs and host signals. Whole genome sequencing and complementation test identified the responsible mutation in the homolog of *Arabidopsis* GT72B1 encoding a UDP-glucosyltransferase, which functions on catalyzing glucosylation of secondary metabolites. GT72B1 is a bifunctional *O*-glucosyltransferase (OGT) and *N*-glucosyltransferase (NGT) catalyzing glucosylation of phenols and anilines respectively. Overexpressing of the mutant PjGT72B1 that only has OGT function complements *spa1* phenotype, while overexpressing of the protein with NGT function cannot complement the *spa1* phenotype, indicating endogenous phenol glucosylation is important in regulating prehaustoria formation. Using the construct of PjGT72B1 native promoter fused with mCherry, we observed weak PjGT72B1 expression in root tip elongation zone, where the prehaustorium formation initiates, while stronger expression was detected in maturation zone where haustoria are rarely produced. We hypothesis that PjGT72B1 regulates prehaustorium formation by glucosylating endogenous phenolic compounds.

Keywords: prehaustoria suppressor, UGT, phenol glucosylation

17. Novel strigolactone formulations and application technology to deplete the seedbank of Striga *Striga hermonthica* (Del.) Benth in infested fields

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Striga hermonthica (Del.) Benth. constitutes a major biotic constraint to cereal production. The control methods developed in the past are focused on emerged plants and do not affect soil seedbank which makes the parasite a permanent threat. This study aims at investigating formulation and application technology to use three strigolactone (SL) analogues (SL1, SL2 and SL3) to induce suicidal germination of *Striga* seeds buried in natural infested fields. Thus, the efficacy of 2 liquid formulations (AG and EC) and 2 granular formulations of SL2 and SL3 to stimulate germination of *Striga* seeds in soil conditions has been evaluated using the Eplee bag technique in mini boxes (1 m^2 area and 40 cm depth) filled with soil at 3/4. The induced germination rate of *Striga* seeds has been evaluated 3, 6 and 9 days after application (DAD) consisted of spraying or incorporation of liquid and granular formulations, respectively. Two application scenarios of the liquid AG formulation of the 3 SLs under field conditions has been assessed in Pearl Millet and Sorghum growth. Scenarios 1 and 2 consisted of applying of SLs during one and two cropping seasons before the cultivation of host crop in treated plots, respectively. *Striga* germination rate induced by the liquid SL formulations in treated mini boxes ranged from 3 to 70%. The performance of the AG and EC liquid formulations of SL2 have been similar while for SL3, only EC liquid formulation has been more effective. *Striga* germination rates ranging from 4% to 39% have been recorded with the two granulated formulations and the SL2 formulation was more effective. In field conditions, the 3 SLs significantly reduced the number (43-65 Striga/plot) and dry biomass (33-202 g/plot) of emerged *Striga* plants in treated Sorghum plots of scenario 1 compared to the control plot. With scenario 2, *Striga* number has been significantly reduced in sorghum plot treated with SL3 and in the millet plots treated with the 3 SLs. These results revealed that the ability of SLs to induce

suicidal germination of *Striga* seeds in soil conditions. However, with regard to the huge amount of *Striga* seedbank in infested soils, a continuous application of more than two (2) cropping seasons without host crop growth will contribute to destroy this seedbank. Exploitation of the properties of Sls may be considered as a component in an integrated *Striga* management approach.

Keywords: *Striga hermonthica*, seedbank, suicidal germination, strigolactone, application technology

18. Can physiological performance of striga-infected sorghum genotypes be rescued by improved plant nutrition?

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A recommended integrated striga management strategy is based on a combination of preventive measures. The effectiveness of the use of fertilisers (mainly N and P) and the use of genotypes with higher levels of resistance (reducing parasite numbers) or tolerance (reducing parasite effects), have been extensively assessed on their individual merits. The effectiveness of a combination of these two remains to be fully explored. Also, the role of micro-nutrients is less understood, and tolerance received much less attention than resistance. In three greenhouse pot experiments (2020, 2021 and 2022), a striga-sensitive sorghum genotype (CK60B) and two striga-tolerant genotypes (Ochuti, from East Africa, Tiemarifing from West Africa) were grown under *Striga hermonthica* infested and free conditions. Four fertiliser treatments were applied, 1. a lower-benchmark level (25% of recommended macro- and micro-nutrients), 2. an upper-benchmark level (100% of both types of nutrients) and two intermediates, i.e. 3. 100% macro + 25% micro, and 4. 25% macro + 100% micro-nutrients. At 60 days after sowing, leaf photosynthesis and photochemical quenching (indicators of Striga tolerance) were measured on sorghum plants, and aboveground striga numbers were counted. On striga-free plants, no inherent differences in physiological performance between genotypes were observed. On striga-infected plants significant effects of genotype and fertiliser treatments on host plant physiology were observed. Physiological performance was dramatically reduced by striga in the sensitive genotype CK60B, and only moderately in tolerant genotypes Ochuti and Tiemarifing. While in tolerant genotypes this loss was fully recovered by fertilisers that included macro-nutrients, in the sensitive genotype these fertilisers did by no means rescue the striga-induced losses. Host plant tolerance appeared the most effective way to prevent striga damage.

