RESPONSES OF *STRIGA HERMONTHICA* SEEDS TO DIFFERENTGERMINATION STIMULANTS PRODUCED BY TEN SOYABEAN, COWPEA AND GROUNDNUT VARIETIES IN NIGER STATE, NIGERIA

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ABSTRACT

Striga hermonthica (Del.) Benth. is a parasite on cereal and a limiting factor in achieving optimum yield in infested areas in Sub-Saharan Africa. The parasite seeds are stimulated to germinate by root exudates from host and non-host plants as well as by synthetic germination stimulants. However, legume cultivars vary in their ability to stimulate germination of S. hermonthica seed of same or different populations. Objectives of this experiments was to determine by laboratory cut roots assays of ten soyabean, cowpea and groundnut differences in their abillty to stimulate germination of S. hermonthica seeds. However, the varieties of the three trap crops used exhibited significant differences for their ability to stimulate the germination of Striga hermonthica seeds. Relative germination percentage was higher in soyabean variety TGX 1448-2E, cowpea variety IT04K-217-5 and groundnut variety RMP-91. The controls (GR-24 and water) recorded the highest and lowest germination percentages. The results obtained for the different legume varieties screened suggested that there is need to determine the inherent genetic differences among these varieties used in this study so that breeding could be done for increased stimulant production.

Keywords: *Soyabean, Cowpea, Groundnut, Stimulant, Striga hermonthica,*

INTRODUCTION

Maize and sorghum are the two most important cereal crops in Sub- Saharan Africa. Among the millions of smallholder farmers in the region, maize and sorghum provide the principal sources of food and income upon which rural livelihoods are based. To avoid continued reliance on food imports, cereal production shortfalls will need to be met through raising crop yields, which has been an on-going challenge. Cereals thus play a critical role in ensuring food security in Africa (Senthilvel et al., 2008).

Root parasitic weeds of the genus Striga constitute a major biotic constraint to cereal production in sub-Saharan countries, in particular pearl millet, sorghum and maize (Yonli et al., 2010). Striga also attack other crops including upland rice, sugar cane and wheat (Amusan et al., 2008). Indeed, Striga are the major and persistent biotic threat to production of these crops mostly grown on the hottest and driest marginal regions of sub-Saharan Africa, Middle East and large part of Asia (Parker, 2012). A Striga plant can produce tens of thousands of tiny seeds and can remain dormant in the soil for many years (Nickrent. D.l and Musselman, L. J., 2004).

West Africa Striga has infested 17 million ha of arable land; 3million ha is reported in eastern and 1.6 million ha in southern Africa (Gressel, 2004). Striga hermonthica is not particular to soil type or pH and can be abundant in lighter soils in West Africa as well as on heavy clay soils in East Africa (Muhammed et al.; 2001). Crop yield losses due to Striga attacks range from a few percent to complete crop failure and depend largely on the cereal host species and variety grown, rainfall distribution, soil fertility and the Striga seed density in soil (Haussmann et al., 2000). The annual yield loss has been estimated to exceed US$10 billion (Pennisi, 2015). More recently, S. hermonthica has been identified as one of the seven most severe biological constraints to food production. Thus Striga present a worrying problem to subsistence farmers with small land holdings (Pennisi, 2010).

Deployment of several management strategies, including quarantine imposed on infested areas, control of movement of farm equipment between infected and uninfected areas, intensive herbicide application has resulted in the control of the witchweed (Van Mourik, 2007). Other control measures developed are breeding crops that cannot be attacked by Striga (Abdalla et al.; 2010), imposition of suicidal germination in the field, before planting the crops. Striga seeds present in the soil are induced to germinate by injecting ethylene gas, which mimics the natural physiological response tied to host recognition and because no host roots are available the seedlings die (Mourik, (2007). Control of ethylene injection is an expensive method and not generally available to many farmers in developing nations of Africa and Asia beside that, the method does not remove all seeds from the soil. Other studies showed that ethylene derived from soil-borne pathogens could be used to induce suicidal germination of the parasite

