

**Molecular markers for the study of pathogen variability: implications for
breeding resistance to *Striga hermonthica***

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Abstract

Striga hermonthica is the most devastating parasitic weed of a group of important *Striga* species which cause damage to crop species. Its parasitic nature has required that it maintains high levels of genetic variation in order to ensure successful parasitism on its host plants and completion of its life cycle. For this reason breeding for resistance to the parasite has been hampered over the years by the parasite's ability to overcome host resistance and success stories have been few. An understanding of the patterns of variability of *S. hermonthica* within and between different regions which are under attack, and the extent of host selection effects, is essential if breeding programmes are to target sources of resistance at different areas and to realise the nature of the resistance required.

Many cross-inoculation experiments have been carried out to study host specificity, but little research has gone into studying geographical variability of the parasite itself. This study aimed to investigate the genetic variability within and between four different sorghum-adapted *S. hermonthica* populations at the molecular level using isoenzyme and RAPD technology. *S. hermonthica* populations were sampled from two sites in Mali and one site in Nigeria, west Africa and one site in Kenya, east Africa. A susceptible sorghum host variety, ICSV111, developed at ICRISAT was used as the host in order that a maximum number of genotypes would be able to parasitise it. Significant differences in variation were found within and between the sampled sites which may reflect fundamental differences in the genotypes present at each site and reinforces the need for multilocation testing of potential resistant varieties.

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Introduction

There are many techniques available today for the study of the genetic variability of populations of organisms from DNA sequence analysis to the study of proteins / isoenzymes. They all share the objective of assessing levels and patterns of genetic diversity, but differ in factors such as resolution, focus, difficulty and expense. All analyses of population variability involve scoring and accumulating the data for each sampled individual of a population, a primary focus of population genetics being on assessment of diversity within or among populations and running comparative tests between individuals and groups of individuals.

The first technique to permit the assessment of genetic variation in populations at the molecular level was introduced by Lewontin and Hubby (1966) when they described enzyme electrophoresis. Although this method provides relatively low resolution, it is simple, fast and the most inexpensive way to study variation in natural and artificial populations. It is by far up until now the most widely used method. The techniques that have advanced the direct analysis of DNA have been revolutionary in their impact, from RFLP (Sambrook et al., 1989) to DNA sequencing to PCR-based technologies and beyond. The RAPD technique (Williams et al. 1990) was one of the most modern PCR-based techniques in the early 1990s, but since then a number of new protocols have found wide applications in population genetic studies, such as minisatellite (Jeffreys et al., 1985) and microsatellite (Dietrich et al., 1996) and AFLP (Vos et al. 1995) technologies. Other techniques that have been described involve DNA conformational and denaturing gradient methods (Orita et. al., 1989) and the study of mitochondrial or chloroplastic DNA (Dowling et al., 1996). The method of choice depends very much on whether the focus of the study is inter- or intra-population variability.

Studies of the molecular variability of populations have their application in the fields such as biodiversity and conservation, population genetics and breeding systems, taxonomy and systematics. Perhaps one of the most important applications is the study of population genetic variability of crop pathogens. This is because an understanding of the variability within and between pathogenic populations from different geographical regions is essential if selection and breeding programmes are to target sources of resistance at different areas. *S. hermonthica* is the most important parasitic weed in the world (Parker and Riches, 1993). The crops it parasitises constitute the staple diet of the majority of people in the semi-arid tropics of Africa, and has its most devastating effect in areas which are already plagued by low soil fertility, drought, locust invasion and other crop diseases. Very few molecular studies on the genetic variation of *S. hermonthica* have been carried out. Most of the work has been concentrated into cross-inoculation experiments to study inter-crop variability for striga virulence, i.e. to test different crop host species against one striga accession; or intra-crop variability, i.e. to test

different populations of striga on one or a few hosts (e.g. Ramaiah, 1987; Parker and Reid, 1979; Vasudeva-Rao et al., 1982). The differences in virulence were usually scored by counting the number of emerged striga plants and scoring chlorotic damage of host leaves.

The present study aimed to investigate the genetic variability at the molecular level within and between four different *S. hermonthica* populations on one sorghum variety classified as a susceptible host. This was done through the application of isoenzyme and RAPD (randomly amplified polymorphic DNA) marker technologies.

Materials and methods

Plant material

Bulked-seed samples (accessions) of *Striga hermonthica* were collected from sorghum field populations at four different sites in Africa: two in Mali, one in Nigeria in West Africa; and one site in Kenya, East Africa. Seed accessions from Dongora, Mali and Mokwa, Nigeria were collected with the help of ICRISAT. The two sample sites within Mali were near Sotuba (coordinates: N 12° 39' 40"; W 7° 54' 43") and Dongora (coordinates: N 12° 7' 33"; W 7° 53' 36"). For the purposes of this paper the two sites within Mali will be referred to as the Sotuba, Mali and the Dongora, Mali sites, though seeds were sampled in the vicinity at the coordinates given. Seed accessions from Alupe, Kenya and Sotuba, Mali were sent by CIMMYT, Kenya and ICRISAT, Mali respectively. Accessions of *S. hermonthica* were given the codes, H1, H2, H3 and H4 for the sampled sites Alupe, Kenya; Sotuba, Mali; Dongora, Mali; and Mokwa, Nigeria respectively. The susceptible sorghum variety seed were given by ICRISAT and was variety ICSV111.

Preparation of S. hermonthica seed, inoculation and growth conditions

The *S. hermonthica* seeds were surface sterilised with 10% v/v of sodium hypochlorite solution for 10 minutes. After rinsing thoroughly with ddH₂O, the seeds were transferred to damp sterile filter paper and left to precondition for 10 days at 30°C, ensuring that the seeds were kept moist.

