CHARACTERIZATION OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* L.) GENOTYPES FOR RESISTANCE TO PARASITIC PLANTS, *ALECTRA* AND *STIGA GESNERIOIDES*

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Abstract
Thirteen Bambara groundnut genotypes and a susceptible cowpea line (IT84S-2246-4) are being evaluated for their response to two legume parasitic weeds, Alecta vogelii and Striga gesnerioides. The haustorial development of Striga gesnerioides on the cowpea line is being demonstrated. The main objective of this project is to identify Bambara groundnut genotypes that are tolerant/resistant to these parasitic weeds and, therefore, select superior genotypes for further genetic improvement of this orphan crop for food, nutrition and environmental security. The cowpea line and 13 genotypes are being screened in triplicates using a random pot experiment design in nutrient-poor soil in a screen house. All the Bambara groundnut genotypes studied are resistant to the parasitic weed Striga gesneroides because there is no single shoot count in the pots containing all 13 genotypes in this study. The parasitic weed, Alectra vogelii, parasitizes all 13 genotypes studied, suggesting that no genotypes are resistant to Alectra in this screen. Significant differences exist in the number of Alectra shoots per pot for the Bambara groundnut genotypes ($p<0.001$).

The number of emerged Alectra plants per pot is high (11-14) on the susceptible cowpea line IT84S-2246-4, readily infected by Striga gesnerioides. The haustoria penetrates the endodermis and becomes established in the susceptible cowpea line. Bambara groundnut genotypes are better tolerant to legume root-parasitic plants than cowpeas in parasitic weeds-infested soil. Improved understanding of parasitic weeds tolerance/resistance mechanisms in Bambara plants, which may be closely related to the amount of endogenous strigolactones produced by Bambara groundnut, is explored in a related TETFund IBR-funded project.

Key words: Bambara groundnut, resistance, tolerance, parasitic weeds, haustorial, *Alectra vogelii*, *Striga generioides*

Introduction

Given the impact of climate change, more food would be needed to feed 10 billion people by 2050, with less use of fertilizers and agrochemicals (Ranganathan *et al*., 2018). However, over-dependence on a few crops (e.g., rice, wheat, maize and soybean) could limit our understanding and options of how best to respond to food security threats (Ebert, 2014; Khoury *et al.*, 2014). An alternative approach is to revisit underutilized crops that staple crops have displaced in recent centuries. Underutilized crops are an integral component of more complex farming systems in the tropics and bear a rich food, nutritional and cultural history for the poorly resourced farmers in sub-Saharan Africa. This is particularly true where the crop has been reported to have trait values beyond the range of those found in modern staple crops, for stress tolerance, for example. In the case of Bambara groundnut, an African native legume, the limited scientific reports and farmer evidence suggest that it is more drought tolerant than the introduced South American peanut. Although considered to be rich in protein, able to fix nitrogen, highly drought tolerant and reasonably resistant to root parasitic plants (Alectra and *Striga gesnerioides*), Bambara groundnut is an example of a tropical crop that is under-researched by the international scientific community and underutilized by a fast-evolving world food system that is overly-reliant on too few crops. In order to tackle the challenges of food and nutritional security, it is imperative to conduct extensive research to unveil the genetic potential of essential crop species, especially in the face of changing climate and the emergence of threats posed by pests and diseases.

Bambara groundnut is an important food security crop in developing African countries. However, the parasitic angiosperm plants *Striga* and Alectra are significant limitations to crop productivity and can severely limit yield by up to 100% (Kamara *et al*., 2008; Mbega *et al*., 2016). These root parasites entirely depend on the host plants' nutrients and water for development and survival. *S. gesnerioides* is known to be highly destructive of cowpea, its primary host crop in West African countries (Parker, 2012). *A. vogelii* parasitizes more host legumes including cowpea (*V. unguiculata*), Bambara groundnut (*V. subterranea*), groundnut (*Arachis hypogaea*), soybean (*Glycine max*), common bean (*Phaseolus vulgare*) and mung bean (*Vigna radiata*) throughout large parts of sub-Saharan Africa (Riches *et al*., 1992; Phiri *et al*., 2019).