d thus deplete the seed reserves (Hassan et al.; 2010). Some practices like trap-crops, use legume plant to mulate suicidal germination of Striga seeds to reduce the seed bank and improve soil fertility (Sculz et al., (2003). e adaptation of obligate parasitic weeds to respond to host plant excreted germination stimulants provide them h an evolutionary benefit that ensures the seeds only germinate in the vicinity of active, viable host plant roots. re recent studies have shown that germination of Striga is not host specific but showed that not only do wild estors of sorghum and millet induce Striga seed germination (Van Mourik, 2007), but also non-host plants, luding some tree species (Yonli et al., 2010). Most of these non-host plants do not permit attachment of the site to their roots with consequence that germinated Striga seeds are not able to survive and reproduce. This ess, often referred to as suicidal germination, contributes to the reduction of the Striga seed population in the so Despite many control methods that have already been proposed, the infestation by this parasitic weed continues (Arianjaka et al.; 2007).

Hever, for most African cereal growers, the most appropriate method would be one that uses a simple and ineensive technique adapted to their farming systems. One such simple, yet promising control method, is the use of n-hosts or trap crops. These crops have root exudates that stimulate Striga seeds to germinate but the germated Striga plants cannot parasitize them.

MAERIAL AND METHODS

Thisudy was carried out in the microbiology laboratory of the Federal University of Technology Minna. The expenent was carried out in three sets involving the ten varieties of each trap crops (soyabean, cowpea or grounut). The varieties and accessions were screened for their ability to stimulate Striga seed germination in vitro,sing the cut-root procedure as described by Lane and Bailey (1990).

The dign of the experiment was a completely randomized design (CRD) with four replications.

Procere of the cut-root techniques

(a) Sterilization (surface disinfection) of seeds

The tracrops and germinable *Striga* seeds were sterilized with 1% solution of sodium hypochlorite to be made up to 100s with sterilized deionized water. The sterilization of test crop was done for each variety separately; 30 mls of the nstituted sodium hypochlorite solution was poured into the seeds in separate beakers and stirred to mix thorougy and left to stand for five minutes. The solution was drained off together with floating seeds and debris.Can distilled water was then added to the seeds and the mixture was poured into funnels lined with filter paper. The seeds were left to dry over night. All the filter papers containing seeds for drying were properly labelled. After drng, the *Striga* seeds were ready for pre-conditioning and the crop seeds ready for planting.

(b) re-conditioning of *Striga* seeds

A standa punch was used to make small disks of glass fiber paper. The disks were put in a wide petri dish and wetted w distilled water. About 180 or more disks from a standard punch were arranged in a 9 cm petri dish for conditiong. Two pieces of 11 cm whatmann filter paper was placed in a sterilized 11 cm petri dish and moistened with distid water. The petri dish was sterilized by wiping with cotton wool moistened with methylated spirit. The moist dis were picked with sterilized forceps and arranged in circles to cover the moistened filter paper in the petri dish The petri dishes were then carried to a sterile hood.The sterilized *Striga* seeds were then dabbed on the pre-arrang glass fiber paper disks in the petri-dish. The lids of the petri dishes were sealed with laboratory film (Para filmM') immediately after dabbing to conserve moisture. The dishes were wrapped with aluminum foil sheets to clude light and kept in the incubator at a temperature of 28^{0}C for conditioning for two weeks (14 days) in the dark.

On the sanday, plastic pots or polybags were filled with 1 kg each of topsoil, watered and labeled and the trap crops seedsown and allowed to grow for two (2) weeks. After which, the seedling was harvested for the experiment he trap crop seeds were watered during the period. Before harvesting the trap crop seedling, 2 cm diameter rit were made with aluminium foil and stored. These rings were made by measuring 3 cm wide foil and folding it in two. The folded aluminum foil sheet was then wrapped round a marker of 2cm circumference ('Mon' AMI super rmanent marker has this circumference). After wrapping the foil around the marker, the excess length was cut offd the edges sealed with cellotape. The ring was carefully pulled off from the tapering end of the marker and red.

