Screening methodologies for resistance of sorghum to the parasitic weed *Striga hermonthica*

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Abstract

Overcoming sorghum [*Sorghum bicolor* (L.) Moench] grain yield losses caused by striga [*Striga hermonthica* (Del.) Benth.] through resistance breeding has been hampered by a lack of reliable screening techniques. Our data supports the use of an agar-gel assay as indirect selection method to screen for low stimulation of striga seed germination, an important component of striga resistance in sorghum. Due to low heritabilities and inconsistent correlations to striga resistance under field conditions, pot screening appears to be of limited use. In field trials, two-row field plots with an empty row between plots, artificial infestation, high number of replicates, and appropriate selection indices offer improved and effective direct evaluation tools. Significant genotype × environment interactions in field experiments stress the importance of multilocational trials under striga infestation to achieve stable resistance.

Keywords: sorghum, striga, resistance, screening techniques

Introduction

The parasitic weed striga [*Striga hermonthica*] constitutes a major biotic constraint to sorghum [*Sorghum bicolor*] production in semi-arid tropical Africa. Resulting grain yield reductions impact most Africa's resource-poor subsistence farmers. Host-plant resistance in adapted cultivars could offer the major background of integrated striga control packages. But progress towards developing striga-resistant cultivars has been limited partly due to the difficulty of evaluating resistance in the field and lack of alternative screening assays. Hence, our objective was to assess striga resistance measures using an *in-vitro* agar-gel assay and pot trials as indirect screening procedures *versus* direct field screening.

Materials and Methods

Genetic materials and experimental design

From two sorghum crosses, (1) IS 9830 × E 36-1 and (2) N 13 × E 36-1, we derived two recombinant inbred populations (RIPs), each comprising 226 $F_{3:5}$ lines. Striga resistance of IS 9830 is based on low production of root exudates required by striga for germination. N 13 has “mechanical” resistance and probably also an antibiosis mechanism. E 36-1 is striga-susceptible. Each RIP was divided into Set 1 (116 $F_{3:5}$ lines) and Set 2 (110 $F_{3:5}$ lines), evaluated in 1997 and 1998, respectively. For each experiment, the individual sets of $F_{3:5}$ lines were randomized together with the corresponding parent lines and three (1997) or nine (1998) checks to fit an 11×11 lattice design. Six replicates were used in all tests.

Resistance tests

RIP 1 was evaluated in an agar-gel assay (Hess *et al.,* 1992), using striga seeds from Kenya, Mali, and Niger (**Table 1**). Both RIPs were tested in striga-infested pots in Kenya, Mali and Niger, and in multi-locational field trials in Kenya and Mali.

In the **agar-gel assays**, striga seeds were surface sterilised, preconditioned for 10 to 12 days, and dispersed in agar-gel in petri-dishes. The radicle of a germinated (24 hours-old) sorghum seedling was inserted into slits in the solidifying 0.7% water agar in each plate. The maximal distance between sorghum rootlet and germinated striga seed was measured after five days of incubation in the dark. Sorghum entries with a maximum germination distance less than 10 mm are usually classified as low-stimulant types. The six replicates of each individual trial with one striga source were performed over three weeks, with two replicates being examined each week.

Table 1: Overview of the resistance tests performed

Type of Experiment	Genetic Material		Test Year	Striga source/Test location[†]					
				Kenya		Mali		Niger	
				Kibos	Alupe	Samanko	Cinzana	Bengou	Sadoré
Agar-gel Assays	RIP 1	Set 1	'97	⊕		⊕		⊕	
		Set 2	'98	⊕		⊕		⊕	
Pot trials	RIP 1	Set 1	'97	⊕					⊕
		Set 2	'98	⊕		⊕			⊕
	RIP 2	Set 1	'97	⊕					⊕
		Set 2	'98	⊕		⊕			⊕
Field Trials	RIP 1	Set 1	'97	⊕	⊕[‡]	⊕	⊕		
		Set 2	'98	⊕	⊕	⊕	⊕		
	RIP 2	Set 1	'97	⊕	⊕	⊕	⊕		
		Set 2	'98	⊕	⊕	⊕	⊕		

[†] ⊕ indicates the respective striga source used for the agar-gel assays or the site and striga source for the pot or field experiments. An exception was the pot trial at Sadoré in which striga seeds collected from Bengou in Niger were utilized.

