GENETIC VARIABILITY, HOST SPECIFICITY AND RESISTANCE IN *STRIGA ASIATICA*-HOST PLANT INTERACTIONS

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ABSTRACT

Genetic analyses were conducted on *Striga asiatica* populations collected from different host species and geographic areas throughout Zimbabwe. These collections had been suspected to differ in their virulence, leading to the breakdown of resistance among resistant sorghum cultivars. Discrete genetic markers analysed in this study included isozymes and arbitrary regions of the genome amplified by the polymerase chain reaction, commonly known as random amplified polymorphic DNA (RAPD). In all, twelve enzyme systems were evaluated; five of these producing well resolved bands and therefore being used for analysis. Polymorphisms were detected at 5 loci; glutamate oxaloacetate-transaminase (*GOT*), *α* -esterases (*α-EST*), leucine amino peptidase (*LAP*), glucose-6-phosphodehydrogenase (*G-6-PDH*) and peroxidases (*PER*). Isozyme variation was absent between populations collected from the same geographical areas but was present between populations from different regions. Much greater genetic variation was detected when populations were analysed with RAPD. Populations were analysed with 32 polymorphic bands amplified by five primers. Cluster analysis indicated strong similarities between populations from the same regions. There was a positive correlation between geographic and genetic distances.

Inter-host specificity of the different strains of *Striga asiatica* was also tested. Using a reciprocal cross-infection experiment, four host species (maize, pearl-millet, finger millet and sorghum) were exposed to *Striga* from the same hosts. The results showed evidence of host specialization as the most severe yield reductions in the respective cereal hosts were caused by *Striga* originating from the same host species. In maize, the *Striga* strain from a maize host caused the greatest yield reduction of 42.5% of the total dry weight and 80.6% of the head weight. In finger millet the strain from the same host caused the greatest grain yield reduction of 59 % as compared to the uninfested plots. There was the same trend in sorghum where the strain from the same host was the most virulent.

Intra-host specificity was investigated using sorghum as the host. Fifteen sorghum varieties were exposed to ten populations of *Striga using* the root exudate technique. There was significant interaction ($P<0.05$) between *Striga* population and sorghum variety. Populations SAR16 x SAR19 and SAR19 x SV-1 had stable resistance across all the *Striga* populations whilst the rest showed different reactions to different populations.

The major findings of this study are that there are physiological strains of the parasitic weed *S. asiatica,* which are adapted to specific hosts within and inter species. Another finding was that molecular techniques such as RAPDs and isozymes can be effectively used to differentiate strains of the parasitic weed, S asiatica however there is need to determine the relationship between molecular differences and differential virulence.

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CHAPTER 1

1.1 Introduction and Justification

Striga asiatica (L) Kuntze is a noxious root parasitic weed which attacks all the major cereals produced in Zimbabwe and the Southern African region. *S. asiatica* is the most wide-spread and economically significant parasitic weed in parts of SADC region, especially in Botswana, Malawi, Mozambique,Tanzania, Zambia, Zimbabwe and Swaziland. Two other species, *S. hermonthica* (in Tanzania) and *S. forbesii* (in Tanzania and Zimbabwe) also can be endemic in specific areas. The main crops affected by *S.asiatica* are sorghum, maize, pearl and finger millets, rice and sugar cane. Crop losses due to *S.asiatica* were reported to be up to 100% depending on the levels of infestation (Mabasa, 1993; Obilana and Ramaiah, 1992). In the case of sorghum, *S asiatica* was reported to be the most important yield reducing factor in Africa (Hess and Ejeta, 1992). This weed therefore is a major constraint to increased cereal production. The effect of *S .asiatica* on sorghum production has significant negative implications on the smallholder sector. This is so because sorghum is one of the most important cereal crops grown by resource poor farmers in Zimbabwe, particularly those in the semi-arid areas.

In order to alleviate the *Striga* problem much effort has been invested in *Striga* control by chemical, mechanical and cultural means. Control of *Striga* has proved difficult with use of cultural practices or herbicides because the weed produces many seeds which can remain viable up to thirty years (Parker and

Riches, 1993). One of the main approaches to decrease yield losses caused by *Striga asiatica* infestation is breeding for resistance. Resistant varieties have long been seen as the main hope for control of *Striga*. Maize grain yields from the *Striga* tolerant/resistant (STR) varieties developed by IITA scientists were 2.5 times higher than those from the susceptible varieties (Lagoke and Hoevers,1993). It may be expected however that, due to genetic plasticity of the parasite, resistance or tolerance would eventually be overcome. The major constraint to breeding for *Striga* resistance is the presence of the so called physiological "strains" or "biotypes" of the common *striga* species (Parker and Reid, 1979). This particularly has been noticed in *Striga hermothica* which is presumed to be more virulent on cereals. The development of specific strains could be attributed to evolutionary changes (Ramaih,1987) presumably encouraged by geographical isolation over a number of years. In the case of *S.asiatica* which is highly self pollinating there is no documented information on why there should be any variation. Although there is some evidence of variability in *S. asiatica*, very scanty information is available on the impact this variability has on the breeding programmes and on the stability of resistant varieties. It is therefore important to have a detailed understanding of the genetic variability of the parasite. This will involve identifying and clearly characterizing the strains, so that the breeding programmes will take this into account.

The genetic variation which exists in *S. asiatica* strains that attack maize, sorghum and millets influences the reaction of the host plant to the weed (King, 1975). Information on the genetic variation of *S. asiatica* should enhance efforts to breed elite sorghum cultivars with broad spectrum and durable resistance

through better understanding of the genetics of host-parasite interactions and identification of different *Striga* virulence genes. The utility of such knowledge on virulence genes or gene-to-gene resistance is the opportunity it will provide to breeders of potentially pyramiding genes for a broader resistance to *Striga*. Information on the genetic diversity of *Striga asiatica* populations from different host plants will also be useful in determining the mechanisms of host specificity in this weed. This information will also give an indication of variability for virulence in the witchweed. Information on variability has implications on the control of the weed. Plant breeders have generated information on host plant resistance with the goal of developing *Striga asiatica* resistant genotypes but the genetics of host and parasite genotypes and their interactions are not clearly understood, yet to fully exploit host-plant resistance a good knowledge of genetic variability in the parasite population is critical.

1.2 Overall objective.

To study the genetic diversity of different S.asiatica populations and to generate information on the interactions of these populations with different host plants.