(c) Setp of the experiment

After conditning the *Striga* seeds and growing test crop seedlings for 2 weeks, the experiment was set up as follows:

Trap crop seting was harvested with care to avoid damaging the roots

The roots were carefully washed with water to remove the soil.
The roots were kept in a petri dish and wetted to avoid drying out of the root and brought into the laboratory.
Before the above steps, sterilized 9 cm petri dishes were lined with double layer 9 cm whatmann filter paper moistened with distilled water and 2 cm diameter aluminium foil ring placed in the centre of the moistened filter paper in the petri dish. The pre-conditioned *Striga* seeds on micro fiber disks were removed from the incubator using a sterilized forcep. The disks containing pre-conditioned *Striga* seeds were then picked and arranged in 4 rows on the double layer moist whatman filter paper. Each row had 5 disks arranged radially from the aluminium foil ring in the center to the edge of the petri dish. The edges of the glass fibre disks were touching each other in the row, and the final disk also touched the aluminium foil ring.

After this, the root of the 4 day old seedlings of the trap crop was cut into tiny pieces (about ½ cm in length) using a sterile surgical blade and 1g root pieces weighed out from the crop. This was wrapped in separate aluminium foil and properly labelled. The 1g weight of root pieces was placed in the centre of the petri dishes. Using a micro pipette, 300ul of distilled water was added to the roots to provide enough wetting to enable diffusion of root exudates across the filter paper to the *Striga* seeds. In other petri dishes, the roots were replaced with either 300µl of a chemical stimulant, (GR-24, one of the *Striga* analogues, which triggers germination of *S. hermonthica* seeds) or sterile deionized water. These represent the positive and negative controls, respectively.All the petri dishes were correctly labelled and sealed with para film 'M' as soon as the stimulant was introduced, the petri dishes were then wrapped in aluminum foil and incubated at 28°C for 72 hours in the dark. The petri dishes were then removed from the incubator after 72 hours and viewed under a dissecting microscope. Both germinated and ungerminated *Striga* seeds were counted using a counter. The last disk in the row was discarded, because they could consistently record very poor or no germination of *Striga* seeds.

Data collection and Analysis

After 72 hours, the Petri dishes were removed from the incubation and viewed under a dissecting microscope. Both germinated and ungerminated *Striga* seeds were counted. From the data collected, the following were calculated:

Actual percentage germination of *S. hermonthica* seeds by test crop (%) or GR-24 (%).

$$= \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ incubated} \times 100$$

Relative germination (%)

$$= \frac{Actual\ \%\ germination\ (\%)}{Actual\ \%\ germination\ with\ GR-24} \times 100$$

Collected data was square-root transformed and analyzed using Genstat. Data was analyzed for each run separately because the percentage germination of *Striga hermonthica* seed by test crop could differ with run.

RESULTS

Among the varieties of soyabean screened in their ability to stimulate in-vitro seed germination in S. *hermonthica*. The relative germination percentage was highest in variety TGX 1448-2E (30.14%). Followed by higher percentage recorded in variety TGX1835-10E (19.40%) and TGX 1019-2EB (18.87%) while the lowest germination percentage occurred in variety TGX 1987-10F (3.92%), TGX 1987-96F (9.45%) and TGX 1937-1F (8.69%) (Table 1).

GR-24 and water gave the highest and lowest relative germination percentage (%) of *Striga* seeds (100% and 0.27% respectively) . In the soyabean three sets (runs) of experiment, the set two (2) had a superior percentage of seed germination (27.29%) when compared to other sets (1 and 3) (17.49% and 14.40%) (Table 1).
In the cowpea varieties screened in their ability to stimulate in-vitro seed germination in S. *hermonthica* (Table 2) The relative germination percentage was highest in variety IT04K-217-5 (10.84%) (Table 2). Higher relative germination percentage was also observed in variety IT07K-25-3-3 (9.50%) and IT04K-339-1 (9.44%) (Table 2) compared to the lowest relative germination percentage observed in variety IT04K-405-5 (2.65%) and lower percentage recorded in variety IT07K-237-2-1 (3.51%) and IT07K-293-3 (3.25%) (Table2). GR-24 and water gave the highest and lowest relative germination percentage of *Striga* seeds (100% and 0.03% respectively) (Table 2).

