

Screening of Tobacco Genotypes for Tolerance/Resistance to *Striga gesnerioides* infestation in Zimbabwe

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Abstract

Witch weed (*Striga gesnerioides*) is a root parasitic weed of tobacco and cowpea in Sub-Saharan Africa. It is very difficult to control and host plant resistance is the most promising effective method affordable to small-scale farmers. This study was done to screen local tobacco genotypes for tolerance and resistance to *Striga gesnerioides* infection. Laboratory screening experiments were conducted during the 2016/17 season using fifteen local tobacco genotypes. The experiments were done using an Agar gel technique at the University of Zimbabwe, with treatments replicated four times in a Randomised Complete Block Design (RCBD). All tobacco genotypes significantly ($p < 0.05$) stimulated the germination percentage and there were significant differences ($p < 0.05$) in the germination distance of *Striga* seeds. The tobacco genotypes K RK66 and T74 had the closest *Striga* germination distance and the lowest germination percentages of *Striga* seeds, indicating their ability to produce low germination stimulants (strigolactones). This study revealed that all the tobacco genotypes were susceptible to *Striga* infection except the genotypes K RK66 and T74 which showed some level of tolerance and could be adopted by farmers in *Striga* infested areas. Additional evaluations, targeting the parental lines of the tolerant cultivars is recommended as further studies.

Keywords: *Striga gesnerioides*; Tobacco; Strigolactones

Introduction

Witch weed (*Striga gesnerioides*) is one of the most devastating parasitic weed of broad-leaved crops in most parts of the world. It is an obligate root parasitic flowering weed that belongs to the *Orobanchaceae* family [1]. The germination of *Striga* is induced by strigolactones which are *Striga* germination stimulants found in root exudates of both host and non-host plants [2]. In Zimbabwe *S. gesnerioides* was reported on tobacco in the Mvuma and Chegutu growing areas, by both small and large-scale growers [3]. However, there is little information on tobacco leaf yield losses caused by this parasite although, 100% yield losses were reported in the Mvuma area under severe infestations [4]. Notably, the biotype of *S. gesnerioides* affecting tobacco in the Mvuma area did not affect cowpea, another known host of the parasitic weed. *Striga gesnerioides* is a problem on cowpea in African countries such as Nigeria, Kenya and Mali [5]. In Botswana, it was reported on sweet potato [6]. In cowpea yield losses resulting from *S. gesnerioides* infestations in the Northern Nigeria ranged from about 83 to 100%. For the control of *S. gesnerioides*, several strategies, including the use of nitrogen (N) fertilizer, host plant resistance, intercropping, crop rotation, suicidal germination in a trap crop system and herbicide control have been proposed [4,5]. However, for the control these measures have not been widely adopted by farmers in Zimbabwe, probably due to lack of resources, especially the smallholder farmers.

Host plant resistance is an effective and affordable option for the control of *Striga*. Cowpea varieties (B301, IT97K-499-35, IT84S-2049 and IT98K-205-8) have shown complete resistance to *S. gesnerioides* [5]. The commercial tobacco cultivars (K RK66, and K RK26) were

reported to be tolerant to *Striga* under field trials in the Mvuma area compared to landraces [3]. The commercial tobacco varieties cultivated by farmers were not bred to be *Striga* resistant and were not evaluated for *Striga* resistance/tolerance. Currently, there is no breeding work being conducted at Kutsaga with the aim to improve tolerance to *Striga*. This research was thus targeted at identifying resistant/tolerant genotypes which can be used in breeding programmes and to elucidate their mechanisms of resistance to *Striga*.

Methodology

Study site and plant materials

The experiment was conducted in the Plant Pathology Laboratory of the Crop Science Department at the University of Zimbabwe. Fifteen local tobacco genotypes from the Tobacco Research Board were used in this experiment. These comprised of eight open release varieties (KM10, K RK22, K RK26, K RK28, K RK29, K RK60, K RK64, and K RK66) and seven limited release tobacco varieties (T70-T76). The experiment was laid out in a Randomized Complete Block Design (RCBD) with sixteen treatments (15 tobacco genotypes and water as a negative control) in two blocks. The treatments were replicated four times in each block.

