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IN-VITRO WHOLE-SEEDLING ASSAY FOR EVALUATING NON-HOST CROP PLANT INDUCTION OF GERMINATION OF WITCH WEED SEEDS

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ABSTRACT

Legume-cereal rotation can reduce density of witch weed (*Striga hermonthica)* seeds in soil. However, legume species and cultivars vary greatly in ability to stimulate germination of *S. hermonthica* seeds of same or different populations, hence the need for simple method for routine characterisation of these species and cultivars for germination of the parasite seeds. A simple and inexpensive technique, *In-vitro* whole-seedling assay; was developed and tested for screening non-host crops for ability to stimulate germination of *S. hermonthica* seeds. In order to compare this new technique with the well established 'cut-root assay', sixteen legume cultivars, comprising of seven cowpea *(Vigna unguiculata)*, six soybean *(Glycine max)*, and three groundnut *(Arachis hypogea)*, in addition to maize (*Zea mays* L.) hybrids 8338-1 and 9022-13 (used as checks) were screened against three *S. hermonthica* populations. The new technique was at least as efficacious as the cut-root assay in detecting differences in ability of cultivars of legume crop species to stimulate germination of *S. hermonthica* seeds. There was less variability in the results obtained in different experimental runs with the new technique, compared with those obtained with the cut root method. The new technique is considered simpler and requires less time, labour and materials. The results obtained for the different legume cultivars screened also suggest that large scale screening of groundnut cultivars for suicidal germination of *Striga* would be worthwhile in the development of control strategies for the parasite in the region.

Key Words: *Arachis hypogea*, cut-root assay,*Glycine max*, *Striga* spp., trap crops, *Vigna, unguiculata, Zea mays*

RÉSUMÉ

La rotation de légumes et de céréales peut réduire la densité de graines de *Striga hermonthica* dans le sol. Cependant, les espèces et les variétés de légumes varient largement en capacité pour stimuler la germination de graines du *S. hermonthica* des mêmes ou différentes populations. De là, la nécessité d'élaborer des méthodes simples de caractérisation de routine des espèces et variétés de germination de parasites des graines. Une technique simple et moins coûteuse, l'essai complet de semi in vitro, était développée et testée pour le dépistage des plantes non hôtes pour leurs aptitudes a stimuler la germination des graines de *S. hermonthica*. Dans le but de comparer cette nouvelle technique avec celle établie d'essai de coupe de racine, seize variétés de légumes, comprenant sept de niébé *(Vigna unguiculata)*, six de sorgho *(Glycine max)*, et trois d'arachide *(Arachis hypogea)*, en plus du maïs *(Zea mays L.)*, les hybrides 8338-1 et 9022-13 (utilisées comme contrôles) étaient évalués contre trois populations de S. hermonthica. La nouvelle technique était au moins efficace comme celle de l'essai de racines coupées dans la détection des différences en capacités des variétés des légumes d'espèces de plantes pour stimuler la germination des graines de *S. hermonthica*. Il y avait une faible variabilité dans les résultants obtenus dans les différentes expériences faites avec la nouvelle technique comparés avec ceux obtenus avec la méthode de coupe

de racine. La nouvelle méthode est considérée simple et demande moins de temps, de travail et matériels. Les résultants obtenus pour les différentes variétés de légumes évaluées suggèrent aussi qu'une grande échelle d'évaluation des variétés d'arachide pour la germination désespérée de *Striga* serait utile dans le développement des stratégies de contrôle de parasites dans la région.

Mots Clés: Arachis hypogea, essai de coupe de racine, *Glycine max*, *Striga* spp., piège des plantes, *Vigna unguiculata*, *Zea mays*

INTRODUCTION

Striga hermonthica (Del.) Benth., the most important parasitic seed plant in the world (Parker and Riches, 1993), is endemic in the African savannahs, infecting and causing serious damage to cereals, including maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), pearl millet (*Pennisetum glaucum* (L.) R. Br.), finger millet (*Eleusine coracana* (L.) Gaertn.) fonio (*Digitaria exilis* (Kippist) Stapf) and upland rice (*Oryza sativa* L.). Moreover, these are staple foods for over 300 million people in sub-Saharan Africa (M'Boob, 1989).