1.3 Specific objectives.

1) To investigate genetic variability in *S. asiatica* populations using RAPD-PCR and the isozyme markers.

2) To determine specificity of *S. asiatica* strains to different host plants.

3) To investigate the variation in virulence of *S. asiatica* strains from different host plants and locations of Zimbabwe.

4) To screen sorghum varieties and populations from crosses for resistance to different *Striga asiatica* strains.

CHAPTER 2

2.1 General Literature Review

2.1.1 Description of the Weed.

Striga asiatica belongs to the genus Scrophulariaceae. The weed grows 15 to 25 cm in height, and has narrow, linear or somewhat lanceolate leaves 0.5 to 5 cm long. The plant has been described by Dogget (1988) as being hairy with its flowers borne in dense or loose terminal spikes, 10 to 15 cm long. Seeds of *Striga* will germinate only in the presence of a stimulant substance produced by the roots of host plants and those of a few non-hosts. The microscopic seed of *Striga* has a long dormant period lasting several months, during which germination stimulants are ineffective. As was described by King (1975) germination of the seed occurs in the soil wherever root exudates from the stimulant-producing plant reach a threshold concentration. *Striga* has no root hairs and must absorb all its water and nutrients from the roots of the host through its haustoria. The aerial parts of *Striga* become green after the parasite emerges from the soil, and it is semi-parasitic thereafter.

Flowering in *Striga* starts about one month after emergence from the soil and seeds are formed in capsules, which burst open to disperse the seeds. Surveys carried out by Obilana (1992) showed that the species is widely distributed in Zimbabwe and probably ranks first in terms of economic importance followed by *S. forbesii*. It has been reported to cause damage to cultivated fields of sorghum, millets and maize in Matopos, Chiredzi, Buhera, Mutare and Darwendale and more recently just outside Masvingo. Other crops affected are

sugar cane and rice. Natural hosts include *Digitaria, Chloris, Andropogon, Heteropogon, Hyperthelia* and various other local grasses. *Striga asiatica* has been collected from a wide range of habitats including cultivated land, vleis, woodlands, grasslands, and granitic outcropping (Williams, 1959).

2.1.2 Breeding for Resistance to *Striga* infestation.

Much effort has been put in developing resistant/tolerant lines of crops so as to combat the S*triga* problem. The main effort using this approach has been in sorghum. In India, Rao, Rao and Pardhasaradhy (1967) attempted to develop varieties of sorghum which are resistant to *S. asiatica* because other methods of controlling this pest were too costly. A resistant variety, designated N13, was released after six years' selection for resistance. Several other Indian varieties of sorghum, including Boganhilo, YK, Bilichigan, Agyalkodal, Illeendi, Nandyaal, Mallemari, No 109 and Co 20 are claimed to have a high level of resistance to *Striga* (Kasasian, 1971). Desai, Khatri and Patel (1972), in studies conducted in India compared the resistance of eight partially resistant sorghum varieties in different seasons and different environments. They concluded that BC-8 showed the most consistent expression of resistance in different situations and could be expected to give a good control of *Striga* even in heavily infested soils. Lagoke (1993) reported that the Institute for Agricultural Research (IAR) Samaru, Nigeria has screened over 200 lines for resistance/tolerance to *Striga*. The research led to the identification of a resistant line, SRN 4841, which is being used to develop more acceptable resistant lines. Currently in Zimbabwe the University of Zimbabwe in collaboration with government's Department of

Agricultural Research and Extension are screening and developing lines for *S. asiatica* resistance. However, as was reported by Ramaiah (1987), there are various limitations to breeding for resistance/tolerance in sorghum. These limitations include low yield and low quality grain in the resistant varieties, hybridisation of *Striga* plants which results in new strains that may overcome resistance/tolerant genes, and great variability in the resistance/tolerance by lines exhibited by developed lines. It is also important to note that the variability exhibited by lines is influenced by other various factors such as level of infection, climatic factors and management practices. This makes the breeding for resistance much more complicated.

2.1.3 Genetic Variation within *Striga*.

The genetics of parasitism can be classified as either simple or complex, depending upon the absence (simple) or existence (complex) of multiple races or strains of the parasite. Races are distinguished by altered virulence or altered specificity. Altered specificity is usually manifested in the form of a new race that overcomes the resistance of certain host genotypes.

A high degree of variability exists within *Striga* as indicated by the variation in germination and pre-treatment requirements and interactions with non host factors. Ramaiah (1987) observed that both *S. asiatica* and *S. hermonthica* have strains which are specific to different crops (intercrop specific strains) and strains within different crops (intra-crop specific strains). Their work showed that the intercrop specificity was mainly observed between sorghum and millet crops. *Striga* strains which attack sorghum do not attack pearl millets and vice-versa.

Even though there are only three species of *Striga* which cause economic losses in sorghum, millet and maize, Ramaiah (1987) reported that virulence variability within these species makes the breeding programmes more complicated. *Striga* is expected to have considerable genetic variability because as resistance mechanisms appear in host populations, new forms of the parasite largely resistant to these mechanisms will most likely get selected for. These resistant *Striga* strains will reproduce and form distinct populations. It is therefore necessary to determine the extent of the variability in *Striga* populations to develop an effective breeding programme.

Ramaiah (1987) reported variability in *Striga* species for (1) germination stimulant requirements (2) preconditioning requirements, (3) chromosome number and (4) pollination systems. Studies by Bharatalakshmi and Jayachandra (1979) in South India revealed that *Striga asiatica* has strains that are specific to sorghum, millet and ragi (finger millet) and their specificity is based on germination stimulant compounds. Variation in chromosome numbers have been reported in the United States and in India. Kondo (1973) reported n = 12 chromosomes in *Striga asiatica* of North Carolina in the U.S. whereas in India, Rao (1965) reported n = 20 chromosomes in *S. asiatica*, *S. densiflora*, *S. angustifolia* and *S. gesneriodes*. The differing chromosome numbers indicated that *Striga* has undergone considerable evolutionary changes and therefore wide genetic variability was expected. The variations which have occurred in these areas dictates the need for investigation of the locally occurring Striga *asiatica* populations.

Although Ramaiah, (1987) reported clear evidence of crop specific strains within each species of *Striga*, there is very little information on the presence of virulence variability in Striga strains attacking the same crop. Some variability in *S. asiatica* attacking sorghum in India was observed. At Akola in Maharashtra State, IS 5603 was resistant, but was susceptible at Patancheru in Andra Pradesh indicating that *S. asiatica* differred in virulence in these two places. These differences in virulence between sites meant that should a relatively resistant crop variety be developed its resistance may not hold in different geographical areas.