Indeed, both parasites can occur in the same cowpea field (Singh & Emechebe, 1991). However, cowpea cultivars that are resistant or moderately resistant to *Striga* are also found to be susceptible to *Alectra* as in the case of APL-1, 87-
2, IT82D-849, Suvita-2, IT97K-205-8andIT98K-1092-1(Singh & Emechebe,1997; Omoigui *et al*., 2010). Such successful parasitism on highly drought-tolerant legumes such as Bambara groundnut and cowpea suggests that *Alectra* in Africa has the potential to undermine the struggle to attain food, nutritional and environmental security.

The principal objective of this TETFund IBR-funded project was the development of Bambara groundnut cultivars that have tolerance/resistance to parasitic weeds by exploiting natural variation in diverse Bambara genetic resources for resistance to root parasitic plants and enhanced capacity to deploy resistance traits into breeding programmes effectively. Characterizing germplasm for resistance to Alectra and *Striga gesnerioides* will facilitate the identification of resistant genotypes and, therefore, contribute towards developing improved Bambara groundnut cultivars with resistance to parasitic plants. The key objectives of this TETFund IBR grant awarded to the PI at the Federal Polytechnic Ekowe were achieved, and this report presents results on the characterization and identification of resistant/tolerant Bambara groundnut genotypes to Alectra.

Materials and Methods

Plant material

Thirteen Bambara groundnut (*Vigna subterranea* L.) genotypes derived from landraces that originated from different locations in Africa and Indonesia were used in the experiments (Table 1), including a susceptible cowpea line (IT84S-2246-4) obtained from the IITA Genetic Resources Centre. Seeds of these genotypes were multiplied and harvested in May 2022 from single plants in a greenhouse in the Niger Delta University, Bayelsa state. Seeds of the yellow witchweed (*Alectra vogelii* Benth.) were obtained from Henderson Research Station, Zimbabwe, via Dr Admire Shanyako, University of Kwa Zulu-Natal, South Africa. Dr Ousmane Boukar, IITA cowpea breeder in Kano station, supplied the seeds of cowpea witchweed (*Striga gesnerioides* (Willd.) Vatke.
Both parasitic weeds seeds are shown in figure1.

Table 1. List of genotypes used in the experiment for *Alectra* and *Striga gesnerioides* infection

Genotype	Location/Source of material
Ankpa 4	Ankpa, Nigeria
DodR	Dodoma, Tanzania
Dip C	Diphiri, Botswana
Getso	Kano, Nigeria
Gresik	Gresik, Indonesia
IITA-686	Tanzania
Kano 2	Nigeria
Mana	Ghana/Zimbabwe
S19-3	Namibia
Tiga nicuru	Mali
Uniswa Red-G	Manzini, Swaziland
Uniswa Red-R	Manzini, Swaziland
TVSu-745	IITA-Nigeria

Assessment of *Alectra vogelii* and *Striga gesnerioides emergence*

Thirteen Bambara groundnut genotypes were used for a pot experiment using a layer of sand infected separately with *Alectra vogelii* and *S. gesnerioides*, respectively, to examine differences in parasitic weed infection. The IITA cowpea breeding line, IT84S-2246-4 and Bambara groundnut accession TVSu-745 were used as positive controls. A mixture of low-nitrogen compost and sand in a ratio of 1:1 (v/v) was prepared. Seeds were germinated in 96-cell module trays for seven days and allowed to grow for an additional two weeks before being transferred to 2-L pots containing one-third of the compost-sand mixture, a thin layer of *Alectra*-or *S. gesnerioides*-infected sand, and then filled with the soil mixture. The *Alectra*-or *S. gesnerioides*- infected layer of sand contained 8 mg of each parasitic weed seed, mixed thoroughly with the sand before being

added to the pot. Plants were grown in a completely randomized design with three replicates under greenhouse conditions. For the remainder of the experimental period, the pots were only watered. *A. vogelii* emergence was first observed on Bambara groundnut genotypes eight weeks after infection, and total *A. vogelii* shoot counts per pot were made 12 weeks after infection. Although *S. gesnerioides* emerged on the susceptible cowpea line, no single *S. gesnerioides* shoot emergence on any of the Bambara groundnut genotypes.