The obligate parasitic nature of *S. hermonthica* and its ability to produce enormous number of seeds (10,000 - 500, 000 seeds plant^{-1}) that can remain viable in soil for up to 14 years (Bebawi *et al.*, 1984), make effective control of the parasite difficult. The seeds are very small (about 200 x 300 microns) and weigh 10^{-5} g each (Saunders, 1993; Berner *et al.*, 1997). After dispersal, and 3-6 months of primary dormancy period, the seeds may germinate only when allowed to imbibe water for 7 -14 days at 20-33 °C and then exposed to exogenous germination stimulant, normally host-root exudate or synthetic germination stimulants (Worsham, 1987; Berner *et al.*, 1997).

Since *S. hermonthica* is an obligate parasite, when the seeds germinate in absence of host roots, the seedlings die within 3-4 days (Worsham, 1987). Hence, a feasible control option for African resource-poor farmers to reduce seed density in the soil is the use of trap-crops, particularly legumes that stimulate germination of the parasite seeds but are non-hosts, in rotation with cereals (Berner *et al.*, 1996a, b). However, there is a wide variation in ability of legume cultivars to stimulate germination of *S. hermonthica* seeds, hence, the need to select most effective cultivars for high levels of stimulant production through screening (Alabi *et al.*, 1994; Berner *et al.*, 1996b; Dashiell *et al.*, 2000; Di Umba *et al.*, 2001). The need for a simple, inexpensive laboratory assay for routine testing of promising legume cultivars for germination of *S. hermonthica* seeds, led to the development of the 'cut-root assay' at the International Institute of Tropical Agriculture, Ibadan (Berner *et al.*, 1996a, b; 1997). This technique had since been adopted by many laboratories. It has become the most commonly used method for screening non-host crops for *S. hermonthica* seed germination in the region because of its simplicity. A positive correlation between germination stimulation in Petri-dish using this method and reduction in *S. hermonthica* emergence in the field by selected legume cultivars have been demonstrated (Berner *et al.*, 1996b; Alabi, 2000; Di Umba *et al.*, 2001). It is, however, hypothesized that intact roots would better mimic the production of germination stimulant in the field. Yet, for any screening method to be adopted by national programmes in sub-Saharan Africa who need continued routine evaluation of non-host crop cultivars for rotation with cereals to reduce the seed bank of this parasite in African soils, such method must be simple and inexpensive. We, therefore, developed a new, simple *in-vitro* screening technique that involves the use of whole seedlings and evaluated the technique in comparison with the 'cut-root assay' (Berner *et al.*, 1997) in screening non-host legume (cowpea, soybean and groundnut) cultivars for efficacy in stimulating germination of *S. hermonthica* seeds.

MATERIALS AND METHODS

***Striga* seeds.** Seeds of three populations of *S. hermonthica* called 'Kano 2000', 'Bida 2000' and 'Zaria 2000' were used for this study. The three populations represent seeds collected from *S. hermonthica* plants growing on sorghum (*Sorghum bicolor* L.) in fields at three different locations in Nigeria, namely Kano (11° 58'N, 8°

30`E), Zaria (11° 07`N, 7° 44`E) and Bida (9° 05`N, 6° 01`E). The seeds were collected between October and November 2000, one year before the experiments were conducted. *Striga* seeds were stored at ambient temperature in polyethylene containers.

To respond to germination stimulants, seeds of *S. hermonthica* must be conditioned by exposing them to water and incubating at favourable temperature for a suitably long period of time (Worsham, 1987). To condition *S. hermonthica* seeds for each day of germination testing, they were surface disinfected for 10 min in an aqueous 1% NaOCl solution. Floating seeds were discarded. The remaining seeds were air-dried aseptically (under laminar flow hood) and placed on 3.5-mm-diameter glass-fibre filter paper (Whatman GF/C) disks, which were in turn, placed on two pieces of moistened 90 mm diameter Whatman no.1 filter paper in 90 mm diameter glass petri-dish. The air-dried surface sterilised *S. hermonthica* seeds were gently sprinkled on the GF/C disks so that 30 – 50 seeds settled per disk and then incubated at 28° C in darkness for 14 days.