2.1.4 Importance of Parasite Variability in relation to durability of resistance.

It is reported in Russell (1978) that the force of the evolutionary direction exerted on major pathogens of cereals by growing of highly resistant varieties has frustrated many attempts to breed for stable resistance. This is because virulent, resistance breaking forms of the pathogens can multiply freely without competition on the varieties which they alone can attack This situation has happened with several pests, for example the level of resistance to the brown plant hoppers is so high in some rice varieties that populations of this insect have been forced to change genetically so that they attack these varieties, or be eliminated (Khush, 1977). On the other hand resistance of rice varieties to the green leafhopper is much less extreme and there is therefore less selection pressure in favour of resistance-breaking biotypes. These differences in selection pressure are reflected in the durability of resistance to these two insect

pests in rice; the resistance to brown plant hoppers of some rice varieties has quickly been broken down by new biotypes, whereas most resistant varieties have continued to give a good control of the green leaf hopper for many years.

The above example and others strongly indicate that the dangers of parasite variation to the breeder are greatest when the expression of resistance in the host plant is high. This can be a problem in plant pests like *S. asiatica* which are obligate parasites and are forced to either be eliminated or change genetically once exposed to resistant varieties.

There are several ways in which plant pathogens can change their forms. Quantitative and qualitative changes in pathogenicity in plant pathogens may occur by mutation. Genetic changes resulting from mutations may be manifested in new strains immediately, or in time, by means of sexual recombination. Inheritance of genes for pathogenicity in *Striga* might follow genetic patterns similar to those governing the inheritance in host plants; that is, dominant versus recessive, independent inheritance or linkage, inhibitor effects and epistatic effects. Such genetic changes will have an impact on the durability of any resistance which will have been bred for in the variety. It would however, be expected that should there be any mutational changes in the pathogen, resistance would disappear quickly, especially for the autogamous species like *S. asiatica* and *S. forbesii*. Grobbelaar (1952) reported loss of resistance in the sorghum variety Radar in South Africa. This loss of resistance could be linked to changes in the virulence of the parasite. Clearly characterising the strains and

their virulence genes will provide a base to develop varieties with durable resistance.

2.1.5 Techniques for the analysis of variation.

A range of plant characters are currently available for distinguishing between closely related individuals. Classical phenotypic features, such as morphological traits, are still extremely useful, but can sometimes be influenced by environmental conditions. More convenient methods of assessing variability have been developed and have been widely used in living organisms. These methods are based on the study of either the DNA or/and proteins of the organisms.

2.1.5.1 Protein markers

The use of individual proteins as molecular markers offers advantages since the proteins are direct (except for post-translational modifications which do not usually vary) products of individual genes (Newbury and Ford-Lloyd, 1993). Protein markers are normally compared by monitoring their migration in gels during electrophoresis; they are detected by using either a general protein stain or a stain to detect a specific enzyme. The range of proteins that can be used as markers is constrained by the number of resolvable protein species that can be visualised as clear bands in an extract from a particular plant organ, or by the number of plant enzymes which can be made to produce a coloured reaction product and so can be used in isozyme analyses. Again, environmental effects can influence results but Newbury (1993) reported that this is not normally regarded as a problem. The isozyme technique was successfully used for

diversity studies in pearl millet by Tostain and Marchais (1989) and Tostein (1992). Lagudah and Hanna (unpublished) also used isozymes to study patterns of variation for seed proteins in the *Pennisetum* gene pool.

The problem with the use of isozyme markers is with interpretation of gels of varying complexity. There is variability in the way different enzyme sysytem can be interpreted, especially when there is no prior information about the number gene loci coding for the enzyme system in that particular organism. In such a situation it is not clear whether the stained banding profiles are products of different gene loci or are of one locus but different alleles. Another limitation of the use of isozyme markers is that a new allele will only be detected as a polymorphism if a nucleotide substitution has resulted in an amino acid substitution, which in its turn affects the electrophoretic mobility of the studied molecule. Because of the redundancy of the genetic code and the fact that not every amino acid replacement leads to a charge difference, only 30 percent of all nucleotide substitutions result in polymorhic fragment patterns (Weising, Nybom, Wolff and Meyer, 1995). Therefore, isozyme analysis underestimates the genetic variability. Another problem is that isozyme markers restrict the study to those parts of the DNA which code for stainable enzymes and this is not necessarily a random sample of the genome.

2.1.2.2 DNA markers

DNA-based markers clearly allow the direct comparison of the genetic material of two individual plants avoiding any environmental influences on gene expression. There are several techniques based on DNA. Restriction fragment

length polymorphism (RFLP) analyses in which mitochondrial, ribosomal, or total DNA were used have provided more rapid methods in a wide variety of species (Coddington, Matthews, Cullis and Smith, 1987; Forster, Oudemans and Coffey, 1990). Alternatively, digested genomic DNA may be probed with random genomic clones (Kistler, Momol and Benny, 1991), simple repeat oligonucleotides (Weising, Kaemmer, Epplen, Weigand, Saxena and Kahl, 1991) or M13 phage (Ryskov, Jincharadze, Prosnyak, Ivanov and Limborska, 1988). Methods involving Southern hybridizations and/or cloning are however, relatively labour intensive and costly. The recent development of random amplification of polymorphic DNA (RAPD) (Williams, Kubelik, Livak, Rafolski and Tingey, 1991) or arbitrarily primed polymerase chain reaction (PCR) has allowed the rapid generation of reliable, reproducible DNA fragments or fingerprints in a wide variety of species, including those in the *Striga* genus (Aigbokhan, Berner, Musselman and Mignouna, 1999) These techniques are based on PCR, but instead of two specific primers, short single primers of arbitrary or random base sequence, with over 50 percent G+C content, are used to amplify genomic DNA under low stringency annealing conditions. Only the sequences that have proximal priming sites in the correct orientation will be amplified; and because of the low stringency, some mismatch annealing may occur between primer and template, giving rise to further products. As well as base sequence changes, length polymorphisms can arise because of insertions, deletions, substitutions, or inversions, either at or between the priming sites.

Milbourne, Meyer and Bradshaw (1997), Newbury and Rord-Lloyd (1993) reported that the RAPD technique offers advantages in speed, technical

simplicity and the frequency of identification of polymorphism. Fregene, Angel, and Gomez (1997) successfully used the RAPD technique to study genetic diversity and genetic mapping of cassava.

