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Assessment of Direct and Indirect Measures of Striga Resistance in Sorghum

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Abstract

The genus *Striga* presents a major biotic constraint to production of cereals and legumes in sub-Saharan Africa. Host-plant resistance in adapted, productive cultivars is recognised as an important component of integrated management of this parasite. However, breeding progress has been largely limited due to the difficulty of evaluating resistance in the field and insufficient information on the genetics of striga resistance. Within the frame of a DAAD- and BMZ-supported project, we evaluated the utility of direct versus indirect measures of striga resistance in sorghum. Laboratory, pot, and field experiments were performed and selected results are presented and discussed.

Key words: striga, sorghum, resistance breeding, indirect selection.

Introduction and objectives

The genus *Striga* poses a major constraint to production of staple cereals and legumes in sub-saharan Africa. Three species, namely *Striga hermonthica* (Del.) Benth., *Striga asiatica* (L.) Kuntze, and *Striga gesnerioides* (Willd.) Vatke are of economic significance. The former two parasitize cereals such as sorghum, maize, and millet, whereas the third attacks dicotyledonous species, mainly cowpea and tobacco. Striga-resistant cultivars would provide a major component of integrated striga control packages if resistance could be incorporated into adapted, high yielding cultivars. Breeding progress has been limited due to insufficient information on genetics of striga resistance, difficulty of evaluating resistance in the field, and lack of alternative reliable screening assays.

This research was designed to compare the suitability of laboratory and pot procedures which are indirect measures *versus* field (direct) measures of resistance to *Striga hermonthica* (Del.) Benth. in sorghum. The experiments also contribute to a study identifying molecular markers for striga resistance.

Materials and methods

Genetic materials

Two recombinant inbred populations (RIPs) of 226 $F_{3:5}$ families each were developed from crosses of IS 9830 × E 36-1 (RIP 1), and N 13 × E 36-1 (RIP 2). The resistant parent IS 9830 produces low amounts of striga seed germination stimulant. N 13 has ”mechanical resistance” and may also possess an antibiosis mechanism. The susceptible parent E 36-1 produces abundant striga seed germination stimulant. Each RIP was divided into two sets. In 1997, Set 1 of each RIP was comprised of 116 $F_{3:5}$ families which were evaluated with their corresponding two parent lines and three local checks. In 1998, Set 2 of each RIP consisted of 110 new $F_{3:5}$ families, tested together with the respective parental lines and nine checks.

Agar-gel assay

In both years, we tested the stimulant production of RIP 1 using the *in vitro* agar-gel assay developed by Hess *et al*. (1992) (See Berner *et al*., 1997 for an alternate description). Seeds of striga were collected from Kenya, Mali, and Niger. These were surface-sterilised and preconditioned for 12 days. They were then dispersed in agar-gel in petri dishes. A 24-hour old germinating sorghum seed was inserted into the agar-gel in each dish. The maximal distance between sorghum rootlets and germinated striga seed was measured in mm after five days of incubation at 28°C in the dark.

Pot experiments

Set 1 was planted in pot experiments at Kibos in Kenya, and at Sadore in Niger. Set 2 was also planted at these two locations, as well as at Samanko in Mali. Pots were laid out in an 11×11 lattice with six replicates. Pots were arranged in single rows on raised beds with footpaths of 1 m in between rows. Within the rows, pots were placed 0.5 m apart. Each pot was artificially infested with 16,000 and 6,000 viable striga seeds in 1997 and 1998, respectively. The reduction in the second year was due to our observation in the first year that under high infestation , highly susceptible sorghum entries supported low numbers of emerged striga due to strongly reduced host vigour. During infestation, a mixture of striga seeds and fine dry sand was incorporated in the top 3 cm of soil. Two non-infested control pots sown with the cultivars IS 8193 (susceptible) and Seredo (tolerant) were prepared for each replicate. Pots were filled up to 2 cm below the top to facilitate watering. Initially the pots were allowed to stand for 7 days with intermittent watering to precondition the striga seeds. After sowing, water was provided up to three times a week, depending on rains and individual pot demand. Thinning was done to allow a stand of three plants in pot trials with Set 1 of both RIPs at Kibos and Sadore, and Set 2 at Kibos. In 1998, at Samanko and Sadore, thinning to a single plant was done in Set 2. Striga counts were taken in each pot at 86 days after planting (DAP). The area under striga number progress curve (ASNPC) was computed using the standard formula for the area under disease progress curve, described by Shaner and Finney (1977).