Keywords: witchweed, fertilisers, defence mechanisms, striga-tolerance, integrated striga management

19. Screening of mutant rice lines against *Striga hermonthica*

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Rainfed rice production is confronted with abiotic and biotic problems, the main ones being water stress and weeds. Striga, a parasitic plant of upland rice, is a particular threat and is more prevalent under water stress and poor soil conditions. The objective of this study is to screen mutant rice lines developed by induced mutagenesis against *Striga hermonthica*. The aim is to develop mutant rice lines with combined tolerance to water stress and *Striga hermonthica* in strict rainfed rice production. Thus, 20 mutants rice lines irradiated with four (4) doses of gamma rays and having an aptitude for tolerance to water stress during the reproductive phase were screened *in vitro* against *Striga hermonthica*. Two lines namely 162/43 and 172/15 irradiated with 300 Gy dose were found to be tolerant to Striga through a Maximum Germination Distance lower than 1 cm and statistically superior to the non-irradiated controls. The lines obtained should be screened in pot and field to confirm their Striga tolerance abilities.

Keywords: Strict rainfed rice, *Striga hermonthica*, Induced mutagenesis, Water stress, Burkina Faso

20. Designing nanoparticles to block root parasite-host communication – environmental friendly weed control

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Problem: The root holoparasitic plant, *Orobanche* and *Phelipanche* spp. (Broomrape), causes severe damage to crops worldwide. Its control is complicated and currently treated by applying herbicides. Broomrape seed germination is elicited by host-derived stimulants, strigolactones, produced by the host plant roots. Main goal is protecting tomato and sunflower crops from broomrape weeds by designing environmental-friendly nano-silica particles which adsorb the strigolactones in the root zone, blocking the communication between the host and the parasite and therefore preventing germination of the broomrape seeds. The nano-particles will be applied by immersing seedlings in the particle solution or via the irrigation system. Methodology: We have successfully grafted silica particles with aminosilanes consisting relevant functional groups. The particles were characterized 1. the loading of the substitute group was determined by thermal gravimetric analysis TGA (10%), 2. the chemical bonding between the particles and the substitute group by confirmed by Fourier Transport Infra-Red FTIR and 3. the surface charge reversal, from slightly negative to positive charge, was indicated by Zeta potential measurement. The particles which were grafted with 3-Aminopropyltriethoxysilane (APTES), had the highest zeta potential, and as a consequence created the most stable suspension. Results: The adsorption of the strigolectones to the APTES particles was higher comparing to the others. A significant decay in germination was achieved, in a petri dish bioassay, upon applying APTES particles. A reduction in the number of broomrape attachments was also found in several treatments in an experiment performed with tomato and sunflower plants, cultivated in a hydroponic system, in which the grafted particles were applied in the root zone. This research shows that the unique approach of adsorbing the strigolactones, using environmental-friendly materials, has a high potential for sustainable broomrape control without using herbicides.

Keywords: *Orobanche*, broomrape, weed-control, sustainable, silica

21. Farmers’ knowledge and perception of finger millet parasitic weeds and its control practices in arid and semi-arid regions of Tanzania