Two-week old sorghum seedlings were inoculated with the preconditioned *S. hermonthica* seed, using approximately 1,000 seeds per host plant. The seedlings were then carefully replanted in 5" pots of John Innes No2 compost and watered daily with sufficient water just to keep the soil moist, thus avoiding water stress and over-dilution of the host root exudate to stimulate germination of the striga seed. The plants were grown in a glasshouse under a 12

hour day / 12 hour night regime, and a minimum temperature of 30°C. Under these conditions striga plants attached and emerged within 4 to 6 weeks. The aim was to sample approximately 30 *S. hermonthica* plants from each site.

Protein extraction and electrophoresis

Approximately 30 mg of leaf material, from the youngest fully expanded leaves, were collected from each plant and ground in liquid nitrogen to a fine powder. The powder was transferred to an 1.5 ml Eppendorf tube and extracted with 2 ml of extraction buffer [50 ml of stock buffer: 15 ml dimethyl sulphoxide, 25 mg dithiothreitol, 500 mg PEG 8000, 500 mg PVP 40, 500 mg egg albumen, 5 g sucrose, ddH₂O to 50 ml; pH to 7.4]. The suspension was spun for one hour at 14,000 rpm at 4°C. The supernatant, containing the active proteins was carefully pipetted off and used for subsequent electrophoresis.

50 µl of each protein sample was electrophoresed using a vertical native polyacrylamide gradient gel system (5 % - 18 % acrylamide) keeping the apparatus and gel at 4°C throughout the run. The gels were pre-electrophoresed at 100 V for 20 minutes before loading and, after loading, at 200V for 2 hours, increasing to 350V for 2.5 hours. The acrylamide stock solution used was 38.7 % acrylamide: 1.33 % bisacrylamide. A continuous buffer system was used with Tris -borate EDTA (TBE) (1 litre of 10x concentration: 109 g Tris, 50 g Boric acid and 9 g NaEDTA, pH 8.3).

Gels were stained for 10 enzymes systems using protocols adapted from Vallejos, C. E. (1983) and Wendel, J.F. e.al. (1989). The enzymes were LAP (leucine aminopeptidase); MNR (menadione reductase); ME (malic enzyme); MDH (malate dehydrogenase); GOT (glutamate oxaloacetate transaminase); ADH (alcohol dehydrogenase); EST (esterase); G6PDH (glucose-6-phosphate dehydrogenase); SKDH (shikimic acid dehydrogeanse); and APS (acid phosphatase). After the banding patterns had developed sufficiently, each gel was scored and photographed.

DNA extraction and amplification

Approximately 40 mg of fresh *S. hermonthica* leaf material per plant was harvested and ground to a fine powder in liquid nitrogen. DNA was extracted based on the CTAB (cetyltrimethylammonium bromide) method of Doyle and Doyle (1990) modified by the use of NaCl to precipitate polysaccharides and PVP40 (polyvinyl pyrrollidone) to remove polyphenols during DNA purification. The protocol was also scaled down to employ the use of Eppendorf

tubes thereby accelerating the speed of extraction yet yielding DNA of a quality and quantity suitable for RAPD-PCR analysis.

RAPD-PCR conditions were described by Williams et.al. (1990). These were modified and optimised for *S. hermonthica* using a few individual samples. Each reaction of 50 µl volume consisted of 1.5 mM MgCl₂, 2 units Taq polymerase, 0.1 mM of each of dATP, dGTP, dCTP, dTTP; 0.2 µM primer, 5 µl of Promega 10x buffer (5 mM Tris pH 9.0, 25 mM KCl, 0.1 % Triton X-100), 50 ng template DNA. Five random 10-mer primers were chosen from a pilot study involving the screening of 80 random 10-mer primers against two *S. hermonthica* populations. They were chosen for their potential to detect polymorphism and also because they yielded a manageable number of bands for scoring purposes which were consistent between repeated reactions. The primer sequences were: 5'-CTGGGCACGA-3' (X1); 5'-TTCCGCCACC-3' (X2); 5'-GGAGCCTCAG-3' (X11); 5'-CCAAGCTTCC-3' (F9); 5'-GGAAGCTTGG-3' (F10).

The cycling parameters used were as follows: Step 1- Dissociation 94 °C, 1 minute; then 45 cycles of the following steps: Step 2- Dissociation 94 °C, 1 minute, ramping rate 6 °C/min; Step 3- Annealing: 38 °C, 1 minute, ramping rate 6 °C/min; Step 4- Extension, 72 °C, 2 minutes, ramping rate 4 °C/min. The PCR products were run with a kilobase ladder marker on a 1.5 % agarose gel made with 0.5 % TBE buffer and stained with ethidium bromide. The bands were viewed and photographed under UV light.

Band scoring and analysis

For both isoenzyme and RAPD data individual plants were scored for presence and absence of band positions, and this data was presented in the format of a series of „1“s and „0“s respectively. For isoenzymes, the bands were numbered according to migration distance from the cathode to the anode for each enzyme system. Only clear consistent bands were chosen for scoring, very faint bands or bands in zones that were not always present from gel to gel were ignored. Electrophoresis was repeated for samples where the presence of one band(s) was inconclusive. RAPD bands were chosen for their consistency throughout experiments and were numbered according to the primer used and the size of the amplified fragment. Small fragments of below 300 base pairs were ignored as they were often inconsistent from gel to gel and were possibly artefacts resulting from, for example, primer-dimers, polymerase slippage or in vitro DNA recombination (Ronning, 1995). Missing data was denoted by either „0.5“ (for SPSS or „9“ (for NTSYS), for both isoenzyme and RAPD data. The isoenzyme and RAPD data were

analysed separately. Each band represented one variable, therefore each plant had a multivariate profile when all bands were combined.