Field experiments

Field experiments were planted in 1997 (Set 1 of both RIPs) and in 1998 (Set 2 of both RIPs) at Samanko and Cinzana (both in Mali), and Kibos and Alupe (both in Kenya) in the Long Rains season, and Alupe in the Short Rains season, amounting to a total of five site/season combinations per testing year. A *hexa* lattice design was employed. Individual plots consisted of two rows separated by one empty row. Artificial striga infestation was done in the on-

station fields at Kibos and Alupe in Kenya, and Samanko in Mali. The number of emerged striga plants was recorded at around 88 DAP in each field plot. This was then expressed per m^2 (S88). ASNPC was also calculated. Sorghum grain yield (GY) was assessed in g m^{-2}.

Statistical analysis

The computer program, PLABSTAT (Utz, 1998) was used for statistical analysis. Raw data were subjected to analysis of variance according to the lattice design with extreme outlayers (as defined by Anscombe and Tukey, 1963) declared as missing values. The latter were iteratively calculated according to Yates (1933) and Healy and Westmacott (1956) such that the error variance became minimal. Effective error variances were estimated as outlined by Cochran and Cox (1957). Operative repeatabilities for the 226 $F_{3:5}$ families of each RIP were calculated with lattice-adjusted plot values, using the formula;

$$Repeatability\,[\%] = \frac{\hat{\sigma}_g^{\,2}}{\hat{\sigma}_g^{\,2} + \frac{\hat{\sigma}_e^{\,2}}{r}} \times 100$$

where $\hat{\sigma}_g^{\,2}$ and $\hat{\sigma}_e^{\,2}$ are the estimated genetic and error variances, respectively, and *r* is the number of replications. Broad sense heritabilities were calculated as described by Becker (1993). Phenotypic and genotypic correlations among traits were calculated as outlined by Mode and Robinson (1959), based on lattice-adjusted entry means from individual environments.

Results

Agar-gel assay

Mean germination distance was highest with striga seeds from Kenya (**Table 1**). Operative repeatabilities for the three striga populations were high, ranging from 84.9 to 94.2%.

Table 1. Means and operative repeatabilities (Rep %) of RIP 1 for the maximal germination distance (mm) in the agar-gel assays conducted with striga from Kenya, Mali, and Niger.

Set	Test year	Parameter	*Striga source*		
			Kenya	Mali	Niger
1	1997	Mean	14.0	8.6	9.0
		Rep [%]	93.2	89.0	93.7
2	1998	Mean	13.3	8.4	8.5
		Rep [%]	84.9	93.0	94.2

Stimulant production in the agar-gel assay differed significantly among the $F_{3:5}$ families of RIP 1 (**Table 2**). The $F_{3:5}$ family variance components were much greater and hence of more importance in determining stimulant production than the $F_{3:5}$ family by striga population interaction. Heritability estimates were 0.92 and 0.95 in Sets 1 and 2, respectively.

Table 2. Components of variance and operative heritabilities (h^2) in RIP 1 for mean maximal germination distance.

Set	*Test year*	*Component of variance due to*			h²
		$F_{3:5}$ families	*$F_{3:5}$ family × striga population*	*error*	
1	1997	30.71 **	4.52 **	3.12	*0.92*
2	1998	38.49 **	2.17 **	3.73	*0.95*

** = significant at the 0.01 probability level.

Striga originating from Mali and Niger were more closely correlated to each other than either was to the Kenyan population (**Table 3**).

Table 3. Phenotypic correlations between striga populations in the agar-gel assay with RIP 1

Set	*Test year*	*Compared striga populations*		
		Kenya/Mali	*Kenya/Niger*	*Mali/Niger*
1	1997	0.73 **	0.76 **	0.92 **
2	1998	0.86 **	0.87 **	0.91 **

** = significant at the 0.01 probability level.

Pot experiments

In 1997 with Set 1 of boths RIPs, the mean number of emerged striga pot^{-1} was much higher at Kibos than at Sadore (**Table 4**). In 1998 with Set 2 of both RIPs, the average numbers of emerged striga was again lowest at Sadore and comparably high at Kibos and Samanko. There were significant differences among sorghum lines for S86 and ASNPC at each individual location and operative repeatabilities for the two traits ranged from 27-61% in RIP 1, and from 35-63% in RIP 2. However, combined across pot locations within the respective years, the variance component due to $F_{3:5}$ families was not statistically significant, whereas high $F_{3:5}$ family by environment interactions were observed. Consequently heritability estimates were low (data not shown).