Crop cultivars. Sixteen legume cultivars, made up of seven cowpea cultivars (IT90K-277-2, IT81D-994, IT90K-284-2, IT89KD-288, IT93K-452-1, IT96D-733 and IT93K-637-1); six soybean cultivars (TGX1740-2F, TGX1830-20E, TGX923-2E, TGX1871-12E, TGX1448-2E and TGX1019-2E); and three groundnut cultivars (M572-801, UGA-2 and UGA-5) were evaluated in this study. In addition, a *S. hermonthica* susceptible maize hybrid, 8338-1, and a tolerant hybrid, 9022-13 (Kim and Adetimirin, 1997) were included in the test. Cowpea and soybean seeds were obtained from the Grain Legume Unit of IITA, Ibadan. Maize cultivars were obtained from the Maize Improvement Program of IITA, Ibadan while groundnut cultivars were from the Savanna Systems Agronomy Unit, IITA, Ibadan.

GR-24. As a positive check in all the *S. hermonthica* seed germination testing trials in this study, synthetic germination stimulant, strigol, 'GR-24' (bis-lactone (XXIII)) [Johnson *et al.*, 1976] was used. It was obtained earlier by IITA, from J. W. Thuring, Department of Organic Chemistry, NSR-Center, University of Nijmegen, Toernooveld, The Netherlands. By dissolving 100 mg of GR-24 in 10 ml of acetone and then diluting with sterile distilled water, a 1-liter stock solution (100mg l^{-1}) was made and kept refrigerated. From the stock solution, a 10-mg l^{-1} solution was made on each day of *S. hermonthica* seed germination testing.

Experimental procedure. All experiments were conducted in laboratory and screenhouse (in the case of cut-root assay) at IITA, Ibadan. For the '*in-vitro* whole-seedling assay', seeds of the crop cultivar to be tested were surface sterilised for 5 min in NaOCl solution and pregerminated for 72 hr on sterilised washed sand (autoclaved for 20 min at 121°C) in 9 cm diameter petri-dish at ambient laboratory temperature (~ 27 °C). Sterile distilled water was used to moisten the sand and the petri-dishes were covered with their lid, but not sealed.

Other petri-dishes, for testing germination of *Striga* seeds, were lined with two pieces of 90-mm diameter Whatman no.1 filter papers, with a small slit made with a pair of scissors at the centre of the upper piece of filter paper. The plates were sterilised by autoclaving dry at 121°C for 15 min. A crop cultivar seedling from the sterilised washed sand was then placed on the layer of filter paper in a petri-dish. To place the seedling, first, the upper piece of filter paper with a slit was picked up using a pair of forceps, and 3.5 ml of sterile distilled water added to the lower piece of paper. Then the radicle of the seedling passed through the slit on the upper piece, which was then gently placed on the lower filter paper, with the radicle sandwiched between the two pieces of filter paper and the cotyledons lying on the upper piece. The petri-dish was then sealed with parafilm M and incubated at 27 °C in a cooled incubator for 24 hr, after which it was opened and GF/C disks containing conditioned seeds of *S. hermonthica* were arranged on the filter paper, round the seedling, in three radial rows, each row containing four disks, as shown in Figure 1. Another 500 µl of sterile distilled water was added through the slit on the upper piece of filter paper and the petri dish sealed with parafilm M and incubated at 27 °C in the dark for 48 hr in an incubator.