CLUSTAN (Wishart, 1987) was used for multivariate comparison of overall variance estimates between sites. NTSYS (Rohlf, 1993) was used for analysing band matrices and cluster analysis. Electrophoretic phenotype similarities were inferred from the cluster analysis employing unweighted pair group method with arithmetic averaging (UPGMA) (Sneath and Sokal, 1973) and phenograms were produced. SPSS (Norusis, 1994) was used for discriminate analysis as a way of emphasising any differences between groups of data. Coefficients were calculated for each variable so that the values of the discriminant function differ as much as possible between groups, or so that for the discriminant scores, the ratio (between-groups sum of squares : within-groups sum of squares) is a maximum. Scatterplots of the data were produced.

Results

Isoenzyme results

The 10 enzyme systems yielded 47 scorable bands (Table 1). The overall „band profile“ obtained by combining all the data for each plant, indicated that there was a very high level of phenotypic, and therefore genetic variation between individuals. No site-specific bands were detected. A total of 137 plants were screened from the four sites in Africa. Sample sizes ranged from 28 to 44 plants sampled per site (Table 2).

Table 1. Enzyme systems used and scored bands.

Enzyme system abbreviation	Number of bands scored
SKDH	3
ADH	3
ACP	4
GOT	9
MDH	3
ME	5
MNR	6
LAP	3
G6PDH	4
EST	7

Table 2. Accession sample sizes for isoenzyme analysis.

<i>S. hermonthica</i> accession	Number of plants screened
H1 Alupe, Kenya	35
H2 Sotuba, Mali	28
H3 Dongora, Mali	30
H4 Mokwa, Nigeria	44
Total	137

Table 3. Variation present within each site from the isoenzyme data.

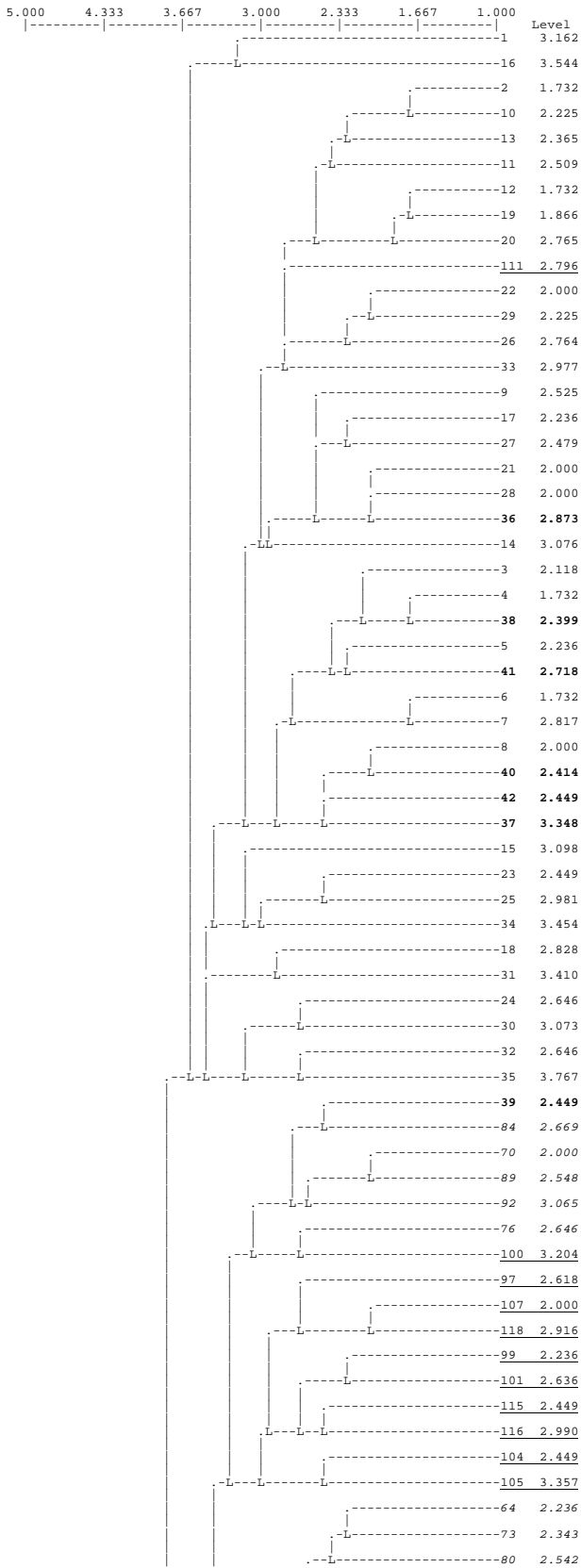
Striga accession	Total Euclidian sum of squares	Site variance
H1 Alupe, Kenya	36.607	6.050
H2 Sotuba, Mali	47.358	6.882
H3 Dongora, Mali	24.094	4.908
H4 Mokwa, Nigeria	15.856	3.908

Table 4. Comparison of the isoenzyme variances for each site.

<i>S. hermonthica</i> accession	F-ratios of within-site variances		
	H1	H2	H3
H2	1.137*		
H3	1.233	1.402*	
H4	1.519*	1.728*	1.233

*Significant difference between site variances observed at P=0.05.

The variances were calculated for each site using squared Euclidean distances as a similarity index, and Ward's method of minimum variance clustering. This provided an estimate of the total variance present within each group and allowed a multivariate comparison of variance between sites to be made (Table 3). Accession H2 (Sotuba, Mali) was the most variable. The F-ratios of the variances for the different sites were calculated (Table 4). These show significant differences between the variances for accession H2 (Sotuba, Mali) compared with all other accessions and between H1 (Alupe, Kenya) and H4 (Mokwa, Nigeria).