Table 4. Environmental means of RIP 1 and 2 in the pot trials

RIP	Set	Test year	Trait†	Kibos (Kenya)	Sadore (Niger)	Samanko (Mali)
1	1	1997	S86	93	12	- ‡
			ASNPC	20	7	-
	2	1998	S86	44	11	38
			ASNPC	17	7	13
2	1	1997	S86	62	14	-
			ASNPC	19	8	-
	2	1998	S86	28	15	35
			ASNPC	9	8	13

† S86 = striga count pot^{-1}, taken 86 DAP; ASNPC = mean area under striga number progress curve divided by 100; GY [$g\ m^{-2}$] = grain yield in grams m^{-2} .
‡ No experiment at this location.

Field experiments

Striga emergence was high at all locations in both RIPs and years (**Table 5**). In the experiments with Set 1 of RIP 1, high S88 (92) was observed at Alupe in the Short Rains of 1997. This site also had the lowest environmental mean for grain yield. The other sites were similar for both striga infestation and sorghum grain yield levels.

Both grain yield and striga infestation were high in Set 1 of RIP 2 at Alupe during the Long Rains season, suggesting optimal conditions for both parasite and host. In addition, mean ASNPC at Alupe in the Long and Short Rains season was the same for these Set 1 entries, but grain yield was much lower in the Short Rains season.

Table 5. Environmental means of RIP 1 and 2 in the field experiments

RIP	*Set*	*Test*	Trait†	Location				
		Year		Kenya‡			*Mali*	
				Kibos	Alupe LR	Alupe SR	Samanko	Cinzana
1	1	1997	S88	22	19	92	21	31
			ASNPC	8	10	32	7	7
			GY[$g\ m^{-2}$]	289	251	39	250	257
	2	1998	S88	39	57	30	16	49
			ASNPC	14	23	10	5	16
			GY [$g\ m^{-2}$]	351	198	178	257	224
2	1	1997	S88	23	60	62	30	41
			ASNPC	9	22	22	10	9
			GY[$g\ m^{-2}$]	145	158	18	42	123
	2	1998	S88	50	54	18	16	46
			ASNPC	18	20	6	6	16
			GY [$g\ m^{-2}$]	252	151	111	103	113

† S88 = striga count m^{-2}, taken 88 DAP; ASNPC = mean area under striga number progress curve divided by 100; GY [g m^{-2}] = grain yield in grams m^{-2} .
‡ LR and SR = Long and Short Rains, respectively.

Analysed across the five field environments, the genetic variance among the $F_{3:5}$ families and $F_{3:5}$ family by striga population interaction variance were highly significant in both RIPs (**Table 6**). Heritability for striga traits varied between 0.61 and 0.74 in RIP 1; and between 0.80 and 0.82 in RIP 2.

Genetic correlations between grain yield and striga traits ranged from -0.30 to -0.40. Hence, high sorghum grain yield in both RIPs was genetically associated with low striga infestation under field conditions.

Table 6. Components of variance and operative heritabilities (h^2) of RIP 1 and 2 in field experiments

RIP	*Set*	*Test year*	*Trait*	*Component of variance due to*			h ²
				$F_{3:5}$ families	*$F_{3:5}$ family × location*	*error*	
1	1	1997	S88	41 **	32 **	103	*0.61*
			ASNPC	4 **	2 **	9	*0.66*
			GY [g m^{-2}]	196 **	1191 **	480	*0.37*
	2	1998	S88	99 **	77 **	97	*0.74*
			ASNPC	12 **	8 **	12	*0.74*
			GY [g m^{-2}]	664 **	1229 **	581	0.65
2	1	1997	S88	255 **	160 **	153	*0.80*
			ASNPC	27 **	16 **	16	*0.81*
			GY [g m^{-2}]	748 **	637 **	333	*0.80*
	2	1998	S88	172 **	70 **	118	*0.82*
			ASNPC	23 **	9 **	15	*0.82*
			GY [g m^{-2}]	562 **	852 **	446	0.68

† S88 = striga counts m^{-2}, taken 88 DAP; ASNPC = mean area under striga number progress curve divided by 100; GY [g m^{-2}] = grain yield in grams m^{-2} .
** = significant at the 0.01 probability level.