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Finger millet (*Eleusine coracana* (L) is the oldest indigenous cereal grown in arid and semi-arid regions of East Africa. The crop is largely cultivated by resource-poor farmers, which serves as a food and nutritional security crop because of rich nutrition and extraordinary storage qualities. However, its productions are extremely low at $\leq$1.3 t ha^{-1} comparison to its potential of up to 10 t ha^{-1}. Various biotic and abiotic constraints are responsible for the low yields. Among the constraints, hardly few studies mentioned parasitic weed invasions in finger millet production systems in Tanzania. The aim of this research is thus to determine the awareness of finger millet farmers on parasitic weed in Tanzania. We surveyed parasitic occurrence and interviewed farmers in seven (7) finger millet growing regions in December, 2020 and June, 2021 in Tanzania. Questionaries’ were developed to know: Whether farmers see parasitic weeds as a problem in finger millet production fields, what are main parasitic weed species? Do farmers know how to manage these weeds? In total, 189 farmers were interviewed in the top 7 finger millet growing regions. Among these farmers, 66% were female. About 55% of the surveyed areas, *Striga* is prevalent and farmers have experienced this weed in their fields. In the southern and central regions of Tanzania *Striga asiatica* is the main species, while in the northern and lake regions *Striga hermonthica* is the dominant species in finger millet fields. About 8% use crop rotation to manage Striga invasions, 5% use intercropping. Furthermore, their awareness of *Striga* control were inconsistent. This study revealed that, most of the finger millet farmers in Tanzania are aware on parasitic weed invasions. The study also exposed that, finger millet smallholder farmers (mainly women), who are the workforce in these regions lacks acceptable control strategies for these weed spp. We therefore, proposed that any mitigation of *Striga* should aim at improving farmers’ adoption of best practices through development of resistant varieties and the use of sustainable management options.

Keywords: *Striga, Finger millet, Nutrition security*

22. Weighted gene co-expression networks explain Striga resistance in sorghum

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Striga resistance in sorghum is believed to be due to diverse mechanisms. However, molecular mechanisms of Striga resistance remain unclear. We performed a comparative transcriptome on five genotypes of sorghum exhibiting diverse Striga resistance mechanisms. using weighted gene co-expression network analysis (WGCNA). We found that out of a total of 43 significant modules, 7 modules were enriched for pathways involving typical Striga/pathogen resistance. General categories of Striga resistance pathways were i) pathogen triggered immunity, ii) cell wall enhancement synthesis of secondary metabolites, and iii) hypersensitive response. Importantly, some modules were enriched for specific cell wall enhancements. For example, cellulose, pectin, and flavonoid biosynthesis. Additionally, some modules were specifically enriched in some genotypes allowing WGCNA to clearly delineate mechanisms of Striga resistance in the sorghum genotypes studied. Based on these results, Striga resistance mechanisms in the sorghum genotypes studied can be summarized as follows: IS1814 has primarily cell wall enhancement mechanisms including cellulose, pectin, lignin, and flavonoid biosynthesis. The main defense mechanisms in IS9830 is pathogen triggered immunity that leads to cell wall enhancement though cellulose, pectin and flavonoid biosynthesis. In IS14963, the primary defense is lignin, flavonoid, and a string HR. N13 appear to have multiple defenses based on the cell wall but not to the high extent of the other genotypes. IS41724 has multiple layers of cell wall defense (cellulose, pectin, and lignin) as well as – albeit to a less extent, HR.

Keywords: Striga resistance mechanisms, Sorghum

23. Control and Management of Dodder (*Cuscuta* spp.) in Ornamental Production in Kampala City

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Dodders (*Cuscuta spp*.) are obligate parasitic plants that pose a great threat to many agricultural crops, including ornamental plants. Blueberries, carrots, alfalfa, potato, tomato, sugar beets, and a variety of ornamentals and weeds are among the economically important plants affected. Due to its rapid growth and increasing harm to many plants, Dodder has recently become the subject of investigation. The nature of attachment and intimate interaction between host and Dodder makes it difficult to achieve effective field dodder control. We conducted a case study on how dodder is being controlled and managed in Kampala city of Uganda and observed that most people are not even aware that dodder is a parasitic plant some people consider Dodder to be an ornamental plant. The vascular connection between Dodder and the host plants necessitates the use of a highly powerful and highly selective herbicide to kill the parasite without causing economic injuries to the host plants. Chemical, biological, physical, and cultural techniques all need to be integrated for dodder management. However, a few people in Uganda try to use physical and cultural techniques. The lack of knowledge on the biology, ecology of Dodder, effective chemicals, and biological control agents in Uganda makes it difficult to control Dodder. An integrated management approach was designed based on the phenotypes and the nature of growth of the dodder since no in-depth research has been performed on this invasive species in Uganda. Due to these knowledge gaps, the landscapers were advised to use clean equipment and use non-host plants such as grass types in landscaping. In the case of the field infestation, the farmer should remove host plants from the landscape, cut off infested sections of plants and burn them and weed tiny infestations by hand. More research should be done to identify dodder species, their distribution, host preferences, and their indigenous biological control agents in Uganda. These shall provide the information that is required for the design of an effective and sustainable integrated dodder management approach. Ugandans should be sensitized about the great threat of dodder parasitic weed to our agriculture sector.