Germination testing by the cut-root assay was

as described by Berner *et al.* (1996 a, b, 1997). Root pieces were obtained from crop cultivar seedlings raised from seeds sown in 200g of sterile sand in 250-ml plastic cups (3 - 5 seedlings per cup), in a screenhouse at IITA, Ibadan. The seedlings, watered as necessary, were grown for 2-wk before the assay. The 2-wk old crop seedlings were gently removed from the cups and the roots washed free of sand. The roots were cut into small pieces (0.5 – 1-cm long) and 1g was weighed. Previously, three radial rows of GF/C disks containing conditioned seeds of respective *S. hermonthica* population had been arranged round a 2-cm-diameter aluminium foil ring centred on two pieces of Whatman no. 1 filter paper moistened with 3 ml of sterile distilled water in petri dish. Then 1 g of root pieces was placed in the aluminium foil ring, and 300 µl of sterile distilled water added to diffuse exudate across the filter paper. The petri dish was sealed and incubated at 27 °C in the dark for 48 hr in a cooled incubator for *Striga* seed germination.

The experimental design was a randomised complete block (RCBD) with a 20 x 3 x 2 factorial combinations, where 20 is the number of germination stimulant source (7 cowpea cultivars, 6 soybean cultivars, 3 groundnut cultivars, 2 maize hybrids, GR-24 as positive check and sterile distilled water as negative check). These were tested against three populations of *S. hermonthica* seeds (Zaria 2000, Kano 2000 and Bida 2000) using the two screening methods (the new *in-vitro* whole-seedling assay and the cut-root assay). There were 6 replications (each replicate represented by a petri-dish) of each treatment, spread over 3 days of testing (experimental runs), with two replications per run.

For both assays, the proportion of germinated *S. hermonthica* seeds (out of the total number of seeds on each GF/C disk in each petri-dish) was determined by counting under a low power binocular light microscope after 48 hr of incubating *Striga* seeds with the germination stimulants. In addition, fresh weight of roots produced by each plant in the *in-vitro* whole-seedling assay was taken after *Striga* germination count.

Statistical analysis. Percentage germination of *S. hermonthica* seeds relative to average *Striga* seed germination by GR24 stimulant in the same

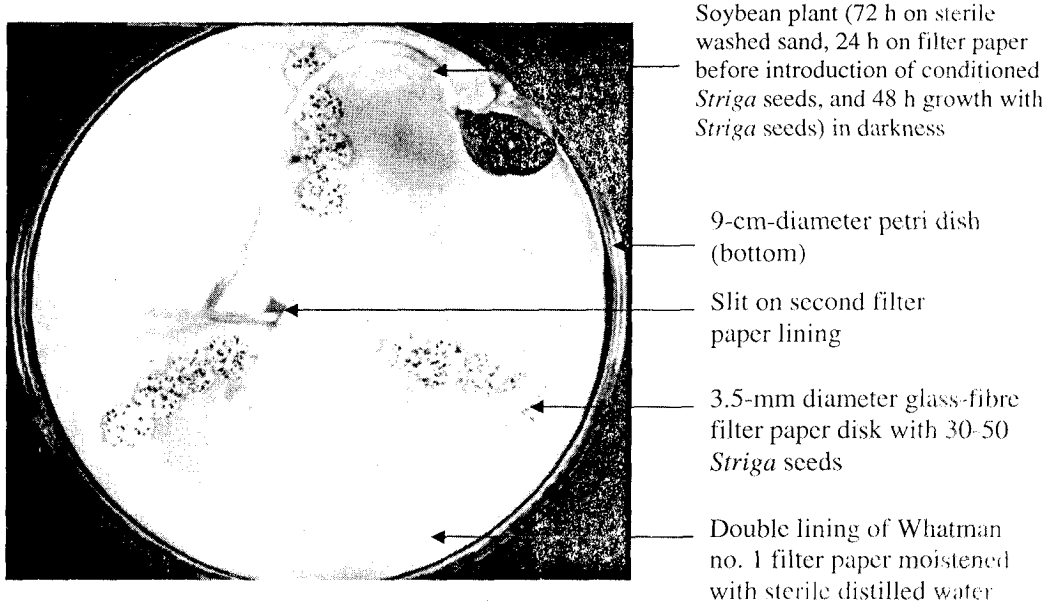

Figure 1. *In-vitro* whole-seedling assay for testing crop cultivars for ability to stimulate germination of *Striga hermonthica* seeds.

experimental run was calculated for each treatment replicate. These were then subjected to analysis of variance, using the 'Generalised Linear Model' (GLM) procedure in SAS (SAS, 1997). Test of significance difference between means was done using single-degree-of-freedom contrast estimate procedure after arcsine '(ASIN(SQRT)' transformation of the data. Means were summarised in bar charts with standard error of means (error bars) given for estimation of difference between means. Percentage variation in germination of *S. hermonthica* seeds according to experimental run, and method of screening was determined by taking a run as a treatment variable and comparing the co-efficient of variation (CV) according to the method.