Surface disinfection of *Striga* seeds

Striga gesnerioides seeds that were collected from the infested fields of smallholder farmers in the Mvuma area were surface disinfested in order to destroy microbial contaminants. This was done by immersing the desired amount of *Striga* seeds in 1% sodium hypochlorite for two minutes. The floating seeds and chaff were discarded and the *Striga* seeds were washed with sterile water on a filter paper fitted in a funnel.

Pre-conditioning of *Striga* seeds

Striga seeds were preconditioned following the method [7] described by Berner et al. Surface disinfested *S. gesnerioides* seeds were placed in a sterile Petri dish with 30 ml of sterile water and stirred until they sunk. The Petri dish was covered with aluminium foil in order to exclude light and incubated at 28°C for 14 days, changing water after every two days using a pipette.

Selecting for hosts with low stimulant production

The agar gel technique [8] was done following the procedure adapted from Reda et al. The surface disinfested *Striga* seeds were used in this technique. Host seeds were germinated by placing them on wet filter papers in petri dishes and incubating at room temperature for 14 days. Water agar was prepared by dissolving 6.8 g of bacto agar in 1000 ml of deionised water and the solution autoclaved at 120°C for 20 minutes to eliminate microbial contaminants. The agar was allowed to cool for an hour before pouring. Fifty micro-millilitres (approximately 150 seeds) of conditioned *S. gesnerioides* seeds were pipetted into sterile petri dishes with a 9 cm diameter. Thirty millilitres of autoclaved water agar was poured over the *Striga* seeds allowing random distribution of the *Striga* seeds on the plate. One tobacco seedling was submerged in the solidifying agar near one edge of the plate with the root tip pointing across the plate. The plates were incubated in the dark at 28°C for 48 hours

Data collection

***Striga* germination percentage:** The germinating seeds were observed using a dissecting microscope at 100X magnification focusing through the bottom of the petri dish. Four sampling areas were randomly selected in each of the petri dishes and the germinated *Striga* seeds were counted and germination percentages calculated.

***Striga* germination distance from the host root:** The distance from the tip of the tobacco seedling to the furthest germinated *Striga* seed was measured using a microscope micrometer calibration ruler on a dissecting microscope at 100X magnification.

Data analysis: Analysis of variance (ANOVA) for the *Striga* germination data was done using Genstat version 14. Treatment means were separated using 5% LSD (Fisher's Protected LSD). Graphs were plotted using ggplot2 of R Statistics version 3.1.3.

Results

***Striga* germination percentage influenced by different varieties:** The tobacco genotypes released *Striga* germination stimulants that significantly ($P < 0.05$) stimulated the germination of *Striga* seeds. An open release tobacco cultivar K RK66 had the lowest *Striga* germination percentage and the tobacco genotypes T75, K RK28, K RK64, T72 and T73 had the highest *Striga* germination percentage as shown in the figure 1. In contrast, in the negative control treatment (water), no *Striga* germination was observed, as expected.

Striga germination distance from the host root

The germination distances of *Striga* seeds were significantly different ($p < 0.05$) among the cultivars. The tobacco cultivars K RK28 K, RK64 K, RK71 and T75 had the furthest *Striga* germination distance (Figure 2). These cultivars were not significantly different in terms of

Striga germination distances. In contrast, the tobacco genotypes T74 and K RK66 had the shortest *Striga* germination distance.

Discussion

Striga germination percentage

The results of the Agar gel assays revealed that the tobacco genotypes K RK28, T75 and K RK64 stimulated high *Striga* seed germination, suggesting that they produced high *Striga* germination stimulants [9,10]. These tobacco varieties are susceptible genotypes as they stimulate high germination of *Striga* seeds, thereby, increasing the chances of many *Striga* plants to be attached to the host roots. In previous laboratory screening trials K RK28 and K RK64 showed above 60% stimulation [3]. The tobacco cultivar K RK66 was associated with low *Striga* germination, suggesting low release of *Striga* germination stimulants [11]. The genotype was, thus, the most *Striga* tolerant of the tested varieties based on *Striga* seed germination and this confirmed previous observations under field conditions [3]. However, in the previous laboratory screening experiment, this tobacco cultivar was comparable with other cultivars evaluated in *Striga* germination studies [3]. The differences in outcome observed could be as a result of the fact that the age of the tobacco seedlings and the screening methods used in the two studies were different. Further laboratory studies are needed to investigate the germination of *S. gesnerioides* seeds stimulated by the host at different ages.