Key to accessions:

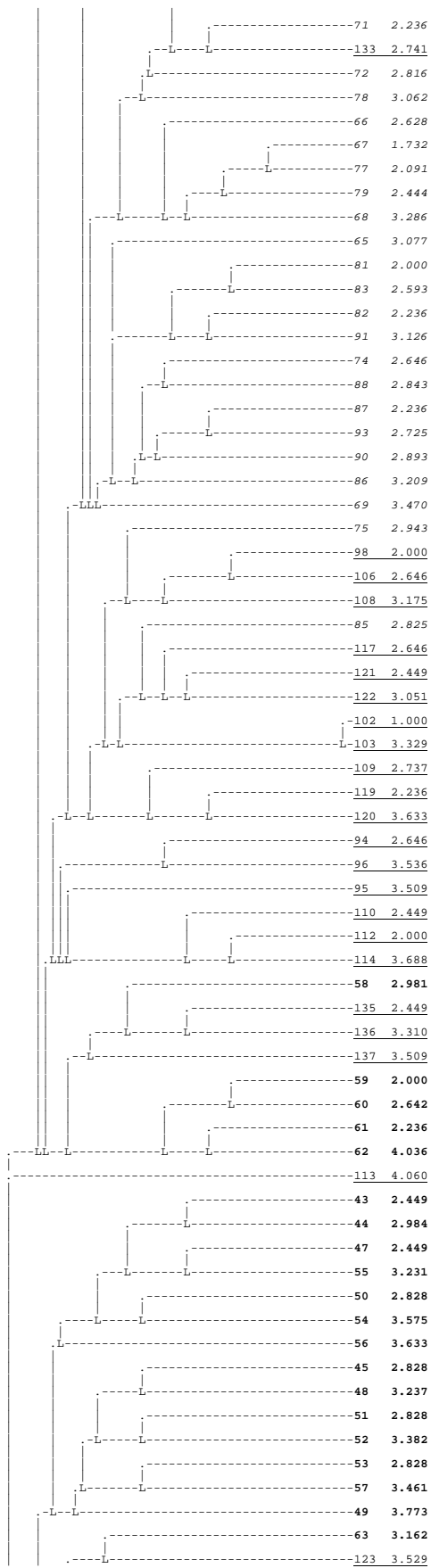
H1: normal

H2: **bold**

H3: *italic*

H4: underlined

Figure 1 (continued on next page).



Key to accessions:

H1: normal

H2: **bold**

H3: *italic*

H4: underlined

Figure 1 (continued on next page).

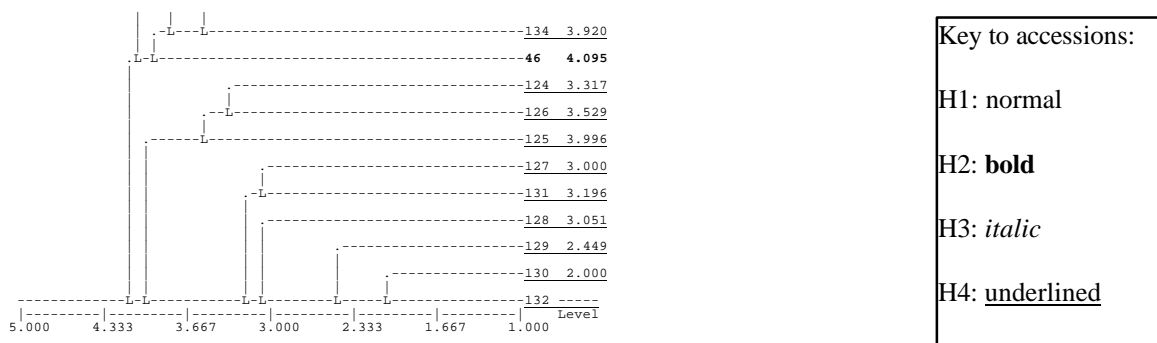


Figure 1. A phenogram based on the cluster analysis of the isoenzyme phenotypes for the H1 (Alupe, Kenya), H2 (Sotuba, Mali), H3 (Dongora, Mali) And H4 (Mokwa, Nigeria) accessions selected by the sorghum host line ICSV111, to show the geographical differences between the sites. Each font refers to a particular *S. hermonthica* accession as given in the key, and each branch represents a single individual (n=137).

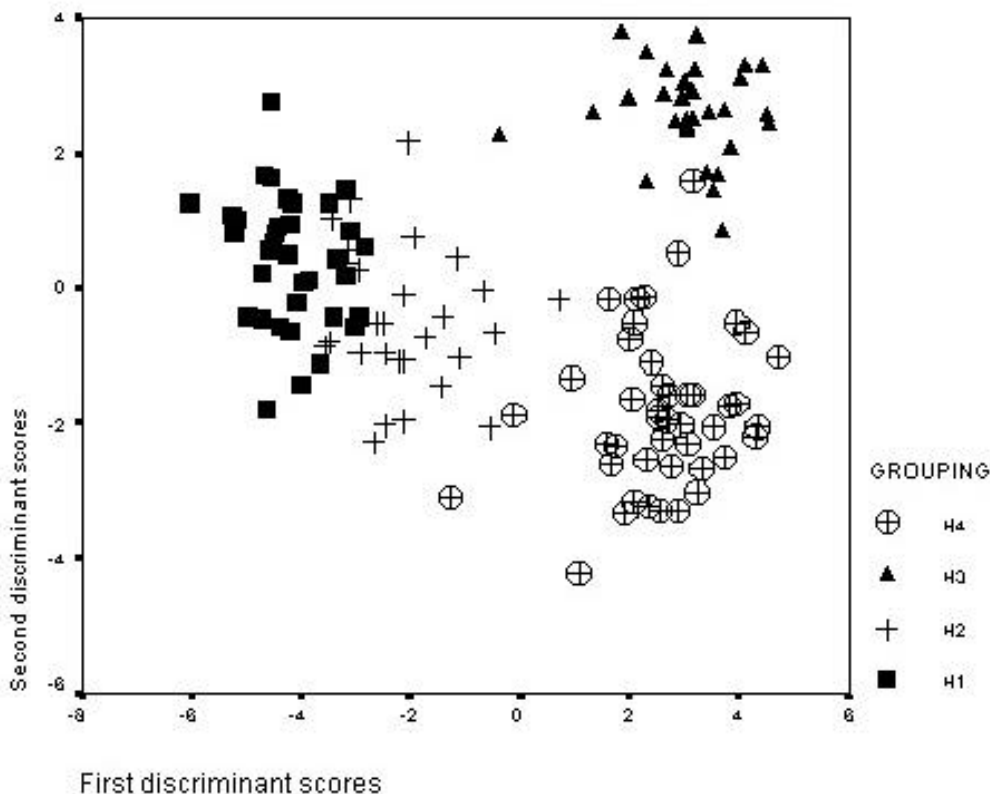


Figure 2. Scatterplot of the discriminant scores for individuals of accessions H1, H2, H3, and H4 grown on the variety ICSV 111.