[‡] Field trials at Alupe were conducted during both Long Rains and Short Rains of each test year.

In the **pot trials**, the 12-litre pots were arranged in serpentine motion in single rows (at Kibos) or double rows (at Samanko and Sadoré) on raised beds with footpaths of 1 m in-between rows. A layer of gravel was placed in each pot before filling with soil/sand mixtures and artificially infesting with a quantified number of viable striga seeds as follows: 16,000 in 1997 trials in Kenya and Niger; 6,000 in Kenya, and 7,000 in Mali and Niger in the 1998 trials. Striga infestation in the 1998 trials was reduced because of the observation in the first year that under high infestation level, highly susceptible sorghum entries supported low numbers of emerged striga due to strongly reduced host vigour. To effect infestation, a mixture of striga seeds and fine dry sand (250 g) was incorporated in the top 3 cm soil. Initially, the pots were left to stand for 7 days with intermittent watering so as to precondition the striga seeds. After sowing, water was provided up to three times a week in Kenya and even daily at Samanko and Sadoré, depending on rains and individual pot requirement. Thinning was performed to allow a stand of three plants in Kenya in both 1997 and 1998, and in Niger in 1997, or single plants in Mali and Niger in 1998. Diammonium phosphate and urea fertilizers were

applied at sowing and after thinning, respectively, to provide the agronomic recommendation of 40 kg N ha^{-1} (in Kenya), and 30 kg N ha^{-1} and 44 kg N ha^{-1} at Samanko and Sadoré, respectively. Two traits from the pot trials will be reported here:

- emerged striga plants pot^{-1} at 86 days after planting (d.a.p.) (S86);
- area under striga number progress curves (ASNPC) based on five striga counts made in two-week intervals during the course of the season, using the formula for area under the disease progress curves (Shaner and Finney, 1977; see also Haussmann *et al.,* 2000).

Each **field plot** consisted of two rows separated from neighbouring entries by one empty row. On-station fields at Kibos, Alupe and Samanko were artificially infested with striga in all seasons **(Table 2)**. Plants were spaced at 75×15 (or 20) cm in Kenya, and 80×20 cm in Mali. Diammonium phosphate and urea fertilizers were applied at sowing and after thinning, respectively, to provide the agronomic recommendation of 40 kg N ha^{-1} in Kenya, and 30 kg N ha^{-1} and 44 kg N ha^{-1} at Samanko and Cinzana in Mali, respectively.

Table 2: Striga infestation details [viable striga seeds m^{-2}] in the individual field experiments in Kenya and Mali

Sets/Year	Kenya			Mali	
	Kibos LR†	Alupe LR	Alupe SR	Samanko R	Cinzana R
1 (1997)	40,000	43,000	43,000	84,000	Natural
2 (1998)	40,000	40,000	40,000	43,000	Natural

† LR, SR = Long Rains and Short Rains Season, respectively.

The following traits are reported from the field trials:

- emerged striga plants m^{-2} at around 88 d.a.p. (S88);
- area under striga number progress curve (ASNPC), calculated as described in the pot trials section;
- sorghum grain yield [g m^{-2}].

Statistical analysis

The computer software PLABSTAT (Utz, 1998) was used for statistical analysis. Raw data were subjected to analysis of variance according to the lattice design. Broad-sense heritabilities were estimated on an entry mean basis (Hallauer and Miranda, 1981). Coefficients of phenotypic correlation were calculated among the agar-gel assays, pot and field experiments. Estimates of genetic correlation among traits were computed as outlined by Mode and Robinson (1959).