RESULTS

An initial analysis of data obtained with the *in-vitro* whole-seedling assay showed that the weights of roots produced in the petri-dish did not differ significantly ($P>0.05$) among cultivars of the same crop species. Also, there was no significant correlation between weight of root produced and percentage germination of *S. hermonthica* seeds induced by the cultivars of maize, cowpea, soybean and groundnut. As a result, root weights were not considered in the analysis of the results presented below.

Among the 16 legume cultivars tested, groundnut cultivars were generally more efficacious in inducing germination of seeds of the three *S. hermonthica* populations (particularly, Zaria 2000 and Kano 2000) than the cowpea and soybean cultivars (Fig. 2). Also, cultivars stimulated germination of *S. hermonthica* seeds, regardless of the screening technique. However, germination percentages induced by crop cultivars varied with screening technique and the population of *S. hermonthica* seeds (Fig. 2). For each screening method, groundnut variety (UGA5) gave the highest germination percentage of Zaria 2000 population of *S. hermonthica*. On the other hand, using the whole seedling assay, the highest germination percentage of Bida 2000 population was obtained with the susceptible maize cultivar, 8338-1, which was closely followed by the cowpea variety, IT96D-637-1 (using the whole-seedling assay for both). However, using the cut-root method, the groundnut variety, M572-801, gave the highest germination percentage followed closely by UGA-5 (another groundnut variety). With respect to the Kano 2000 population, the highest germination percentage was induced by groundnut variety M572-801 (in the cut-root assay), followed by the maize cultivar, 8338-1, and the groundnut cultivar (UGA-2), using the whole-seedling assay in both cases. For each of the three populations of *S. hermonthica*, the highest germination percentages induced by soybean varieties were obtained in the whole-seedling assay with the variety, TGX 1830-20E.

Among the cowpea cultivars, IT90K-277-2 stimulated the highest germination percentage of *S. hermonthica* Zaria 2000 seeds with the whole-seedling assay. It also induced the highest germination percentage of the same population with the cut-root assay, but this was significantly higher than that induced by only two of the varieties (IT93K-637-1 and IT89KD-288) (Fig. 2). By contrast, IT93K-637-1 and IT93K-452-1 gave the highest germination percentage of the Kano 2000 population in the whole-seedling and the cut-root bioassays, respectively, but these were not significantly ($P>0.05$) different. As for the Bida 2000 population, IT93K-637-1 also induced the highest ($P<0.05$) germination percentage in the whole seedling assay among the varieties, with the exception of IT96D-733, which also gave the highest germination percentage among the varieties, in the cut- root assay.

Among the soybean cultivars, TGX1830-20E gave the highest germination percentage of population from Zaria (except TGX 1871-12E) and Kano, using the whole-seedling technique (Fig. 2). The highest germination percentage of Bida 2000 was produced by TGX 1448-2E in the whole-seedling assay but this was not significantly higher than that induced by the other cultivars with the exception of TGX1871-12E.