In the cowpea three sets (runs) of experiment, the set two (2) had a superior percentage of seed germination (17.65%) when compared to other sets (1 and 3) (10.94% and 12.51%) (Table 2). In the groundnut varieties screened in their ability to stimulate in-vitro seed germination in *S. hermonthica*. The relative germination percentage was highest in variety RMP-91 (13.04%) (Table 3). Higher relative germination percentage was also observed in variety RMP-12 (11.95%) and TE3 (10.13%) (Table 3) compared to the lowest relative germination

percentage observed in variety Groundnut-11 (1.21%) and lower percentage recorded in variety K-H241D (1.69%) and RRB (3.79%) (Table 3).

GR-24 and water gave the highest and lowest relative germination percentage of *Striga* seeds (100% and 0.14% respectively) (Table 48). In the groundnut three sets (runs) of experiment, the set two (2) had a superior percentage of seed germination (16.54%) when compared to other sets (1 and 3) (11.20% and 13.90%) (Table 3). On the basis of their efficacy to stimulate seed germination of *Striga hermonthica,* the soyabean, cowpea and groundnut varieties tested may be grouped into three, High stimulant, moderate stimulant and low stimulant production as shown in Tables 4 for soyabean varieties, Tables 5 for cowpea varieties and Tables 6 for Groundnut varieties. The varieties of soyabean, cowpea and groundnut selected as high stimulant producers need to be further investigated with *S. hermonthica* strains from other locations in Nigeria due to reports of strain variation in *S. hermonthica.*

CONCLUSION AND RECOMMENDATIONS

Among the soyabean varieties, (TGX 1448 - 2E, TGX 1835 - 10E and TGX 1019 - 2EB) TGX 1448 - 2E showed best performance in the laboratory. In cowpea (IT04K -217-5, IT07K-25-3-3, IT04K- 339-1) IT04K-217-5 performed best and among the groundnut high stimulant varieties (RMP-12 and RMP-91) RMP 91 showed the best performance.

Among the soyabean, cowpea and groundnut trap crops screened soyabean variety TGX 1448 – 2E, cowpea varieties IT04k – 339 – 1 and IT04k – 217-5 and groundnut variety RMP-91 could be recommended as best varieties for use to control *Striga* under intercropping system. It is also recommended that screening of the potential trap crops varieties be done for different *Striga hermonthica* populations from different location representing variable *Striga* genotypes in order to determine their ability geographically.

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Table 1: Actual percentage germination of *Striga* as induced by Soyabeans varieties, GR-24 and water (Square-root transformation Y+ 0.5).

	Variety	Actual Germination (%) Set (run)				Relative Germination (%) Set (run)			
		1	2	3	Mean	1	2	3	Mean
1	TGX 1937-1F	2.92	12.97	3.58	6.49	4.12	16.29	5.67	8.69
3	TGX 1987-10F	0.67	4.61	3.17	2.82	0.94	5.79	5.02	3.92
4	TGX 1990-45F	6.00	14.55	3.03	7.86	8.46	18.28	4.79	10.51
5	TGX 1987-62F	6.31	14.09	3.42	7.94	8.89	17.70	5.41	10.67
6	TGX 1987-96F	3.42	14.17	3.62	7.07	4.82	17.80	5.73	9.45
7	TGX 1448-2E	15.74	38.06	12.90	22.23	22.20	47.81	20.42	30.14
8	TGX 1835-10F	13.04	20.75	8.68	14.16	18.39	26.07	13.74	19.40
9	TGX 1830-20E	10.13	21.21	3.87	11.74	14.29	26.65	6.13	15.69
10	TGX 1019-2EB	10.66	33.02	5.31	16.33	15.04	41.48	0.08	18.87
	GR-24	70.90	79.60	63.18	71.23	100.00	100.00	100.00	100.00
	WATER	0.16	0.29	0.14	0.20	0.23	0.36	0.22	0.27
	MEAN	13.25	22.39	9.54	14.80	17.49	27.29	14.40	20.00

Table 2: Actual percentage germination of *Striga* as induces by Cowpea varieties, GR-24 and water (Square-root transformation Y + 0.5).