Results

Agar-gel assay

Mean maximal germination distance was significantly higher with striga seeds from Kenya (13-14 mm) compared to Mali or Niger (8-9 mm). Genetic variances among the $F_{3:5}$ lines (Φ^2_g) of Sets 1 and 2 of RIP 1 were highly significant (P#0.01). The interaction variances between $F_{3:5}$ lines and striga populations ($\Phi^2_{g\times s}$) were also highly significant (P#0.01) but less important than the genetic variances. Ratios of Φ^2_g : $\Phi^2_{g\times s}$ were 1 : 0.15 in Set 1, and 1 : 0.06 in Set 2. Accordingly, the agreement between the agar-gel assays using different striga sources was high (coefficients of correlation between 0.73 and 0.92). Bimodal frequency distributions of the $F_{3:5}$ lines for the maximal germination distance coupled with an overlap of high- and low-stimulant fractions suggested the involvement of one major gene and an unknown number of minor genes in stimulation of striga seed germination.

Pot trials

In the pot trials, $F_{3:5}$ lines of both RIPs differed significantly at each individual location (P#0.05 or P#0.01). However, in the combined analysis of variance, the genetic variance among the $F_{3:5}$ lines was non-significant while the $F_{3:5}$ line × environment interaction was highly significant and

much larger than the genetic variance (**Table 3**). Consequently, heritability estimates were low in the pot trials, though slightly higher in RIP 2 compared to RIP 1.

Table 3: Relative importance of genotype × environment interaction in pot trials, expressed as the ratio of Φ^2_g: $\Phi^2_{g\times e}$, and broad sense heritabilities (h^2) of Sets 1 and 2 of RIPs 1 and 2 for number of emerged striga at around 86 d.a.p. (S86) and area under striga number progress curve (ASNPC), estimated from the combined ANOVA across two or three environments, namely Kibos and Sadoré for Sets 1; and in addition, Samanko for Sets 2

RIP	Set/Year	S86		ASNPC	
		Φ^2_g: $\Phi^2_{g\times e}$	h^2	Φ^2_g: $\Phi^2_{g\times e}$	h^2
1	1 (1997)	1 : 22.0	0.04	1 :12.83	0.06
	2 (1998)	-	-	1 : 9.20	0.12
2	1 (1997)	1 : 7.70	0.11	1 : 4.84	0.14
	2 (1998)	1 : 2.87	0.27	1 : 1.80	0.43

- negative estimate for variance components.

Field trials

In the field trials, the genetic variance among the $F_{3:5}$ lines of both RIPs and their interaction with locations were highly significant (P#0.01). Estimates of broad-sense heritability were moderate to high with both RIPs (**Table 4**).

Table 4: Relative importance of genotype × environment interaction in field trials, expressed as the ratio of Φ^2_g: $\Phi^2_{g\times e}$, and broad-sense heritabilities (h^2) of Sets 1 and 2 of RIPs 1 and 2 for number of emerged striga at around 88 d.a.p. (S88), area under striga number progress curve (ASNPC) and sorghum grain yield (GY), estimated from the combined ANOVA across five site-season combinations, namely Kibos Long Rains, Alupe Long and Short Rains, Samanko and Cinzana

RIP	Set/Year	S88		ASNPC		GY	
		Φ^2_g: $\Phi^2_{g\times e}$	h^2	Φ^2_g: $\Phi^2_{g\times e}$	h^2	Φ^2_g: $\Phi^2_{g\times e}$	h^2
1	1 (1997)	1 : 0.77	0.61	1 : 0.50	0.65	1 : 6.09	0.37
	2 (1998)	1 : 0.77	0.74	1 : 0.67	0.74	1 : 1.85	0.65
2	1 (1997)	1 : 0.63	0.80	1 : 0.55	0.82	1 : 0.85	0.79
	2 (1998)	1 : 0.41	0.82	1 : 0.43	0.82	1 : 1.52	0.68

Genetic correlations between area under striga number progress curve and sorghum grain yield were –0.27 and –0.31 for Sets 1 and 2 of RIP 1, and –0.37 and –0.38 for Sets 1 and 2 of RIP 2, respectively.