Among the three groundnut cultivars, cultivar UGA-5 was significantly better than both UGA-2 and M572-80I against Bida 2000, and better than only M572-80I against Zaria 2000, but it was significantly inferior to M572-801 against Kano 2000, when whole-seedling assay was used (Fig. 2). Using the cut root assay, cultivar M572-80I and UGA-5 were significantly better than UGA-2 against Bida 2000. Only M572-80I was

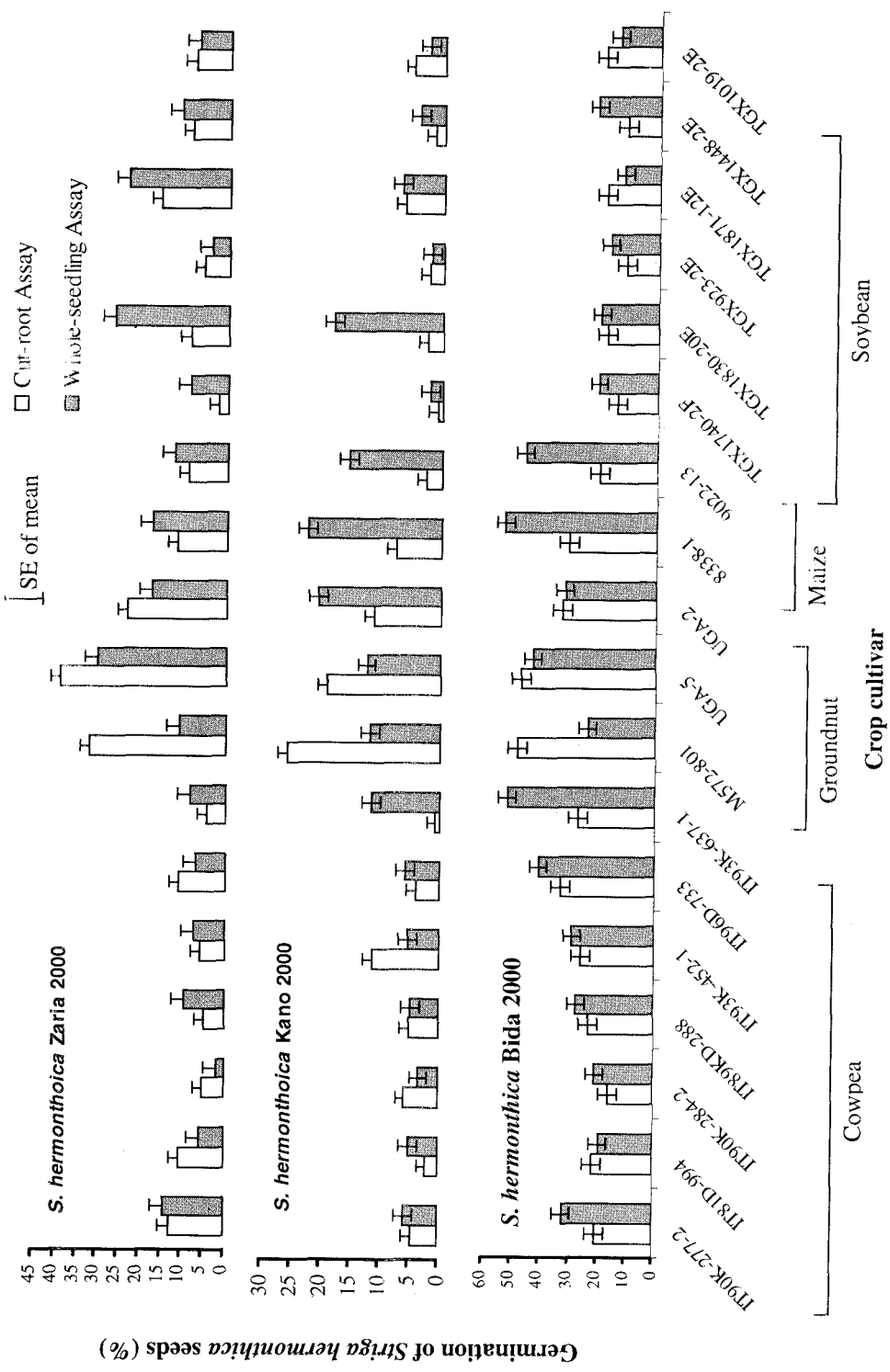

Figure 2. Germination of seeds of three populations of *Striga hermonthica* (Zaria 2000, Kano, 2000 and Bida, 2000) in response to stimulation by cowpea, soybean, groundnut, and maize cultivars using cut-root and whole-seedling assays.

significantly better than UGA-2 against Kano 2000. There were no significant differences among the three cultivars against Zaria 2000.