Although RAPD-PCR has been useful in detecting genetic differences there are some complications with this technique. In RAPD phenotypes there is always a continuum of band intensities observed. Some RAPD bands are intense while on the other hand some bands are very faint. When scoring such bands it becomes very subjective, which bands to score and which not to score. Other than subjectivity on the scoring, a slight change in the RAPD-PCR conditions might result in a completely different banding profile making the technique difficult to replicate across laboratories.

CHAPTER 3

3.1 General Materials and Methods.

Collection of plant material and seeds

Seeds and vegetative tissues of *Striga asiatica* populations were collected from infested fields of maize, sorghum, pearl and finger millets at 24 sites across Zimbabwe as shown in Figure 1 and Table 1. Collections of these *striga* populations were done from as many parts of the country as possible to capture as much diversity as possible and each collection site was designated by the name of the nearest town or village (Figure.1). Vegetative tissues (leaves and stems were collected separately from between 10 and 15 individual *Striga asiatica* plants at each location. The tissues were washed and then freeze dried in liquid nitrogen and then transported to the University of Zimbabwe and Kutsaga Research Station for DNA and isozyme extraction. Seeds were also collected separately from each of the individual plants in the sampled population. Sampling was done at random at each site with some preference being given to plants with fresh leaves and large number of mature intact seed capsules.

Figure 1. Map of Zimbabwe highlighting the collection sites for S*triga asiatica.*

Table *1* *S. asiatica* samples, locations where collected and hosts from which they were collected from.

Sample	Location collected	Host	Field conditions
A	Mazoe	Maize	weed free except for striga.
B	Mt Darwin	Maize	Communal setup, sandy soils
C	Guruve	unknown	Maize/sorghum intercrop
D	Mt Darwin	Maize	Rocky, hillside field
E	Mazoe	Maize	
F	Mutoko	F. millet	
G	Murewa	Maize	Maize and bambara-nut intercrop
H	Mutoko	Maize	
I	Mutoko	Sorghum	
J	Murewa	P. millet	
K	Makoni	Maize	Heavy red soils, high potential
L	Zimunya	F. millet	Marginal, sandy fields
M	Gutu	Maize	
N	Chikomba	F. millet	
O	Tsholotsho	Maize	Low rainfall area, shallow soils
P	Dlamini	P. millet	
Q	Tsholotsho	P. millet	Low rainfall area, shallow soils
R	Tsholotsho	Sorghum	Low rainfall area, shallow soils
S	Dlamini	Maize	
T	Hunters	Maize	Large irrigated commercial area
U	Barry	Sorghum	Large irrigated commercial area
V	Gutu	Maize	
W	Guruwe	Maize	
X	Zimuto	Maize	

The collected seeds were sown in pots with the hosts from which they were collected from and then characterized using the isozyme and RAPD techniques. The leaf material was harvested, freeze-dried and was then used for isozyme and RAPD analysis. The characterized *Striga* seed populations were then used for the inter-crop specificity trial (pot experiment) and the intra-specific experiment (laboratory). For the intra-specific experiment *Striga* resistant lines and their crosses with local cultivars were used. Root exuded technique developed by Parker and Reid (1979) was modified and adopted for this experiment. For the inter-crop specificity experiment, host specialization of the annual parasitic plant *S. asiatica* to four of its host plants, the grass species *Zea mays, Pennisetum glaucum, Eleusine corocana* and *Sorghum bicolor* was tested. Using a reciprocal cross-infection experiment, host plants were exposed to four populations of *S. asiatica* collected from the same host plants. To estimate specialization measurements of Striga counts, dry matter and grain yield reduction, and root to shoot ratios of the host plants were taken.

The detailed materials and methods for each and every experiment are described in the coming chapters.

CHAPTER 4

Isozyme Polymorphism in populations of *S.asiatica* found in Zimbabwe.

4.1 Introduction

There has been ample demonstration that genetic variability often is distributed in nonrandom patterns in space. Highly structured distribution on micro- and macro geographical scales have been described for morphological (Jain, 1973 Hiesey, 1958), cytological (Stebbins, 1971), physiological (Bradshaw, 1972) and secondary chemical (Mabry, 1973) variants within large numbers of species. Protein polymorphism in plants also appears to be distributed in a nonrandom fashion within and among populations (Allard, 1975). This study therefore intends to use the isozyme technique to explain the specialisation of certain populations of *Striga* Hypotheses about the origin of host-specific populations in the parasite can be tested using data on isozyme variation (Werth, Riopel and Gillespie, 1984).

Evaluation of the genetic diversity within and between host-specific populations will reveal the degree of specialization of the pathogens. Werth *et al.* (1984) reported that one of the best techniques to evaluate genetic diversity is analysis of the isozyme patterns obtained for different enzymes by electrophoresis. This technique was reported to be appealing as it is simple, cheap and correlates directly with genes, and so is a measure of genetic divergence. This technique was therefore one of those used to evaluate genetic diversity in the *S. asiatica* in this study.

Objective: To use the isozyme technique to reveal genetic diversity in *Striga asiatica* populations collected in Zimbabwe.

Hypothesis: Genetic diversity which can be revealed by isozyme variation exists in *S. asiatica* strains.

4.2 Basis of the Isozyme technique using Isoelectric Focusing

Isozymes are different proteins with distinct genetic origins that catalyse the same reaction. The proteins differ in one or more amino acids, indicating a difference in the DNA which specifies them. Isozymes are the last great reservoir of genetically controlled qualitative differences and can therefore be used for diversity studies. These proteins can be separated by electrophoresis on the basis of their charge and size. Assumptions made when using isozymes in molecular systematics are; (1) enzyme mobility in an electrical field reflects changes in the encoding DNA sequences and (2) the enzyme expression is co dominant, i.e. all alleles are expressed as phenotypes.

Isoelectric focusing takes place in a pH gradient, and enzymes (amphoteric substances) move towards the anode until they reach a position in the pH gradient where their net charge is zero. The pH at this point is referred to as the isoelectric point of the enzyme and since the enzyme has no charge, it no longer migrates in the electric field.