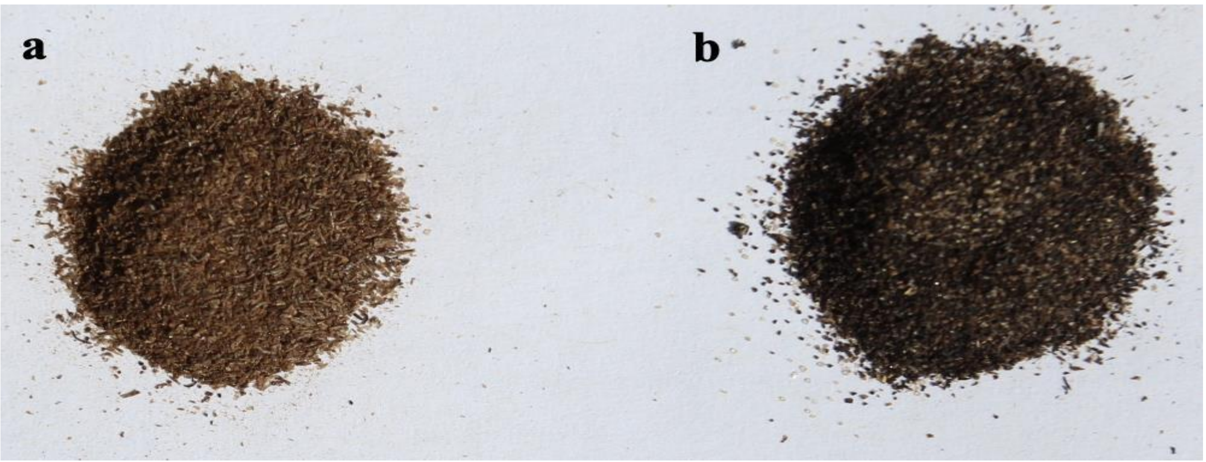


Figure 1: Seeds of the root-parasitic plants (a) *Alectra vogelii*, (b) *Striga gesnerioides*

Striga gesnerioides seed preconditioning and infection of susceptible cowpea roots

Striga gesnerioides seeds (300 mg) were surface sterilized with 2% (v/v) NaOCl containing 0.02% Tween 20 for 5 min. After rinsing 3-5 times with Milli-Q water and surface drying for 1 h in a laminar hood, *S. gesnerioides* seeds were pretreated (conditioned) on moistened glass- fibre filter paper (Whatman, Maidstone, Kent,UK) in Petridishes for 9-11days at 30°C before use. Preconditioned seeds were treated with aliquots of the synthetic strigolactone analogue (GR24), and sorghum root exudate (SRN39) or a 100-fold diluted for 2-6 hours. After 2weeks of the susceptible cowpea line growth in a rhizotron chamber, plants were inoculated with *S. gesnerioides* seeds by carefully placing the preconditioned seeds next to the roots of the cowpea plants.

Anatomical sectioning of *Striga gesnerioides infection of susceptible cowpea roots* Attachment for susceptible cowpea line, IT84S-2246-4 was studied for *Striga gesnerioides* parasitization (figure 2). To assess vascular connection,

roots infected with were randomly selected, segments were cut and immediately fixed overnight in a four °C fixation solution containing 4 % paraformaldehyde in 1xPBS (pH 7·2), 0.1 % Triton X-100 and 0.1 % Tween 20. Following fixation, parasite-infecting roots were processed through a 30-minute vacuum treatment and were left overnight in a vertical rotor at 4C. The following day, samples were washed for 2x30min in 1xPBS at 4°C at room temperature. Samples were dehydrated in a graded ethanol series and embedded in Spurr's low-viscosity resin (TAAB, S024/D). Embedded ultra-thin (500-800 nm thick) sections were cut with glass knives on a Leica Ultramicrotome EMUC6 (Leica) and dried onto coated microscope slides (Fisher Scientific Co., Pittsburgh, PA, USA). Sections were stained with 0.25% toluidine blue O in 1% Sodium Borate and observed with a Leica DM5000B microscope (Leica). Digital microscopic images were taken using a Leica DFC420C camera(Leica).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the GenStat software 16th Edition (VSN International Ltd, Hemel Hempstead, UK)