Phenograms were generated from the Euclidean distance matrix using the clustering method UPGMA. Figure 1 shows the resulting phenogram for the isoenzyme data. There are essentially three main clusters of phenotypically related individuals; one consisting mainly of the H1 (Alupe, Kenya) accession, another containing all individuals from the H3 and H4 accessions with a few H2 individuals, and finally a cluster consisting of the majority of individuals from the H2 accession. The cluster consisting of the H3 and H4 accessions contains smaller subclusters of each accession showing that, although the two accessions contain similar isoenzyme variants, there are variants which are characteristic of each accession.

The discriminant scores were calculated with respect to each *S. hermonthica* accession grown on the sorghum variety ICSV111 resulting in a scatterplot, Figure 2. The scatterplot is supportive of the cluster analysis with phenotypes characteristic for each population forming separate groups. The east African accession (H1) phenotypes overlap slightly only with those of the H2 (Sotuba, Mali) accession; the Nigerian accession (H4) has phenotypes bearing similarity to the other two West African accessions. The West African H3 and H4 accessions bear no similarity to the east African accessions and are closer to each other and the H2 accession.

RAPD-PCR results

Table 5 gives the approximate molecular weight of the scored amplified fragments. On average between 6 and 7 polymorphic bands were detected per primer giving a total of 33 scored bands. No bands were found that were unique to a particular accession. Table 6 shows the total number of plants sampled for each site. The site variances were estimated in the same way as for the isoenzyme data. Table 7 shows that site H2 (Sotuba, Mali) is more variable than the rest; H3 (Dongora, Mali) is the least variable site; and sites H1 (Alupe, Kenya) and H4 (Mokwa, Nigeria) have similar intermediate amounts of RAPD variation. The F-ratios of the site variances showed that there was no significant differences between the amounts of RAPD variation detected at each site (Table 8).

The phenogram resulting from the UPGMA clustering analysis is shown in Figure 3. The H1 (Alupe, Kenya) accession forms a discrete group, quite separate from the west African accessions, i.e., it joins the rest of the phenogram at the highest level of dissimilarity (3.304). This accession appears to consist of two subclusters of genotypes. The H2 (Sotuba, Mali) accession forms a distinct group at a level of 3.144 with an overlap of two individuals grouping with the H4 (Mokwa, Nigeria) accession cluster. The H3 (Dongora, Mali) accession also forms a discrete cluster, but appears to contain genotypes more closely related to the H4 accession than to the other Malian accession (H2). The branching occurring within the H1 and H2 clusters

suggest that the genotypes of individuals in these accessions are highly variable, whereas the genotypes within the H3 and H4 accessions are more similar to each other.

Table 5. The approximate molecular weights of the RAPD bands produced for each primer.

Primer	Band number and molecular weight (base pairs)							
	1	2	3	4	5	6	7	8
X1	875	950	750	525	510	370	1,0181	450
X2	5,090	1,300	900	750	600	-	-	-
X11	3,054	1,800	1,550	1,018	850	506	450	700
F9	8,000	2,200	1,900	1,100	450	-	-	-
F10	2,500	1,800	1,300	800	600	450	370	-

Table 6. Sample sizes used for RAPD analysis.

<i>S.hermonthica</i> accession	No. of plants screened
H1 Alupe, Kenya	20
H2 Sotuba, Mali	20
H3 Dongora, Mali	5
H4 Mokwa, Nigeria	20
TOTAL	65

Table 7. RAPD variation present within each site.

Striga accession	Total Euclidean sum of squares	Site variance
H1 Alupe, Kenya	55.198	7.429
H2 Sotuba, Mali	77.254	8.789
H3 Dongora, Mali	32.781	5.725
H4 Mokwa, Nigeria	54.059	7.352

Table 8. Comparison of the RAPD variances for each site.