4.3 Materials and Methods.

4.3.1 Plant materials

S. asiatica seeds accessions were collected from fourteen locations in Zimbabwe as indicated in the map (Fig.1). Thirty natural population samples of *S. asiatica* were collected. Collection sites for the species are shown in Fig. 1. The different habitats in which the twenty four populations were collected are described in Table 1. Generally the collection sites were agricultural land. The altitude of the various collection sites varied, and some sites were on flat ground whilst others were on slopes of differing gradients.

4.3.2 Electrophoretic procedures.

Enzymes were extracted from *S. asiatica* leaves as described by Werth (1985) but with some modifications. Plant tissue was ground in liquid nitrogen in a mortor. The ground tissue was transferred into an Eppendof tube containing 300 µl of buffer [0.01M Tris, 0.1M KCl, 0.005M EDTA, 0.1 M 2-mercaptoethanol, pH 7.0]. The homogenate was centrifuged for 10 min at 13000 rpm and 4 ^{o}C. Supernatant was aliquoted into 20 µl vials and stored at -80 ^{o}C for subsequent use in the different enzyme systems. For each sample, 16-20 µl was loaded onto a native polyacrylamide gel and electrophoresed at 600V, 20 mA , 10 watts and 4 ^{o}C for 45 min.

4.3.3 Stain assay procedures for enzyme activities

The following staining protocols which were adopted for this study are modifications of the methods described by Pasteur, Pasteur, Bonhomme, Catalan and Britton-Davidian (1987). Pasteur et-al (1987) described the staining procedures for starch gels and modifications had to be made to use the protocols for the much thinner polyacrylamide gels. The Fixing Solutions 1 and 2 which were used are described in Appendix C.

Aconitase (ACO)

Thirty milliliters of 0.5 M Tris.HCl buffer (pH 8.0) was mixed with 68.8 mg cis-Aconitic acid solution (4 ml cis-AA), 100 mg $MgCl_2$ (1 ml) and just before staining 20 mg NADP (2 ml),100 units Isocitric dehydrogenase (1.5 ml IDH), 15 mg MTT (1.5 ml) and 4 mg PMS (0.8 ml) were added. The above staining solution was poured onto the gel and incubated at 37 ^{o}C for 2h. Bands were scored the following morning after rinsing and fixing in Fix. 1 solution.

Alcohol dehydrogenase (ADH)

A solution containing 1 ml 95% Ethanol, 20 mg NAD (1 ml), 20 mg MTT (2 ml) and 5 mg PMS (1 ml) were added to 50 ml 0.05 M Tris.HCl buffer (pH 8.0). The solution was poured onto the gel slice and incubated at 37oC for 30 min. The gel was rinsed and fixed in Fix. 1 solution.

α-Esterase (α-EST)

The staining solution with 50 ml Phosphate assay buffer (pH 6.0), 2.5 ml N-propanol, 20 mg β-Naphthyl acetate (1 ml β-NA) and 25 mg Fast Garnet GBC salt (0.5 ml GBC) was poured onto the gel slice. After 5 min, 30 mg α-Naphthyl acetate (1.5 ml α-NA) was added and the gel incubated for 45 min. The gel was rinsed and fixed in Fix. 2 solution.

Glutamate dehydrogenase (GDH)

The staining solution with 50 ml 0.1 M Tris-HCl buffer (pH 8.5), 50 mg L-Glutamic acid (1.5 ml GA), 50 mg $CaCl_2$, 20 mg NAD (1 ml), 15 mg NBT (1.5 ml), and 5 mg PMS (1 ml) was poured onto gel slice and incubated overnight at room temperature after 60 min at 36 °C. The gel was rinsed and stored in water before scoring of bands.

Glutamate-oxaloacetate transaminase (GOT)

Fifty milliliters of substrate solution (pH 7.4) [400 ml water, 146.1 mg α-ketoglutaric acid, 532.4 mg L-aspartic acid, 2 g PVP-40, 200 mg EDTA and 5.68 g Na_2HPO_4] was mixed to 50 mg Fast Blue BB salt (0.5 ml BB) just prior to staining [Adding approximately 1 mg β-Naphthyl Acetate (4 drops b-NA) will stain for enzymes associated with Est8 locus]

The above staining solution was poured onto the gel and incubated in the dark at room temperature for 2 hours. Bands start appearing after 1 hr and improve in intensity up to 2 hours.

Isocitrate dehydrogenase (IDH)

An agar overlay procedure was used for staining this enzyme locus. Two solutions were prepared. Solution 1 had 15 ml 0.05 M Tris-HCl buffer (pH

8.0), 50 mg $MgCl_2$ (0.5 ml), 150 mg DL-Isocitric acid (1.5 ml ISCA), 5 mg NADP (0.5 ml), 5 mg NBT (0.5 ml) and 1 mg PMS (0.15 ml). Solution 2 had 15 ml 0.05 M Tris-HCl buffer (pH 8.0) and 200 mg Agar.

Solution 2 was heated until agar was dissolved and then cooled to 60 °C. Solution 1 was added to solution 2, swirled, and applied over the gel slice. The gel was incubated for 60 min. Stain appeared after I hr and it darkened overnight. Fixing was done in Fix 1 solution.

Malate dehydrogenase (MDH)

The solution consisting 50 ml 0.1 M Tris-HCl (pH 9.1), 100 mg DL-Malic acid (neutralised (2 ml MA), 20 mg NAD (1 ml), 10 mg NBT (1 ml) and 1.25 mg PMS (0.25 ml) was poured onto gel slice and incubated for 60 min. The gel was rinsed and fixed with Fix 1 solution. (See appendix).

Malic enzyme (ME)

The solution comprising, 50 ml 0.1 M Tris-HCl buffer (pH 8.5), 50 mg DL-Malic acid, neutralised (1 ml MA), 50 mg $MgCl_2$ (0.5 ml), 15 mg NADP (1.5 ml), 10 mg NBT (1 ml) and 1 mg PMS (0.2 ml) was poured onto the gel and incubated overnight at room temperature after 60 minutes at 36 °C. Gel was fixed in Fix 1 and the bands scored.

Shikimic acid dehydrogenase (SAD)

A solution containing 60 ml 0.1 M Tris.HCl buffer (pH 9.1), 60 mg (-)-Shikimic acid, 10 mg NADP (1 ml), 5 mg MTT (0.5 ml) and 1.33 mg PMS (0.33 ml) was

poured onto the gel and incubated for 2 hrs. The gel was fixed with FIX1 and the bands scored immediately.