Keywords: Management, Control, Dodder, Ornamental production, and City

24. The Inheritance of Resistance to *Striga hermonthica* in Provitamin A-rich maize in Uganda

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The development of maize varieties combining high yield, provitamin A (PVA), biotic, and abiotic stress tolerance is an effective and affordable strategy to contribute to food security in Sub-Saharan Africa (SSA) where maize is a staple food crop. The objectives were to determine the inheritance of yield and Striga-resistance in PVA-rich hybrids in Uganda and to identify hybrids that combine high yield, Striga resistance, and stability across environments. Six females (Striga resistant) and Six males (Provitamin-A rich) were crossed using a North Carolina II design to generate 36 single-cross hybrids. The hybrids were evaluated with 4 hybrids checks during the 2021B growing season at 4 locations under three on-farm Striga-infested fields and one optimal growing location in Uganda. Additive gene effects were moderately greater than non-additive effects for grain yield. The gene action controlling Striga resistance on Striga infested farmer-fields showed a predominance of additive gene effect for the number of Striga plants that emerged by 12 weeks after planting. Hybrids STR1004xCLHP0046 and STR1004XCLHP0352 supported fewer emerged Striga plants and were the top two highest yielding single crosses. They should be further tested for consistent performance for possible commercialization in Uganda. Hybrids STR1003xCLHP0286, STR1003xCLHP0476, STR1162xCLHP0352, CLHP1259xCLHP0046, and STR1003xCLHP0005 were identified as Striga-tolerant because they had reduced Striga damage and have maintained relatively good yield despite supporting the growth of a high number of Striga plants.

Keywords: Striga Hermonthica, Provitamin A, gene action

25. Inheritance of resistance to witchweed (*Striga hermonthica* (del.) Benth) in maize under low and optimum nitrogen levels in Uganda

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Striga (*Striga hermonthica* (del) Benth) and low nitrogen are major yield reducing factors in maize production in Uganda. Four Striga resistant inbred lines, four low nitrogen stress tolerant inbred lines and two inbred line testers of heterotic group A and B were crossed in a 10 x 10 half-diallel mating design. The seed of 42 hybrids successfully generated were evaluated in a 6 x 7 alpha-lattice design, during 2018A. The trials were planted on-farm under natural Striga infestations in Kasese and Bukedea districts; 3-meter plots were used with three replications. In each location, two levels of nitrogen (i.) no nitrogen added, and (ii.) 70 kg ha^{-1} of nitrogen were added. Data on Striga emergence, Striga damage syndrome and grain yield were recorded. The General combining ability (GCA) of the parents and Specific combining ability (SCA) of hybrids for resistance to Striga, and grain yield were estimated. Both additive and non-additive gene actions were found

to condition the inheritance of resistance to Striga under low (no nitrogen added) and optimum (70kg ha^{-1} of nitrogen) nitrogen. However, non-additive gene action predominantly controlled *Striga* emergence, Area under Striga number progress curve (AUSNPC) and additive gene action controlled *Striga* damage rating under low nitrogen. Under optimum nitrogen, additive gene action controlled Striga damage at 10WAP and AUSNPC while non additive conditioned Striga emergence. Grain yield was predominantly controlled by non-additive gene action under both environments. Maize parental line TZISTR1146 displayed negative GCA effects for resistance to Striga and positive GCA effects for grain yield under both low and optimum nitrogen environment hence could be used as a source of genes for resistance to Striga. Also, hybrids TZISTR1146/CML443, and CML395/CML312 exhibited resistance to *S. hermonthica* under the two environments. We gratefully acknowledge Alliance for Green Revolution in Africa (AGRA) for funding this research and National Crops Resources Research Institute (NaCRRI), Makerere University Regional Centre for Crop Improvement (MaRCCI) and Makerere University for their collaboration which has eased conducting this research.