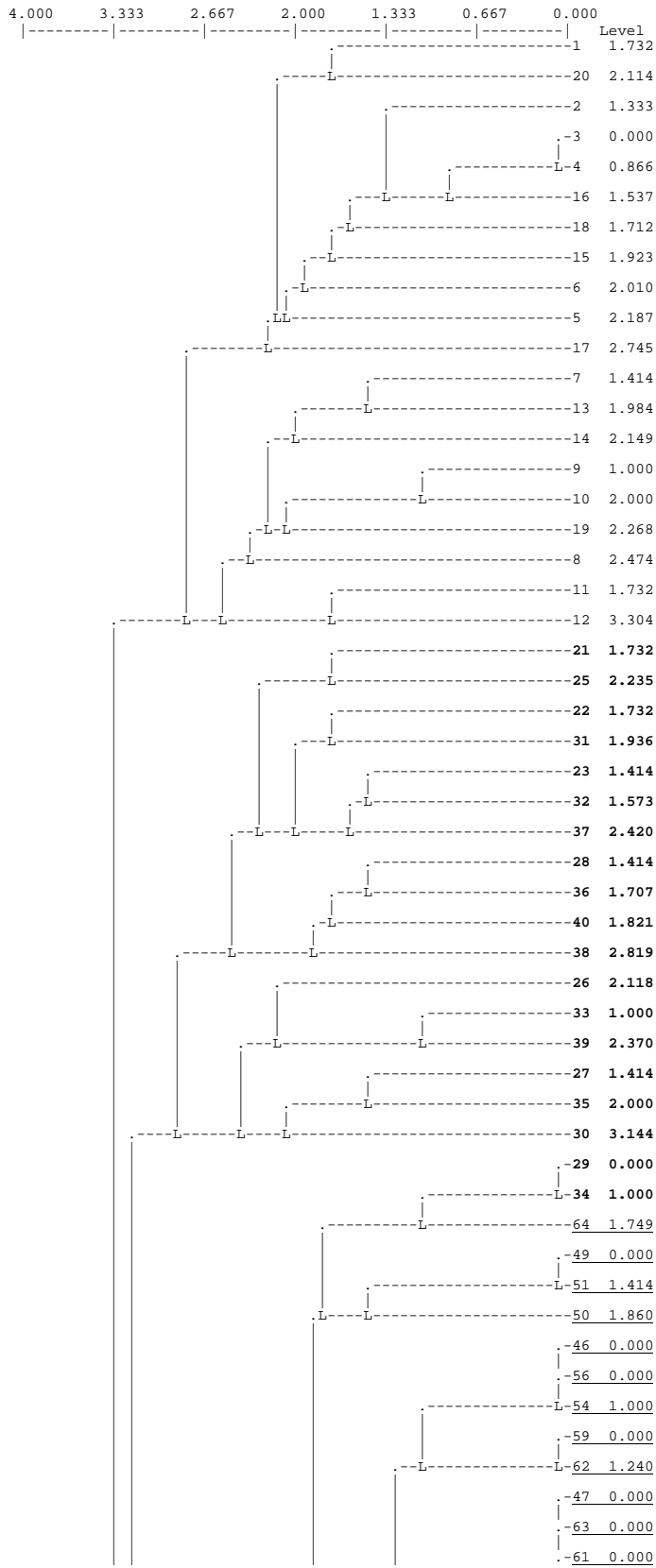
<i>S. hermonthica</i> accession	F-ratios of Within Site Variances		
	H1	H2	H3
H2	1.183		
H3	1.297	1.535	
H4	1.010	1.195	1.284

Figure 4 shows the scatterplot of the RAPD data resulting from the discriminant analysis. As in the isoenzyme results the scatterplot is supportive of the results of the cluster analysis in that all accessions for each population form distinct groupings. However, because discriminant analysis emphasises the differences between groups there is no overlap of accession groups. The scatter of points within each group reflects the level of variation found at each site

Discussion

The genetic basis of the isoenzyme phenotypes observed does not affect the main purpose of this study, which was to determine the extent of isoenzyme polymorphism within and between sites. Individual plants were scored for the presence and absence of isoenzyme bands which were used as simple taxonomic / phenetic markers.

RAPDs have been reported as being sensitive to amplification conditions (Ellsworth et al. 1993). There is also the problem that bands of similar sizes are not always homologous (Yli-Mantilla et al., 1996; Thormann et al. 1994). The verification of RAPD markers in *S. hermonthica* was made in the F1 generation of selected individuals from the four populations (data not shown). Controlled crosses were made between parents of known genotype and the segregation ratios of each marker band in the progeny was studied. Allowing for the fact that parents having a RAPD marker were either heterozygous or homozygous for the presence of the dominant marker allele, the ratio of the observed progeny genotypes were in concordance with expected ratios for Mendelian inheritance.



Key to accessions:

H1: normal

H2: **bold**

H3: *italic*

H4: underlined

Figure 3 (continued on next page).

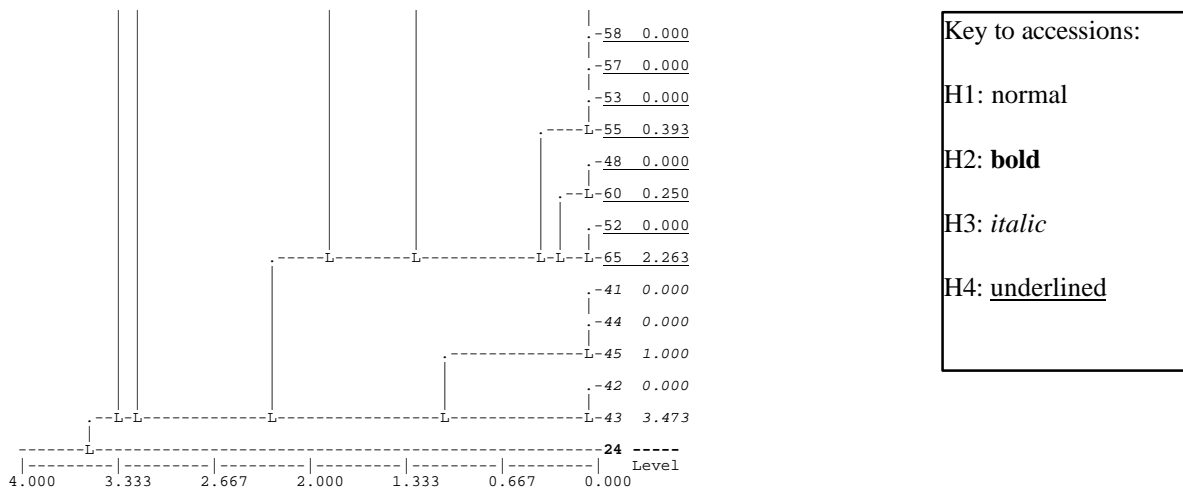


Figure 3. A phenogram based on the cluster analysis of the RAPD phenotypes of the H1, H2, H3 and H4 accessions as selected by the sorghum host line ICSV111, to show geographical differences between the sites. Each font refers to a particular host variety \times *S. hermonthica* accession combination, as given in the key, and each branch represents a single individual (n=65).