Using the whole-seedling assay, there was no significant difference between the two maize cultivars, 8338-1 and 9022-13, in inducing germination of seeds of Bida 2000 and Zaria 2000. However, 8338-1 stimulated higher percentage germination of Kano 2000 (Fig. 2). With the cut-root assay, the two maize cultivars were not significantly different from stimulating germination of Zaria 2000, but 8338-1 gave higher germination percentage of both Bida 2000 and Kano 2000 than 9022-13.

Generally, variation that occurred in the whole-seedling assay was less (80%) than the variation in the cut-root assay (93 %) (Fig. 3). In terms of variations within each *S. hermonthica* population among the three experimental runs, variation in any of the three populations was lower in the whole-seedling assay than in the cut-root assay (Fig. 4).

DISCUSSION

The three groundnut cultivars tested were generally better in inducing germination of *S. hermonthica* seeds than cowpea and soybean cultivars (Fig. 2). This indicated the need to devote more attention to screening groundnut cultivars for capacity to stimulate suicidal germination of *Striga* seeds. The focus of researchers on screening legume crop cultivar for use in rotation with cereals for *S. hermonthica* seed bank reduction has mostly been on soybean (Alabi *et al.*, 1994, 2000; Berner *et al.*, 1996b; 2000; Dashiell *et al.*, 2000; Di Umba *et al.*, 2001). Groundnut receives little or no attention. Recently, Bothe (2001) used the cut root and root exudates methods to evaluate several forage legumes (including two varieties each of cowpea, groundnut and soybean) and found that the groundnut cultivars gave consistently higher *S. hermonthica* seed germination percentage than soybean and cowpea varieties. Since groundnut is a traditional cash crop in northern Nigeria and many other savannah areas of West Africa, farmers may easily adopt rotation systems involving the use of groundnut cultivars selected for their efficacy in causing suicidal germination of the parasite's seeds.

Differences in germination response of the three *Striga* populations tested, to germination stimulants produced by cultivars of crop species, indicate that a highly effective cultivar in causing germination of one *Striga* population may not necessarily be effective in inducing germination of another population. This supports an earlier recommendation by Berner *et al.* (1996b) that to ensure deployment of the most effective legume cultivars in legume-cereal rotation systems,

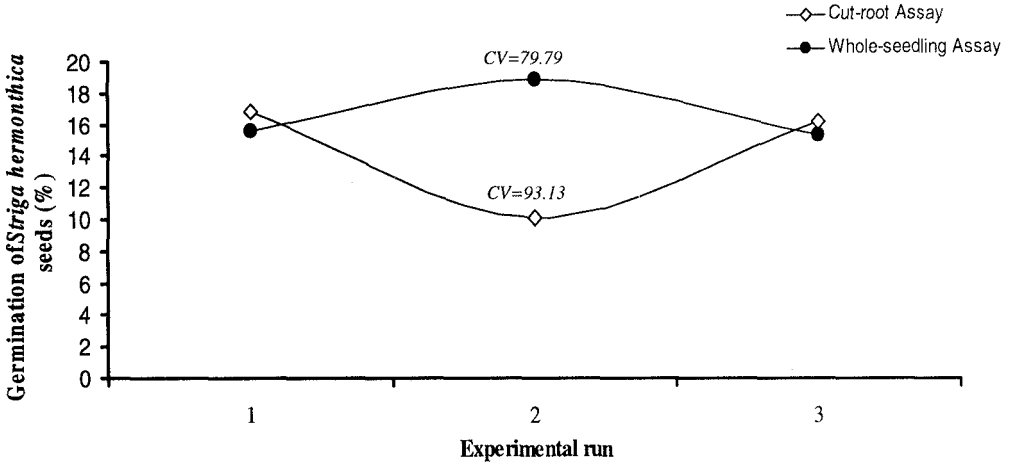

Figure 3. Germination response of seeds of three populations of *Striga hermonthica* (Zaria 2000, Kano 2000 and Bida 2000) according to experimental run, using cut-root and whole-seedling assays.

legume cultivars should be screened for efficacy at the local levels to stimulate germination of the population of *S. hermonhtica* present in the area.