Leucine Aminopeptidase (LAP)

The stain solution [40 ml Tris/Malate-0.2 M (pH 5.5), 100mg L-Leucyl-β-Naphthylamide, 5 ml $MgCl_2$ (0.5M) and 30 mg Black K Salt] was poured over the gel and incubated at 37 ^{o}C for 1 h. Violet bands appeared after 30 minutes. Fixing was done with FIX 1.

4.4 Isozyme data analysis.

Bands on the electrophoretic gel were recorded as present (1) or absent (0) and scores assembled in a data matrix. Allele frequencies were calculated for each locus and population. The following four measures were used to quantify genetic variation within a population: (1) the expected heterozygosity (Nei, 1975) at each locus were calculated as:

$$H_e = 1-\sum^{k}_{i=1} P^2_i$$

where P^2_i is the frequency of the i^{th} allele, summed over k alleles; (2) the mean number of heterozygous loci per individual was calculated as was done by Nei, (1973); and (3) the mean number of alleles per locus were calculated by averaging over all polymorphic and monomorphic loci. Nei's (1978) unbiased genetic identity (In) and genetic distance (Dn) were used to quantify the degree of differentiation among populations.

4.5 Results

Active and well resolved bands were produced in five of the twelve enzyme systems tested (Figures 2 to 6). The five enzyme systems which stained, GOT, α-EST, LAP, PER and G-6-PDH were all polymorphic with a minimum of three and a maximum of five groups (grouped by banding patterns).The enzymes MDH, ACO, ADH, GDH, IDH, ME and SAD had too low or no activity and did not produce well resolved bands. The most anodal band (fastest mobility) is designated "F", the least anodal (slowest) "S", and an intermediate between fast and slow is designated "M". The extremely fast or slow band whose frequencies were quite low, are designated "FF" and "SS" respectively. The null allele which makes no enzymatic product was shown as "N" in GOT, EST and LAP.

4.4.1 Glutamate oxaloacetate- transaminase (GOT)

The enzyme system produced 4 different banding patterns (Fig.2). Collections in lanes 1, 11, 16, 19, and 22 had two faint slow migrating bands, and these collections were collected from different areas but all from the same Northern region of Zimbabwe. Collections in lanes 2 to 5, 8, 14, 17, 21 and 23 produced four bands, two slow migrating and two fast migrating ones. All except for one sample were collected from the north-eastern Zimbabwe. The one exception is sample in lane 14 which was from the Eastern region of Zimbabwe. The third group produced two big slow migrating bands (lanes 6, 9 to10, 12 to 13, 20 and 24). All these samples were collected from different areas but in the same south-western part of Zimbabwe. The fourth group in lanes 15 and 18 had four bands,

two slow migrating and two fast migrating and both were from the south-central part of the country (Gutu and Zimuto).

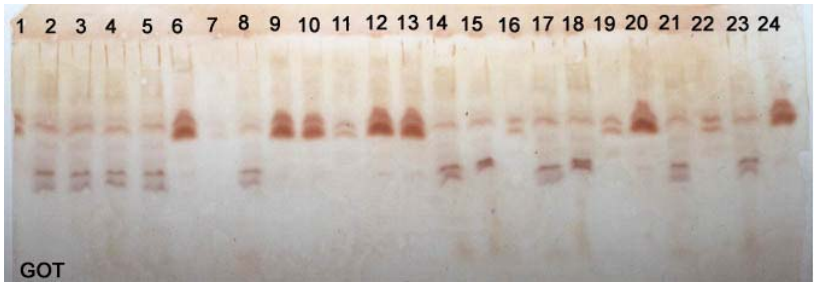

Figure 2 Gel photograph showing isozyme patterns for glucose-transaminase loci in *Striga asiatica* populations, lane 1= population A; 2=G; 3=I; 4=J; 5=M; 6=O; 7= K; 8=N; 9=T; 10=U; 11=B; 12=P; 13=Q; 14=L; 15=V; 16=C; 17=W; 18=X; 19=D; 20=S; 21=H; 22=E; 23=F; 24=R.

4.4.2 α-Estarases (EST)

The collections made in the northern Zimbabwe (lanes 1, 4, 6, 21, 24) which gave the same banding patterns with GOT gave two different banding patterns on the α-EST loci (Fig. 3). Collections A, B and C in lanes 1, 21 and 24 gave two slow migrating bands whilst Collections D and E in lanes 4 and 6 gave four bands, two slow migrating and two fast migrating ones. Collections F to J from the north-eastern zone in lanes 10, 15, 18, 19 and 20 respectively showed very weak activity with no distinct banding at all (null allele). Sample K (lane 16) from the same zone however gave a different pattern with 4 distinct bands. The same banding pattern as in K was found in sample L (lane 7) from eastern zone and M, N (lanes 22-23) from the central zone (Gutu and Chikomba, respectively). Collections from the south-western zone (O-R in lanes 3, 5, 12 and 17, T-U in lanes 9 and 8) gave the same banding pattern of two big slow migrating bands

and one fast migrating band. Sample S (lane 13) from the same zone however gave a different banding pattern of four bands.

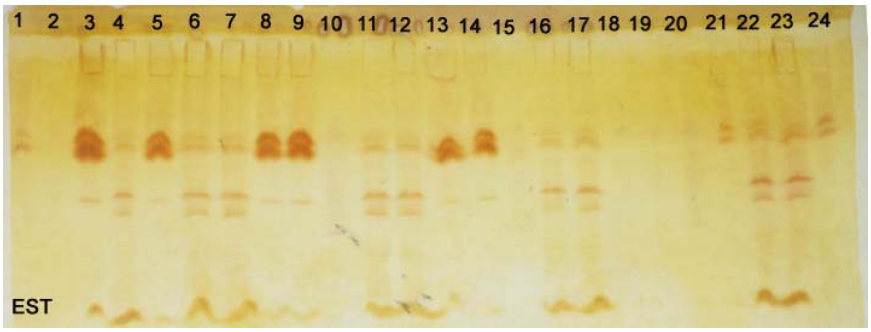

Figure 3. Gel photograph showing isozyme patterns for α- Esterases loci in *S. asiatica populations, lane* 1= population A; 2=V; 3=O; 4=D; 5=P; 6=E; 7=L; 8=U; 9=T; 10=F; 11=W; 12=Q; 13=S; 14=U; 15=G; 16=K; 17=R; 18=H; 19=I; 20=J; 21=B; 22=M; 23=N; 24=C.