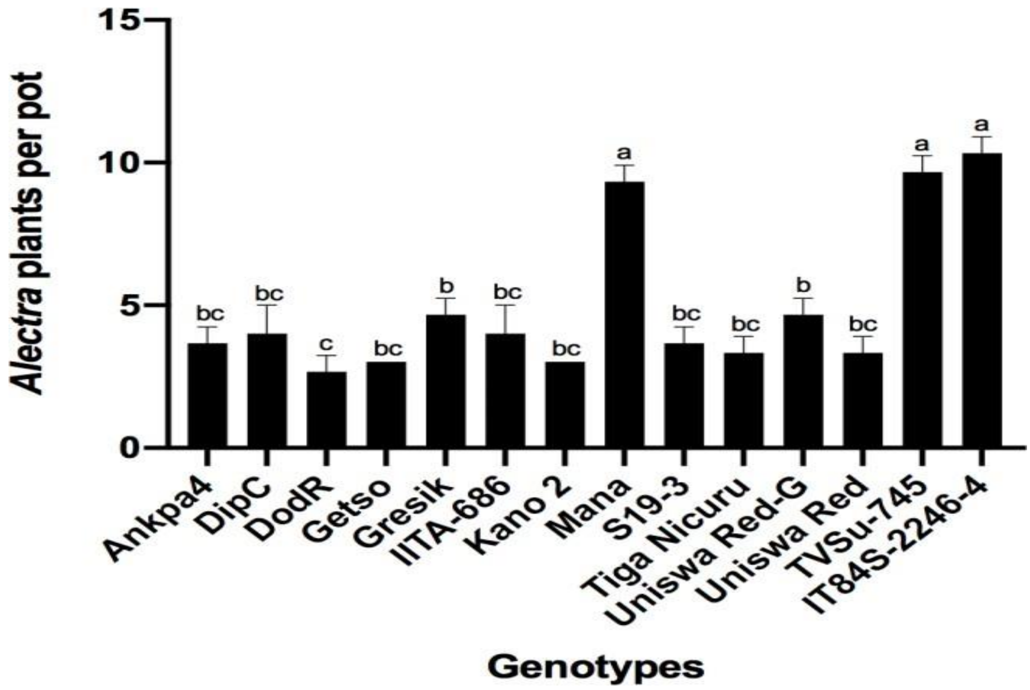
and GraphPad Prism version 8.03 for Windows (GraphPad Software, Sandiego, California USA) to observe the difference between genotypes and Alectra emergence. Means were compared for significant differences using LSD at a significant level of5%.

Results and Discussion

Alectra vogelii infection

To assess the effect of *A. vogelii* infection on Bambara groundnut, 13 Bambara groundnut genotypes and a susceptible cowpea line were grown in pots with soil infected with *A. vogelii* seeds. *Alectra* parasitized all the 13 genotypes studied, and the infection resulted in high (9 – 10) *Alectra* shoot count for some of the genotypes (Figure2). There were significant differences in the number of *Alectra* shoots per pot for the Bambara groundnut genotypes ($p<0.001$). The number of emerged *Alectra* plants per pot was high (11-14) on cowpea line IT84S-2246-4, and Bambara groundnut TVSu-745 but negligible (2 – 3) on DodR (Fig.2).

Figure 2: Emergence of *A. vogelii* plants in a greenhouse experiment.