Furthest *Striga* germination distance

A high stimulation of *Striga* seed germination and a furthest germination distance of *Striga* seeds was associated with K RK28, suggesting the host produced high amounts of strigolactones [11]. This implies susceptibility to *Striga* infection since the host could stimulate *Striga* seeds that were located far away from the roots to germinate, potentially resulting in many *Striga* attachments. The least *Striga* germination distance was recorded from T74 and K RK66. Both stimulated low *Striga* seed germination suggesting low production of *Striga* germination stimulants. Tobacco genotypes with low germination stimulant (LGS) produce insufficient amounts of the exudates required for germination of conditioned *Striga* seeds. Genotypes producing low levels of germination stimulants were found to be resistant to *Striga* in field tests, and this low germination stimulant (LGS) trait was found to be inherited as a single, nuclear, recessive gene with largely additive gene action [2]. In studies done with sorghum, all highly susceptible genotypes were high producers of the germination stimulant. Most *Striga* seeds close to the growing tip of the tobacco seedling germinated better when compared to seeds that were close to the older part of the roots. This indicated that root exudation was active in the root tip of the tobacco seedling. This is in line with the report by Okonkwo [12] which states that smaller quantities of strigolactones are produced by the older root cells of the host plant.

Conclusions and Recommendation

The study revealed that the tobacco genotypes K RK66 and T74 were the most tolerant to *S. gesnerioides* infestation as these produced low *Striga* germination stimulants. The most susceptible tobacco genotypes were K RK28, T75, K RK64, T72 and T76; and these genotypes produced more germination stimulants. The root exudates from susceptible tobacco genotypes induced enhanced *Striga* seed

germination. The tobacco genotypes K RK66 and T74 can be grown in *Striga* infested areas since these genotypes have shown to be tolerant to *S. gesnerioides* infection. Host avoidance of *Striga* can be implemented as a control measure in *Striga* infested areas of the country. However, there is need to conduct more laboratory studies to investigate the germination of *Striga* seeds using the parental lines of the tolerant tobacco genotypes and field evaluation is recommended to validate the results obtained from the laboratory studies.

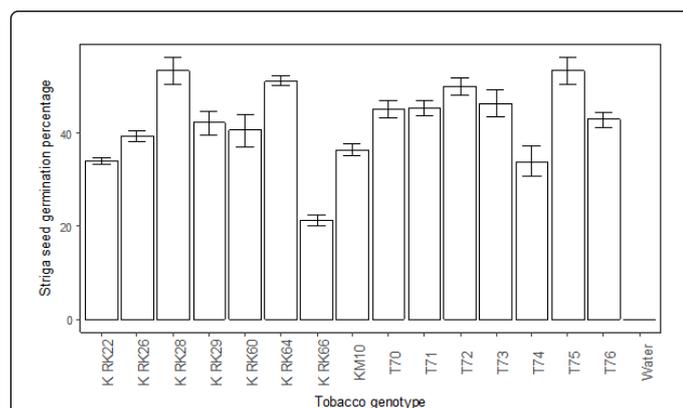


Figure 1: *Striga* germination percentages induced by different tobacco cultivars.

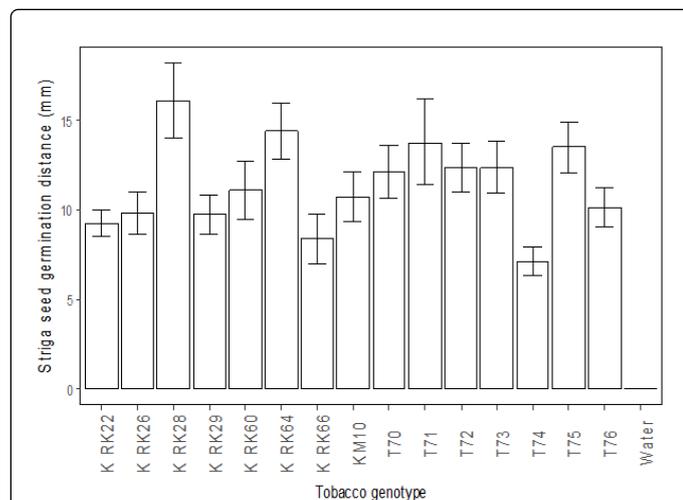


Figure 2: *Striga* germination distance from root tip of different tobacco genotypes.

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Disclosure Statement

The authors declare no conflict of interest.

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