Correlations among experiments

Phenotypic correlations between germination distance and field resistance were positive and generally higher in Mali than in Kenya (**Table 5**). Correlation coefficients between pot and field trials were low and inconsistent, though higher for RIP 2 than for RIP 1 (data not shown).

Table 5: Coefficients of phenotypic correlation between area under striga number progress curve (ASNPC) in the field trials and maximal germination distance in the agar-gel assay (GD5) (using the corresponding Kenyan or Malian striga population) for the $F_{3:5}$ lines of Sets 1 and 2 of RIP 1

Field experiments		Correlation coefficient between ASNPC in the field and GD5	
Country	Location	Set 1 (1997)	Set 2 (1998)
Kenya	Kibos	0.24**	0.21*
	Alupe Long Rains	0.19*	0.32*
	Alupe Short Rains	0.04	0.13
Mali	Samanko	0.45**	0.29**
	Cinzana	0.63**	0.51**

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Discussion

Screening techniques in laboratory or pot trials (indirect selection methods) are preferable over direct selection in the field if the indirect traits are quick and cheap to measure, highly heritable, and closely correlated with the direct trait.

Indirect screening using the agar-gel assay

The agar-gel assay allows an easy and quick screening of sorghum entries for low stimulation of striga seed germination, one mechanism of resistance to striga. Because of its simplicity and the positive correlation between *in-vitro* germination distance and field resistance, the agar-gel assay is a useful indirect selection tool in breeding sorghum for resistance to striga.

Higher germination distance was attained with the *Striga hermonthica* population from Kenya, indicating its greater responsiveness to germination stimulants than the populations from West Africa. Thus, the capacity to stimulate low levels of striga seed germination may be less effective in Kenyan striga-infested fields, a conclusion which is also supported by the lower correlations between *in-vitro* germination distance and area under striga number progress curve in the Kenyan field trials. It remains unclear whether striga seeds from Kenya are more sensitive to lower concentrations of the major germination stimulant sorgolactone, or to sorgoleone and strigol, stimulants thought to be of minor importance (Ejeta *et al.,* 1992).

Other laboratory methods are still needed to permit efficient indirect screening for resistance mechanisms other than low stimulation of striga seed germination.

Pot screening

Striga resistance measures in the pot trials had a low heritability and were weakly and inconsistently correlated to field resistance. Hence, pot screening appears to be of limited use in breeding programs. The low correlation between pot and field trials may be partly attributed to the rather artificial and highly limited root environment in pots.

Direct screening in the field

We achieved high heritabilities in the field trials. This feat was possible through implementation of several measures described by Haussmann *et al.* (2000), namely: the two-row plot with an empty row between plots, high number of replications (six in our case), artificial inoculation of test rows of on-station fields with striga to reduce heterogeneity; and use of up to five emerged striga counts to compute the area under striga number progress curves. Hence this study confirms the usefulness of these improvements towards improving reliability of field screening.

The area under striga number progress curve is an excellent measure of progressive striga emergence. We encourage its use in breeding work. When funding is prohibiting, we suggest that using the third and/or fourth emerged striga count (normally at around 70-85 d.a.p.) may be sufficient. At this stage, the number of emerged striga plants is near its peak.

The observed highly significant $F_{3:5}$ lines × environment interaction in field trials underline the importance of multilocational trials under striga infestation to achieve stable resistance to this parasitic weed. Breeding materials should therefore be evaluated against different striga morpho-types, host specific races and various locations / under differing environmental conditions in order to obtain stable, polygenic resistance (Ramaiah, 1987).

Striga resistance and grain yield were genetically positively correlated. This should facilitate selection of materials with low striga emergence and high grain yield for striga-infested areas in Kenya and Mali.

The discussed modifications in screening techniques and the quantitative-genetic information derived from the present study contributes to a more efficient development of productive sorghum cultivars for farmers living in striga-infested areas.

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