Keywords: low and optimum nitrogen, combining ability

26. Identification of Molecular Markers for Deployment in Accelerated Breeding of Maize Resistant to *Striga*

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Striga hermonthica is a widespread, destructive parasitic plant that causes substantial yield loss, ranging from 60 to 100%, to maize productivity in sub-Saharan Africa (SSA). To accelerate maize genetic improvement for *Striga* resistance through molecular tools, a genome-wide association study (GWAS), comprising 150 diverse maize inbred lines with contrasting responses to *Striga,* was performed. The lines were genotyped using Genotyping-by-Sequencing (GBS) platform and phenotyped for resistance to *Striga hermonthica* for two years at two locations in Nigeria. Thirty quantitative trait nucleotides (QTNs) were identified across the multiple environments to be significantly associated with *Striga hermonthica* resistance, and 88% of the lines showed resistance/tolerance to *Striga* infestation with high heritability values. Three of the identified QTNs (5_216138908, S7_173626143 and S10_4274477), located on chromosomes 5, 7, and 10, were found to be strongly associated with *Striga* resistance-related traits based on marker prediction analysis. These QTNs were used to develop Kompetitive allele-specific PCR (KASP) assays. Following a successful verification of the assays, over 1500 maize lines, including early generation materials, fixed lines and families segregating for *Striga* resistance, were genotyped. The KASP markers were very effective in differentiating the resistant (> 86%) and susceptible (<13%) genotypes. Functional analysis of the genomic regions harboring the QTNs is underway. This finding corroborates the benefit of integrating marker-assisted selection (MAS) at the early generation selection. Genetic improvement of maize can be accelerated by integrating such genomic tools into the breeding scheme, thereby improving the efficiency of *Striga* resistance breeding and significantly reducing the expenses in field inputs, maintenance, and evaluation of material over the years.

Keywords: Maize, *Striga,* Marker-assisted selection, Quantitative trait Nucleotide, Kompetitive allele-specific PCR (KASP)

27. Microbe-induced barriers to Striga parasitism in sorghum roots

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Sorghum bicolor is one of the most important cereal crops in the world, predominantly grown in subSaharan Africa by smallholder farmers. Despite its outstanding resilience to the abiotic stresses, around 20% of sorghum yield is annually lost on the African continent due to the infestation with the parasitic weed, Striga. Existing Striga management strategies often show low efficiency and are not easily integrated into current agricultural practices. Microbial-based solutions may prove an effective, low-cost mode for reducing Striga parasitism in sub-Saharan Africa. We identified a field soil whose microbiome component suppressed Striga infection. This soil microbiome promotes endodermal suberization as well as formation of aerenchyma, both coinciding with fewer Striga attachments to sorghum root. Moreover, in a presence of microbes we observed a depletion of haustorium inducing factors (compounds essential for Striga to establish the host-parasite association) and an increased level of the products of their degradation. With modeling approach, we predicted which microbial strains reduce Striga parasitism via identified mechanisms. Individual strains of identified microbes were tested for their capacity to degrade haustorium inducing factors, induce formation of aerenchyma and deposition of suberin. Our results provide a framework for future developments of microbial-based solutions for Striga-infested fields.

Keywords: Bicolor, resilience, Striga, Suberization

28. Deploying Striga Smart Sorghum

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Crop losses caused by parasitic plants of the genus Striga pose a great danger to livelihoods of millions of smallholder farmers in Africa. The most efficient and cost-effective way to control Striga infestations would be to develop crops that are resistant to Striga. We have done extensive searches that have led to the identification of cultivars and wild relatives of crops that are resistant to Striga parasitism (Striga Smart Sorghum). The overarching aim of this project is to develop an innovation platform for deployment of sorghum varieties with durable resistance to Striga. Firstly, we are working with local farmers and extension officers to select – from a set of Striga resistant varieties already tested under laboratory and field conditions, sorghum varieties with preferable traits. In this participatory variety selection process, farmers will be able to grow sorghum varieties in their farms before making their selection. As a result, we will be able to identify farmer preferred, locally adapted sorghum varieties with improved potential to maintain resistance in farmers' fields. Secondly, we are using genomics to determine how Striga adapts to the environments and determines host compatibility. This data will help develop a well-informed deployment strategy for Striga resistant sorghum based on; i) eco-geographic regions with the most aggressive (described as virulent) Striga and ii). Compatibility/incompatibility between Striga ecotypes and selected sorghum varieties. Selected

varieties will then be disseminated as “StrigaSmartSorghum”. Finally, we are using nutrition to enhance Striga resistance and tolerance in sorghum. Our preliminary results reveal the important role played by nutrients in overall crop well-being and specific roles in maintenance of crop defense.