This study has shown that the *in-vitro* whole-seedling assay is as efficacious as the cut-root assay (Berner *et al.*, 1996a, b) currently in use as a simple laboratory screening method for testing ability of non-host trap crops to cause suicidal *Striga* seed germination. In this new technique, a whole, live, and growing young seedling is used instead of root pieces cut from plants grown in pot culture as practised in the cut-root assay. The new method is petri-dish based, and needs less time and labour. In contrast to cut-root assay, it does not require growing seedlings in pot culture for 2-3 weeks in a glasshouse and subsequent washing and cutting of roots, making aluminium rings, and weighing of root pieces. This could lead to more efficiency and cost reduction in screening of several crop cultivars for the national and international research institutes, compared with the cut-root assay.

Our present *in-vitro* whole-seedling assay compares favourably with the cut-root assay in satisfying important requirements of simplicity and affordable costs. In addition, the use of undamaged roots in the whole-seedling assay ensures that the stimulants are produced by intact roots and not from cut root surfaces, thereby simulating more closely what occurs naturally in the soil.

In the *in-vitro* whole-seedling assay, seedlings are used 2-3 days after germination compared to 2 weeks seedlings used in the cut-root assay. This study did not indicate any obvious difference in overall *Striga* germination percentages using the two assays. This suggests that both the new whole-seedling assay and the cut-root assay are relatively robust *Striga* seed germination testing methods and that plant age difference in the two assays has no significant influence on results. In addition, the data suggest that cutting the roots in the cut-root assay may not influence absolute germination rate of *Striga* seeds but germination variability. From our results (Figs. 3 and 4), the new *in-vitro* whole-seedling assay would produce less variability among repetitions of the assay by researchers separated over time and space than with the cut-root assay. High variation in results among experimental replications and repetitions

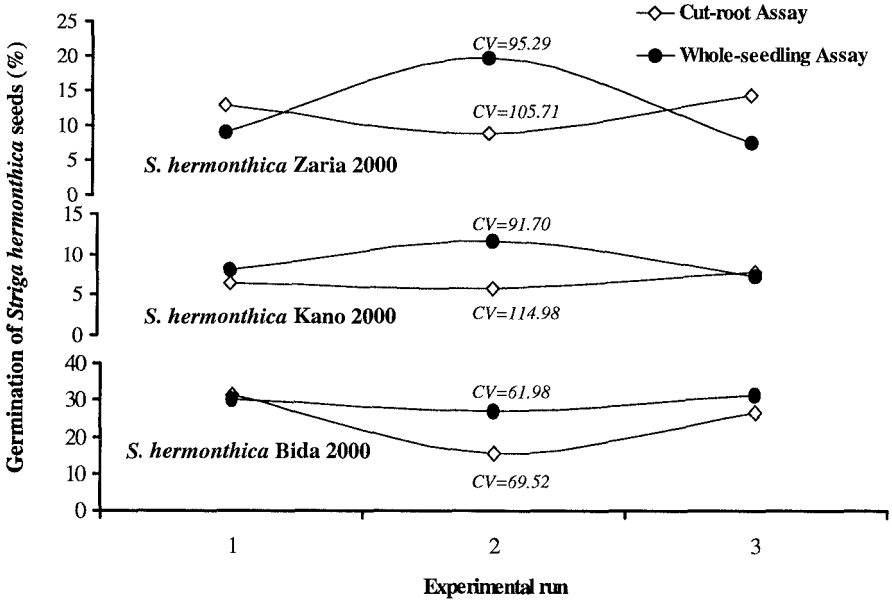

Figure 4. Germination response of *Striga hermonthica* seeds according to experimental run and *Striga* population, using cut-root and whole-seedling assays.

is a well known characteristic in *Striga* research as also shown in this study. Therefore, continued research on the understanding of the sources of variability in results of *Striga* experiments is necessary.

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