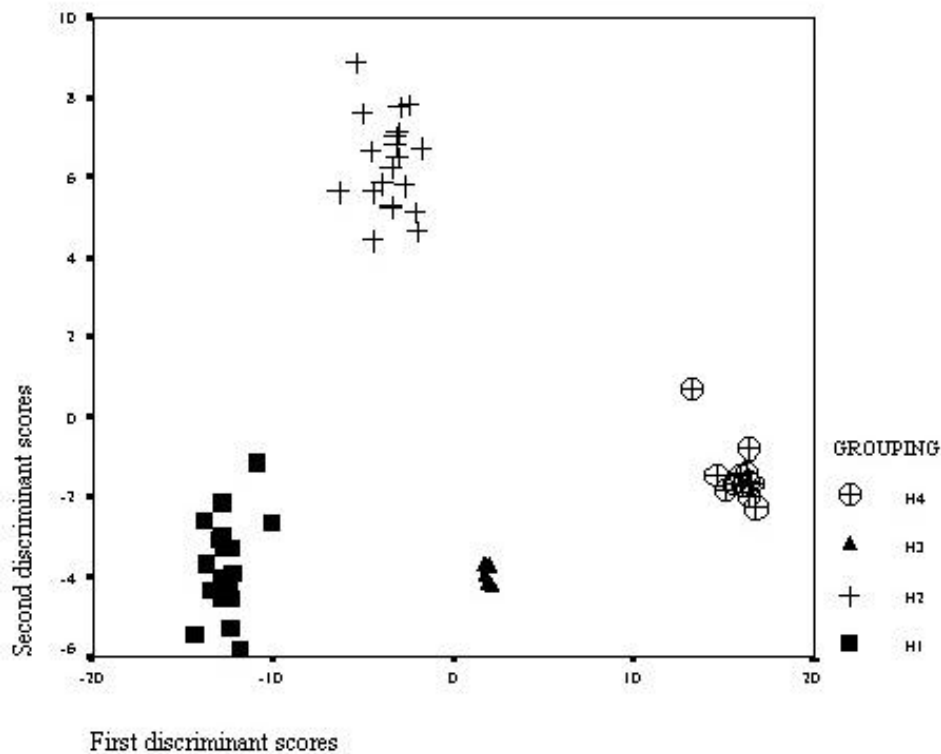


Figure 4. Scatterplot of the accession groups grown on variety ICSV 111 using RAPD data.

The isoenzyme results showed significant differences in amounts of variation detected between sites (even within country), whilst the RAPD results showed no significant differences. The expected pattern observed for outbreeding populations (Hamrick, 1989) is that most of the variation usually occurs within populations rather than between, however, these observations were made for non-parasitic plant populations. The fact that the isoenzyme results detected significant differences between sites may reflect both the fundamental differences in genotypes present at each site and adaptation of populations to a particular sorghum varietal selection pressure.

The variance estimates within each *S. hermonthica* population range from 3.908 to 6.882 for the isoenzyme study, and from 5.725 to 8.789 for the RAPD study. The RAD technique is expected to detect higher levels of variation due to the sampling of non-coding regions which are prone to mutation than the more highly conserved coding regions of the isoenzyme survey. The trends in the amount of variation at each site compared with other sites was consistent between the two techniques. These variance estimates are high, especially when compared with variance estimates from a similar study conducted by Shawe and Ingrouille (1993). Three populations of the inbreeding *Striga gesnerioides* were surveyed for isoenzyme polymorphism with variance estimates ranging from 0.024 to 0.077. These estimates are expected to be low as predominantly self-pollinating species usually have less variability within populations compared to between populations (Levin, 1978).

The cluster and discriminant analysis of both the RAPD and the isoenzyme data show clear separation of the site specific groups as shown in the phenogram and scatterplot. As expected the RAPD data produced more distinct groupings. This indicates that genotypes characteristic of particular sites exist, although no site-specific markers were detected. Similarity coefficients showed that there were distinct differences between the West African sites and the east African site, and also between the Malian sites and the Nigerian site within west Africa.

Using isoenzyme studies, Musselman et al. (1991) demonstrated that genetic divergence between populations of *S. hermonthica* in different areas of Africa is greater than the divergence between populations with a different host preference, for sorghum or pearl millet, in one country. This provides an insight to the maintenance of variability which allows adaptation to a particular host crop, in that adaptation can be achieved without sacrificing useful genetic variation that can be utilised in the parasitisation of a new host.

The success of producing resistant varieties is based on the degree of control which man can exercise through selection, or breeding, for high yield and striga resistance. Doggett (1965) reported that the cultivars Dobbs and Framida of east Africa were resistant to striga infection, but the inconsistency of performance of host resistance to striga infestations in different

geographical areas has made many investigators reluctant to put forward comparable claims which may later prove to be unfounded. This may be because, at that time, investigators did not consider the possible effects of physiological variants among the species (Parker and Reid, 1979). Therefore, the physiological variability of *Striga* species, expressed by differences in host range and between regions, is of agricultural significance.

Recently much effort has been put into breeding sorghum varieties with desirable grain characteristics and the low-stimulant recessive gene from SRN39 (Ejeta, 1997). The first resistant variety containing this gene was produced in 1995, now new varieties have been produced and released with the estimation that some 200,000 farms should be growing this striga-resistant sorghum in 12 African countries by this year. However, these varieties still support the life-cycle of a few *S. hermonthica* plants, thus the danger still exists that this resistance may eventually be broken down. In view of the high levels of variability existing in *S. hermonthica* populations that allows the parasite to quickly adapt to new crops / varieties, the target should be to produce multigenic resistant varieties with a broad selection pressure together with the use of multilocation trials to verify resistance in the field as an absolute necessity.