4.4.3 Leucine amino peptidases (LAP)

Collections A to E (lanes 8, 4, 16, 7 and 21 respectively), all from the northern zone of the country produced very weak activity at this locus and did not show any distinct banding (Fig. 4). Collections from F-J from the north-eastern zone (lanes 24, 1, 10, 2 and 11) showed wide variation in their banding patterns with collections F and G giving one slow migrating band, collections H and J produced no bands and collection I produced five bands.

Collections K (lane 22) and L (lane 15) both from the eastern zone did not produce the same banding profiles. Sample K had two bands and sample L, no bands. Collections from the central zone M and K (lanes 13 and 12) also did not show any similarity with sample M having 5 bands and sample N nil. Collections O-U collected from an area ranging from the central to the south-southern zone

all had the same banding patterns (2 big, slow bands and 1 faint, fast one) except for collection Q which had five bands.

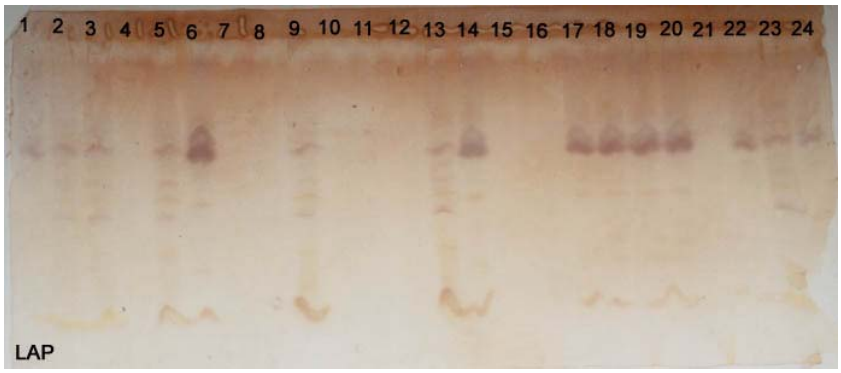

Figure 4. Gel photograph showing isozyme patterns for the Leucine amino peptidases loci in *S. asiatica* populations, lane1= population G; 2=I; 3=V; 4=B; 5=W; 6=O; 7=D; 8=A; 9=X; 10=H; 11=J; 12=N; 13=M; 14=P; 15=L; 16=C; 17=U; 18=S; 19=R; 20=T; 21=E; 22=K; 23=Q; 24=F.

4.4.4 Glucose 6 phospho-dehydrogenase (G-6-PDH)

This enzyme locus (Fig. 6) was weakly polymorphic with most of the collections producing one band except for collection A in lane 18 with three bands and collection U in lane 21 which also had 3 small bands. The enzyme activity at this locus was however weak for all the samples.

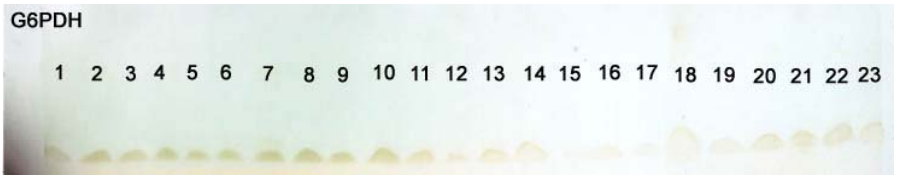

Figure 5. Gel photograph showing isozyme patterns for the Glucose-6-phosphodehydrogenase loci in *S. asiatica* using PAG electrophoresis, lanes 1= population F; 2=J; 3=P; 4=E; 5=N; 6=D; 7=O; 8=I; 9=V; 10=M; 11=X; 12=W; 13=G; 14=H; 15=Q; 16=S; 17=K; 18=A; 19=T; 20=L; 21=U; 22=B; 23=C.

4.4.5 Peroxidases (PER)

The enzyme locus (Fig. 5) was weakly polymorphic producing predominantly two bands, one slow migrating and the other fast migrating. Collections Q and P both from the south-western zone had one extra faint band after the fast migrating band. Collection R from Tsholotsho had an extra big band after the fast migrating band. The enzyme loci were then used to detect polymorphism in the strains.

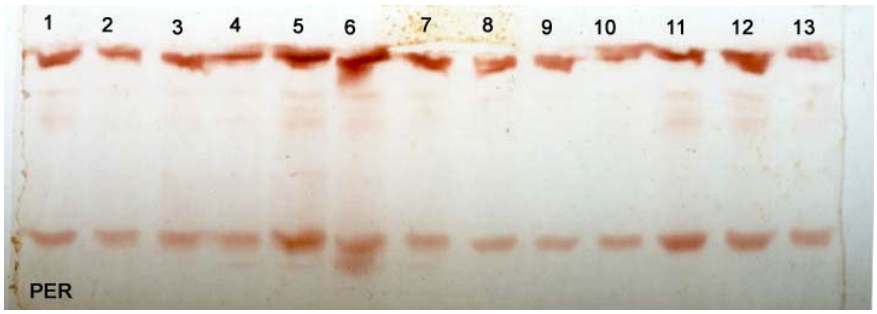

Figure 6. Gel photograph showing isozyme patterns for the peroxidases loci in *S. asiatica strains,* 1=D; 2=T; 3=U; 4=P; 5=Q; 6=R; 7=V; 8=X; 9=B; 10=K; 11=W; 12=D; 13=A.

Table 2. Locations and hosts of *S. asiatica* samples used for isozyme analysis

Sample	Location collected	Host
A	Mazoe	Maize
B	Mt Darwin	Maize
C	Guruve	unknown
D	Mt Darwin	Maize
E	Mazoe	Maize
F	Mutoko	F. millet
G	Murewa	Maize
H	Mutoko	Maize
I	Mutoko	Sorghum
J	Murewa	P. millet
K	Makoni	Maize
L	Zimunya	F. millet
M	Gutu	Maize
N	Chikomba	F. millet
O	Tsholotsho	Maize
P	Dlamini	P. millet
Q	Tsholotsho	P. millet
R	Tsholotsho	Sorghum
S	Dlamini	Maize
T	Hunters	Maize
U	Barry	Sorghum
V	Gutu	Maize
W	Guruwe	Maize
X	Zimuto	Maize

Discussion

These results demonstrate what has long been suspected; there are genetic differences between the *S. asiatica* populations collected from different areas and different hosts. The sources of this variation could be anything from mutations, hybridization with related species or it is a result of a long term adaptive co-evolution with the different hosts. Most of the isozyme differences were found between the *Striga* populations of Tsholotsho and those of Mt Darwin area. The *Striga* from Tsholotsho showed the most distinctness probably because the main host crop grown in the area is Pearl-millet, which is not the case with the other areas where maize is the major hosts followed to a lesser extend by finger millets. Mutikainen *et-al (*2000) reported that co-evolution may lead to local adaptation of parasites to their sympatric hosts. This might actually be detected by the different alleles/loci of some enzyme systems as reflected in this study.