Keywords: Striga Smart Sorghum, resistance, virulence, nutrition, farmer preference

29. Germination of the mistletoe *Phoradendron quadrangulare* (Santalaceae): effects of substrate type, presence of fruit coat and host species

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Mistletoes are the most diverse and widespread group of parasitic plants worldwide, being key species in some ecosystems, infesting tree canopies and causing great losses in tree growth and wood production. However, the ecology of mistletoes is still poorly understood. Mistletoe seeds typically show high germination rates (close to 100%) and can germinate on substrate types such as concrete, wood, rocks, and several trees. Regardless of that it is not clear why some seedlings can successfully connect and grow haustoria in some tree species and not in others. We performed two experiments in order to analyze the germination, survival, and attachment rate of *Phoradendron quadrangulare* (Kunth) Griseb. (thereafter referred as *P.q.*): one experiment testing the presence/absence of fruit parts and other comparing the germination success among substrates and tree species. We collected fruiting branches of the parasite and manually selected 800 mature fruits. These seeds were set to germinate in two different substrates: *i*) styrofoam trays with filter paper, including 100 seeds still attached to the squeezed fruit coat (exocarp+mesocarp+viscidic layer) and 100 seeds where the fruit coat was removed and the sticky viscin was kept. Both settings were observed weekly, and the germination stages were recorded using a stereomicroscope for three months. Such experiment was conducted on a greenhouse and on a laboratory bench; *ii*) three different potential host species, commonly found in São Paulo city. We used 800 seeds, attaching 20 seeds presenting viscin per potential host. The potential host species were: *Handroanthus chrysotrichus* (Bignoniaceae), a native species seldom found parasitized by *P.q.*; *Melia azedarach*, an exotic species to Brazil, very frequently parasitized or completely infested by *P.q.*; and *Schinus mole*, a native species never parasitized by *P.q.*, but whose family (Anacardiaceae) is a frequent host to *Phoradendron* spp in the Neotropics. The germination of these seeds in the trees was recorded for four months. Our hypothesis was based that: 1- pericarp removal positively affects germination rate; 2- host species affects seed/seedling survival. The germination and mortality rates using different substrates were compared by chi-square tests. Our results showed that the germination rate after 50 days is 37-45% in trays and 19-32% on host trees. The removal of the fruit coat resulted in twice the mortality (12%), which might be explained by a potential physical damage to the embryos during coat removal. The germination rate on *M. azedarach* was, the greatest 86% after 50 days and 96% after 100 days while the mortality was the lowest among all hosts, 4% after 50 days, 13% after 100 days. The germination rate on *H. chrysotrichus* was 67%, after 50 days, and mortality rates were 33% after 50 days and 47% after 100 days. In *S. molle* the germination was almost 20% after 50 days and 53% after and100 days. The development of adhesive disc occurred first in seedlings on *M. azedarach* and after 100 days they were formed in 41%, while 32% in *H. chrysotrichus* and 14% in *S. molle.* The transference of seedlings from trays to trees was a complete failure for all tree species. All the results coincide with what is seen in the nature and explain partially the finding that *M. azedarach* gets to be a host very infested in the areas where it is an invasive species while *H. chrysotrichus* is rarely parasitized. *S. molle* was never found parasitized in nature and no seedling survival occurred in our experiments, suggesting that there is something in its bark that avoids seedling development and enhances mortality.