Conclusion

This study has demonstrated high levels of geographical variation within and between *S. hermonthica* populations. This allows the rapid adaptation of *S. hermonthica* populations to new host crops / varieties and emphasizes the need for an integrated approach to control using treatments and cultural methods which eliminate or minimize parasite seed production, leading to reduction of the seed bank, and which improve soil quality (not discussed here). Farmer training (Shaxson, 1993) will also be important in the acceptance of an integrated approach by the farming community in the struggle against this very successful parasitic plant.

References

- Dietrich, W.F., J. Miller, R. Steen, M.A. Merchant, D. DamronBoles, Z. Husain, R. Dredge, M.J. Daly, K.A. Ingalls, T.J. OConnor, C.A. Evans, M.M. DeAngelis, D.M. Levinson, L. Kruglyak, N. Goodman, N.G. Copeland, N.A. Jenkins, T.L. Hawkins, L. Stein, D.C. Page, and E.S. Lander. 1996. A comprehensive genetic map of the mouse genome. *Nature* 380: 149.
- Doggett, H. 1965. *Striga hermonthica* on sorghum in East Africa. *Journal of Agricultural Science* 65: 183-194.
- Dowling, T.E. et al. 1996. p. 249-321. *In* *Molecular Systematics*, 2nd Edn. Sinauer, 150

Sunderland, MA.

- Doyle, J.J., and J.L. Doyle. 1988. Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- Ejeta, G. 1997. Saving sorghum by foiling the wicked witchweed. *Science* 227: 1040.
- Ellsworth, D.L., K.D. Rittenhouse, and R.L. Honeycutt. 1993. Artfactual variation in randomly amplified polymorphic DNA banding patterns. *Biotechniques* 14: 214-217.
- Hamrick, J.L. 1989. Isozymes and the analysis of genetic structure in plant populations. p. 87-105. *In* D.E. Soltis and P.S. Soltis (eds): *Isozymes in Plant Biology*. Chapman and Hall Ltd. Jeffreys, A.J., Wilson, V., Thein, S.L. 1985. Hypervariable minisatellite regions in human DNA. *Nature*, 314: 67.
- Levin, D.A. 1978. Genetic variation in annual Phlox: self compatible versus self-incompatible species. *Evolution* 32: 245-263.
- Lewontin, R.C., and J.L. Hubby. 1966. *Genetics* 54: 595.
- Musselman, L.J., et al. 1991. Recent Research on the Biology of *Striga asiatica*, *S. gesnerioides* and *S. hermonthica*. p. 31-41. *In* Kim, S.K. (ed.): *Combating Striga in Africa*. IITA, Nigeria.
- Norusis, M.J. 1994. SPSS Professional Statistics 6.1. SPSS UK Ltd., SPSS House, 5 London Street, Chertsey, Surrey, KT16 8 AP.
- Orita, M., Y. Suzuki, T. Sekita, and K. Hayashi. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 5: 874-879.
- Parker, C., and D.C. Reid. 1979. Host specificity in *Striga* species - some preliminary observations. p. 79-90. *In* L.J. Musselman, A.D. Worsham, and R.E. Eplee (eds.): *Supplement to Proceedings, 2nd International Symposium on Parasitic Weeds*, Raleigh, USA.
- Parker, C., and C.R. Riches. 1993. p. 1-73. *In* *Parasitic Weeds of the World: Biology and Control*. CAB International, Oxon, UK. Chapter 1.
- Ramaiah, K.V. 1987. Breeding cereal grains for resistance to witchweed. p. 227-242. *In* L.J. Musselman (ed.): *Parasitic Weeds in Ariculture*. Vol. 1. CRC Press, Boca Raton, FL.
- Rohlf, F.J. 1993. NTSYS-pc Numerical taxonomy and multivariate analysis system, version 1.8. Publishers: Exeter Software, New York.
- Ronning, C.M., and R.J. Schnell. 1995. Inheritance of random amplified polymorphic DNA (RAPD) markers in *Theobroma cacao* L. *J. Amer. Soc. Hort. Sci.* 120: 681-686.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning - a laboratory manual* (2nd edition). Cold Spring Harbour Laboratory Press, U.S.
- Shawe, K.G., and M.J. Ingrouille. 1993. Isozyme analysis demonstrates host selection of parasite pathotypes in the association between cowpea and *S. gesnerioides*. *Brighton Crop Protection Conference- Weeds* 2: 919-924.
- Shaxson, L.J., et al. 1993. Incorporating farmer knowledge in the design of weed control

- strategies for smallholders. Brighton Crop Protection Conference - Weeds 3: 1149-1154.
- Sneath, P.H.A., and R.R. Sokal. 1973. Numerical Taxonomy. Freeman, San Francisco. 573pp.
- Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang, and T.C. Osborn. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88: 973-980.
- Vallejos, C.E. 1983. Enzyme activity staining. p. 469-516. In S.D. Tanksley and T.J. Orton (eds.): Isozymes in plant genetics and breeding, Part A, Elsevier, Amsterdam.
- Vasudeva Rao, M.J. et al. 1982. Genetic control of *Striga asiatica* in sorghum. p. 22. In R.V. Vidyabhushanam et al. (eds.). ICRISAT-AICSIP (ICAR) Working Group Meeting on Striga Control. ICRISAT, Patancheru.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van der Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids research 23: 4407-4414.
- Wendel, J.F., and N.F. Weeden. 1989. Visualization and Interpretation of plant isozymes. p. 5-45. In: D. E. Soltis and P. S. Soltis (eds.): Isozymes in Plant Biology. Chapman and Hall Ltd., London.
- Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids. Res. 18: 6531-6535.
- Wishart, D. 1987. Clustan User Manual. Fourth edition. Computing Laboratory, University of St. Andrews, UK.
- Yli-Matilla, T., S. Paavanen, A. Hannukkala, P. Parikka, R. Tahvonen, and R. Karjalainen. 1996. RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathology 45: 126-134.