Relationships among the experiments

Moderate to tight phenotypic correlations were observed in RIP 1 between germination distance *in vitro* and field resistance in Mali, especially at Cinzana (**Table 7**). In contrast, non-significant or weak associations existed between *in vitro* germination distance and field resistance in Kenya.

Reduced striga emergence in the pot trials was generally inconsistently associated with germination distance in the agar-gel assay. Correlation coefficients between the pot and field trials were also generally low and inconsistent, although higher for RIP 2 than for RIP 1 (data not shown).

Table 7. Phenotypic correlations between striga emergence in the field trials and germination distance of the corresponding striga population (from Kenya and Mali) in the agar-gel assay with RIP 1.

Set	*Field trait*[†]	*Kenya*[‡]			Mali	
		Kibos	Alupe LR	Alupe SR	Samanko	Cinzana
1	S88	0.23 *	ns	ns	0.47 **	0.61 **
	ASNPC	0.24 *	0.19 *	ns	0.45 **	0.63 **
2	S88	0.22 *	0.27 **	ns	0.34 **	0.51 **
	ASNPC	0.21 *	0.32 **	ns	0.29 **	0.51 **

† S88 = striga count m^{-2}, taken 88 DAP; ASNPC = mean area under striga number progress curve divided by 100.
‡ LR and SR = Long and Short Rains, respectively.
*,** = significant at the 0.05 and 0.01 probability levels, respectively.
ns = not significant at the 0.10 probability level.

Discussion

The striga population from Kenya was more responsive to germination stimulant than the populations from West Africa. The low stimulant character may therefore be less effective in Kenyan striga-infested fields. 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. Presently, production of the sorgolactones and sorgoleones by sorghum roots has been documented (Hauck *et al*., 1992; Netzly and Butler, 1986). The response of the $F_{3:5}$ families to the striga seeds from the three countries suggest that striga samples from Mali and Niger populations were more closely related to each other than either was to the Kenyan population. Recent results of Koyama (1998), using isozyme and random amplified polymorphic DNA (RAPD) marker techniques, support these findings. Significant genotype by striga population interactions and the specific reaction of the striga population from Kenya in the agar-gel assay indicate the existence of parasitic variability among the tested striga populations. Doggett (1965), Parker and Reid (1979) have also acknowledged existence of variability of striga within species and geographical regions.

In addition, highly significant $F_{3:5}$ family by environment interaction in pot and field trials underline the importance of multilocational resistance trials to achieve stable resistance to this parasitic weed. As Ramaiah (1987) noted, breeding materials should be evaluated against different striga morphotypes, host specific races and various locations/under differing

environmental conditions in order to obtain stable, polygenic resistance. Based on our results, the agar-gel assay provides a useful indirect selection method, especially for target environments in Mali, to screen for striga resistance in sorghum. Striga resistance and grain yield were genetically and positively correlated. This facilitates selection of materials with low striga emergence and high grain yield for striga-infested areas in Kenya and Mali.
In this study striga resistance measures in the pot trials were weakly correlated to field resistance. Hence, pot screening appears to be of limited use in breeding programs. The ASNPC is under strong genetic control and offers a suitable measure of progressive striga emergence in the field. We therefore encourage its use in screening work. However, it is important to note that these results were obtained with five striga counts and six replications. We were thus able to obtain high heritabilities which was also important to enhance the accuracy of our search for QTL for striga resistance (as additional objective). For national agricultural research centres with limited funding, we suggest that using the third and/or fourth striga count (normally at around 70-85 DAP) may be sufficient. At this stage, the number of emerged striga plants is at near peak levels and screening large numbers of sorghum entries would be more cost-efficient.

Conclusions

From our results we found that:

i. The agar-gel assay provides a useful indirect selection method to screen for striga resistance in sorghum, especially for Cinzana and Samanko environments.

ii Pot experiments were inconsistently correlated to mean maximal germination distance and striga traits in the field experiments. Hence, pot screening appears to be of limited use in breeding programs.

iii. Significant genotype by environment interactions in pot and field trials stress the importance of multilocational resistance trials to achieve stable resistance to *Striga hermonthica*.

iv. Significant genotype by striga population interactions and specific reaction of the striga population from Kenya in the agar-gel assay indicate existence of parasitic variability among the tested *Striga hermonthica* populations.

v. The area under striga number progress curve offers a suitable measure of progressive striga emergence in the field. We encourage its use in screening work.

vi. Other laboratory methods are still needed to enable efficient screening for resistance mechanisms other than the low stimulant production.

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