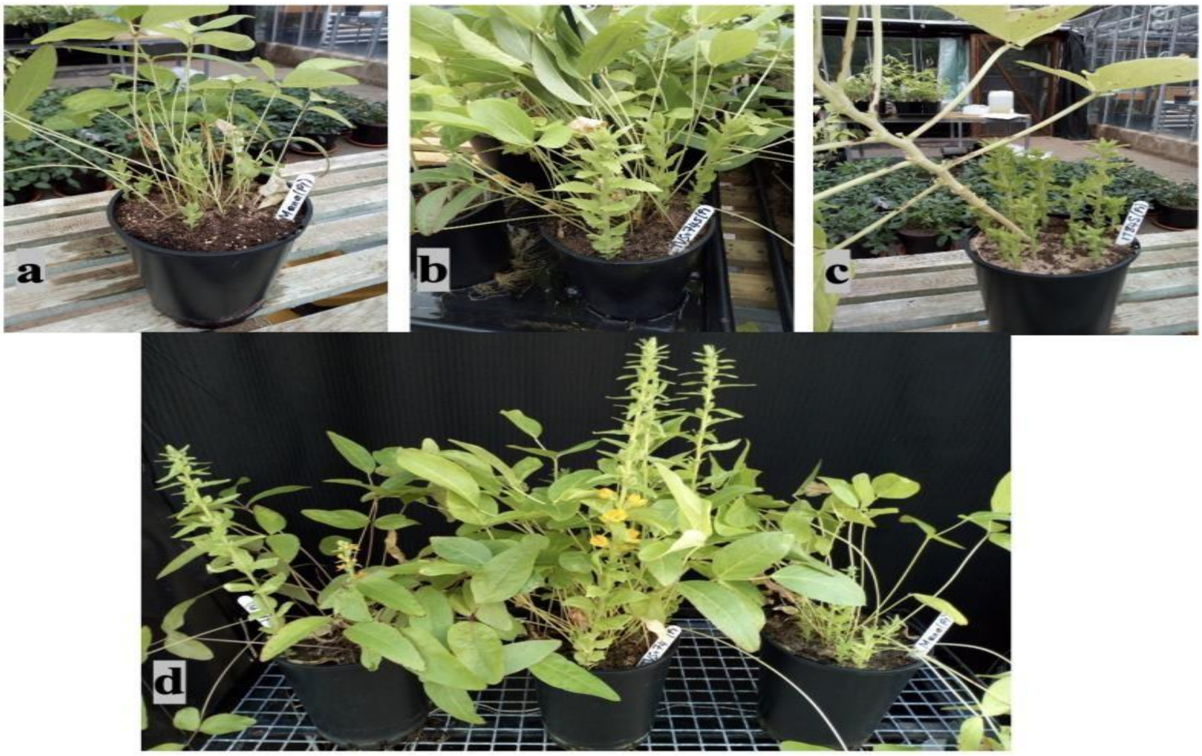


Figure 3: *Alectra vogelii* parasitizing Bambara groundnut and cowpea in pot experiments, (a) Mana, (b) TVSu-745, (c) cowpea line IT84S-2246-4, and (d) DodR, TVSu-745 and Mana

Figure 4: *Alectra vogelii* and *S. gesnerioides* parasitizing susceptible cowpea lineIT84S-2246- 4 in pot experiments (a) *Alectra vogelii* growing on cowpea roots, (b) *S. gesnerioides* growing on cowpea root.

Parasitization of susceptible cowpea line IT84S-2246-4 by S. gesnerioides

To observe *S. gesnerioides* infection processes with the host cowpea line, we employed an observation chamber rhizotron system (Gurney *et al*., 2006). Preconditioned seeds were placed next to the host cowpea line in the rhizotron chamber. Within a few days, S. gesnerioides seeds had germinated. At 2 weeks post-infection, the parasite continued development after the formation of the haustorial (figure 5). The germinating striga seed, the attachment to the host, and the invasion of the host root by a haustorium are depicted in Figure 5. A bulboushaustorium, formed at the tip of the radicle, made contact with the endodermis of the host root and pressed against cortical cells which became distorted as the haustorium penetrated into the cortex and ultimately came into contact with the endodermis.