Keywords: germination, host preference, viscin, Santalales

30. Thiourea derivatives as novel and potent inducers of *Striga hermonthica* seed germination

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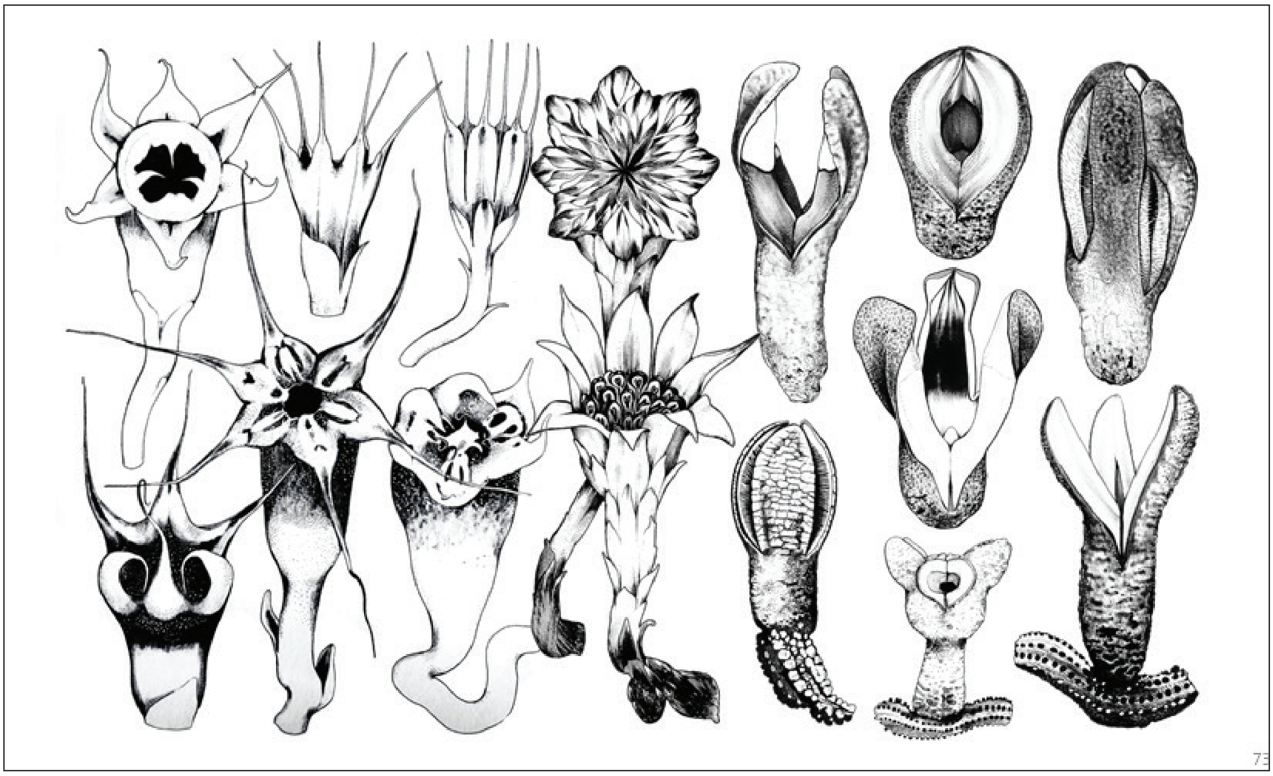
Striga hermonthica, or whichweed, is a kind of root parasitic weeds which confers serious damages on crops in Africa. In sub-Saharan Africa, up to 40 million hectares of farmland is infected by *Striga*, reducing yields by 20 % to 100 % [1]. *Striga* Suicidal germination is one of the options for combating *Striga*, which is attracting attention nowadays. Utilizing the nature of *Striga* that cannot survive in the absence of host, this strategy contributes to reducing the seedbank of *Striga* by applying synthetic germination stimulants in the absence of the host [2]. Ethylene is a gaseous plant hormone that is well known for promoting ripening of fruit and senescence of flower. Ethylene is also known to promote germination of *Striga* and has been used to cope with *Striga* in America. However, the gaseous nature of ethylene makes its application difficult and expensive. Therefore, we targeted the discovery of solid compound which shows ethylene-like-activity on plants and carried out exploration of lead compounds by screening, analysis of mechanism of action, and structural development of lead compound. Firstly, selection of compounds which promotes *Striga* seed germination from chemical library (11,000 compounds, Maybridge) was carried out and 12 candidates were selected. Secondly, ethylene-like activity of candidates was evaluated using etiolated seedlings of *Arabidopsis thaliana*. So-called triple response was used as the indicator of ethylene-like activity, and one compound with thiourea skeleton was selected. The compound was named ZT1. Then, whether ZT compounds activate ethylene signaling pathway was tested using *A. thaliana* ethylene insensitive mutant (*ein2*), ethylene biosynthesis inhibitor (AIB), and ethylene action inhibitor (STS). The result indicated that ZT compounds could act upon ethylene receptor. Lastly, structural development from ZT1 was carried out and their activity of inducing *Striga* seed germination was examined. As a result, compounds that show higher activity than ethylene precursor 1-aminocyclopropane-1-carboxylic acid were obtained.

Keywords: *Striga*, Ethylene, Suicidal germination



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