	Variety	Actual Germination (%) Set (run)				Relative Germination (%) Set (run)			
		1	2	3	Mean	1	2	3	Mean
1	IT04K-217-5	3.70	13.3	4.93	7.31	5.51	19.83	7.19	10.84
2	IT04K-227-4	2.60	8.10	3.79	4.83	3.87	12.08	5.52	7.16
3	IT07K-210-1	1.49	6.90	3.34	3.91	2.22	10.29	4.87	5.79
4	IT07K-25-3-3	3.58	11.96	3.66	6.40	5.34	17.83	5.33	9.5
5	IT07K-273-2-1	0.44	3.87	2.81	2.37	0.66	5.77	4.09	3.51
6	IT04K-333-2	2.36	10.13	3.87	5.45	3.52	15.10	5.64	8.09
7	IT04K-339-1	3.66	11.47	3.95	6.36	5.45	17.10	5.76	9.44
8	IT04K-405-5	0.24	3.23	1.90	1.79	0.36	4.82	2.77	2.65
9	IT07K-293-3	2.89	2.42	2.96	2.76	4.31	3.61	4.65	4.19
10	IT07K-318-2	0.10	3.54	2.74	2.13	0.15	5.28	4.31	3.25
	GR-24	67.10	67.07	68.56	67.58	100	100	100	100
	WATER	0.03	0.03	0.00	0.02	0.05	0.05	0.00	0.03
	MEAN	7.34	11.83	8.54	9.24	10.94	17.65	12.51	13.70

e 3: Actual percentage germination of *Striga* as induced by Ground nut varieties, GR-24 and water are-root transformation Y+ 0.5)

Variety	Actual Germination (%)				Relative Germination (%)			
	Set (run)				Set (run)			
	1	2	3	Mean	1	2	3	Mean
TE 3	3.95	9.87	7.68	7.17	5.62	13.37	11.4	10.13
KH241D	0.27	3.42	0.05	1.25	0.38	4.63	0.07	1.69
QH243C	3.87	7.57	4.47	5.3	5.51	10.25	6.68	7.48
CN94C	0.56	6.68	2.12	3.12	0.79	9.05	3.17	4.33
RRB	0.46	1.57	5.7	2.58	0.65	2.12	8.61	3.79
RMP-12	4.84	11.47	8.97	8.43	6.89	15.54	13.41	11.95
RMP-91	4.38	14.63	8.74	9.25	6.23	19.82	13.06	13.04
Groundnut-23	3.91	6.47	3.54	4.64	5.57	8.77	5.29	6.54
Groundnut-11	0.16	2.19	0.29	0.88	0.23	2.97	0.43	1.21
GR-24	70.23	73.8	66.9	70.31	100	100	100	100
WATER	0.02	0.16	0.11	0.096	0.03	0.22	0.16	0.14
MEAN	7.85	12.2	9.3	9.01	11.2	16.54	13.9	13.87

Table 4: Grouping of soyabean varieties on the basis of their efficacy to stimulate seed germination in S. *hermonthica*.

High stimulant production varieties (Group 1)	Medium stimulant production varieties (Group 2)	Low stimulant production varieties (Group 3)
TGX 1448-2E	TGX 1830-20E	TGX 1987-10F
TGX 1835-10E	TGX 1986-2F	TGX 1937-1F
TGX 1019-2EB	TGX 1990-45F	TGX 1987-96F
	TGX 1987-62F	

Table 5: Grouping of cowpea varieties on the basis of their efficacy to stimulate seed germination in S. *hermonthica*.

High stimulant production varieties (Group 1)	Medium stimulant production varieties (Group 2)	Low stimulant production varieties (Group 3)
IT04K-217-5	IT04K-227-4	IT07K-273-2-1
IT07K-25-3-3	IT07K-210-1	IT04K-405-5
IT04K-333-2		IT07K-293-3
IT04K-339-1		IT07K-318-2