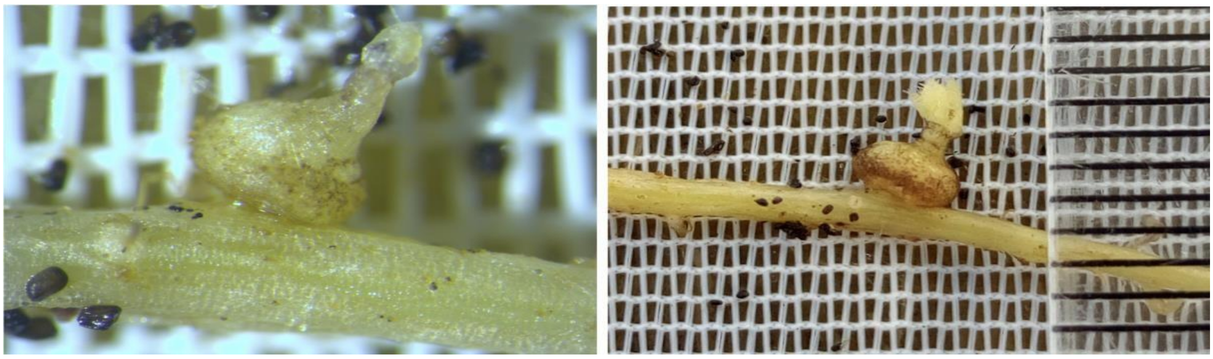


Figure 5: Growth and development of *S. gesnerioides* attached on susceptible cowpea line IT84S-2246-4 in rhizotron experiment (a) early stage, (b) advanced stage

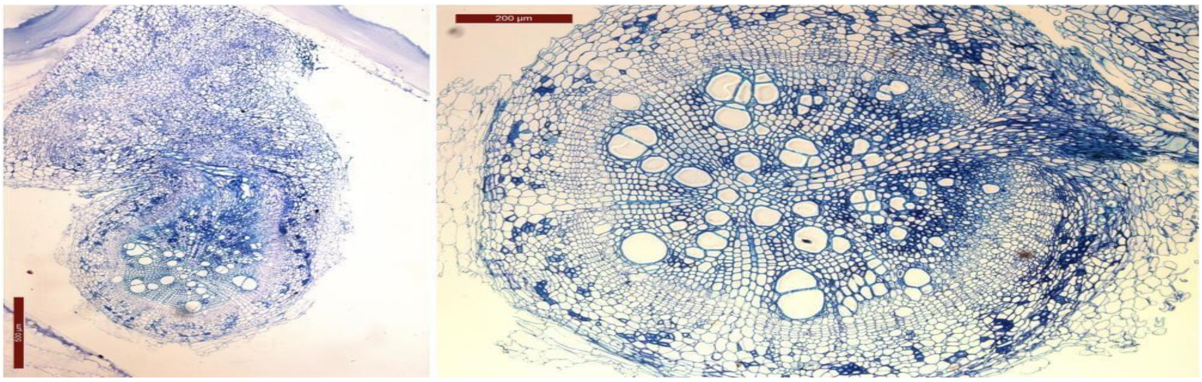


Figure 6: Anatomical sections of the penetration of roots of susceptible cowpea line IT84S- 2246-4 by *S. gesnerioides*

~~Our study has demonstrated that Bambara groundnut is resistant to S.~~

gesnerioides, but susceptible to *A. vogelii* based on the number of root-parasitic weeds infection. This finding is in good agreement with the parasitism of a broad range of legumes by an *A. vogelii* collection from Southern Africa (Riches *et al*., 1992). In general, complete resistance to all identified strains of *A. vogelii* is lacking in host species (Riches *et al*., 1992; Phiri *et al*., 2018). Therefore, the identification and understanding natural variation in response to parasitic weeds may extend the knowledge of the initial steps of parasitism by *Alectra* and *S. gesnerioides* and ecological interactions of plants with their biotic environment.

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