Despite the polymorphism which has been shown by the different enzyme systems, genetic interpretation of gels from organisms that have not been previously studied is always a problem as success cannot be guaranteed in advance. In this experiment it was not clear therefore whether the different banding patterns were products of different gene loci, products of different alleles of the same loci or a combination of both. In such a case only assumptions were made and the reliability of the interpretations were therefore questionable.

CHAPTER 5

Use of RAPD markers to study genetic variability of S. asiatica populations collected from Zimbabwe.

5.1 Introduction

During recent decades several techniques have been introduced that detect molecular variability within and among several species. Three of these widely applied techniques are the use of restriction fragment length polymorphisms (RFLPs), (Helentjaris *et al*., 1986), DNA fingerprinting (Jeffreys *et al*., 1985) and specific amplification of polymorphic DNA fragments with PCR (Weining and Langridge, 1991). Prior knowledge of the DNA composition of the species and/or the presence of useful probes, is however required. In the case of S. asiatica no useful probes have been developed as yet so the use of a technique which uses random primers will be most suitable. The Random Amplified Polymorphic DNA (RAPD) technique which uses the polymerase chain reaction (PCR) and random primers can discriminate between individuals, varieties or biotypes of the same species.

As was described by Weising, Nybom, Wolff and Meyer (1995), the RAPD technique depends on there being differences, or polymorphism, between plants in the sequence of bases in their genomic DNA, and this can arise from different mechanisms (base pair substitutions, deletions, insertions or repetions). All these can result in different strains of a species (Watson, Hopkins, Roberts, Steitz and Weiner 1987). A polymorphism is detected when there is a difference

between two or more plants in the length, or the presence/absence, of a DNA fragment on an agarose gel. A change in the length of a fragment occurs when the number of base pairs between two PCR primer sites (RAPD) is different which can be brought about by deletion, repetition, or insertion. The absence of a band occurs when the change in the base sequence leads to the loss of a priming site.

The strategy of RAPD analysis using arbitrary primers was utilised in this study.Genomic DNA from the plants of interest (*Striga* strains) was extracted and subjected to PCR using arbitrary primers, thermostable DNA polymerase and nucleotide precursors. The arbitrary primers will anneal to anonymous target sequences in the template genomic DNA. If two primers anneal in opposite directions and at suitable distances from each other, the fragment between the two primers is amplified.

Objective: To use RAPD markers to study genetic variations in S. asiatica populations from Zimbabwe.

Hypothesis: Polymorphism in *Striga* populations can be detected by means of the RAPD technique.

5.2 Materials and Methods

5.2.1 DNA Preparation

Total cellular DNA was prepared from 0.5 g of young *Striga* plant material. Leaves were frozen in liquid nitrogen, ground to a fine powder in a morter and pestle. The ground plant tissue was then added to 500 µl of extraction buffer [0.1 M Tris-HCL (pH 9.0), 1M NaCl, 10 mM EDTA, 50 mM B-mercaptoethanol

and 100 ug.ml Proteinase K], incubated at 50 ^{o}C for 2 h. After adding 160 µl of 5M potassium acetate the homogenate was extracted with 400 ul of chloroform and centifuged in an Eppendorf 5415C microcentrifuge at 10000 rpm for 5 min. The nucleic acid was precipitated from the aqueous phase by adding 400 µl of ice cold isopropanol and pelleted by centrifugation at 10000 rpm for 5 min in the microcentrifuge. The dried nucleic acid was dissolved in 400 µl TE-RNAse (10 µg/ml) and incubated for 30 min. at 37 ^{0}C. The DNA was recovered by extracting once with phenol and twice with phenol/chloroform (1:1,v/v) and precipitated with absolute ethanol at -20 ^{o}C. The DNA was pelleted by centrifugation at 10000 rpm for 10 min, dried and dissolved in 100 µl TE buffer (10 mM Tris-HCL, pH 8.0, 1 mM disodium EDTA).

5.2.2 Polymerase Chain Reaction

PCR conditions for RAPD reaction with the GeneAmp 9700 thermal cycler (Perkin-Elmer Corporation, Norwalk, Conn. USA) are described as follows: Each sample, comprising 50 mM Tris-HCL buffer (pH 8.5) containing 20 mM KCL, 1.5 mM $MgCl_2$, 0.5 mg/mL BSA, 200 um each of dATP, dCTP, dGTP, dTTP, 0.4 uM 10-base primer, 60 ng of template DNA and 1.7 units of Taq DNA polymerase (Boeringer Mannheim Biochemica) at a final volume of 20 µl was heated to 94 ^{o}C for 2.30 min and then subjected to 40 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 38 ^{o}C for 1 min, and polymerisation at 72 ^{o}C for 1.30 min and a final extension at 72 ^{o}C for 5 min. The amplification products were stored at 4 ^{o}C before analysing by gel electrophoresis.

A total of 240 primers were tested and only 5 gave well resolved repeatable polymorphic bands.

Table 3. Sequences and codes of random primers that amplified to give polymorphic bands.

Primer	Sequence(5'--3')
OPK -05	TCTGTCGAGG
OPK -19	CACAGGCGGA
OPJ - 18	TGGTCGCAGA
OPB- 19	ACCCCCGAAG
OPG- 19	GTCAGGGCAA

5.2.3 Analysis of PCR products

Amplification products were separated by electrophoresis on a 1.5 per cent agarose gel and visualised by staining with ethidium bromide. Polymorphism was detected by the absence or presence of certain bands for each of the *Striga* strains. Different fragments produced with each primer were numbered sequentially. Individuals from the same row on the gel were compared with each other. Fragments with a medium or strong signal were taken into account as these fragments are fully reproducible. Fragments with the same mobility on the gel but with different intensities were not distinguished from each other when strains were compared with each other. The actual procedures for this work was done as was described by Hoisington, Khairallah and Gonzalez-de-Leon (1994).

5.2.4 RAPD Data Analysis

Variability among strains was expressed as the similarity *S*. This is calculated as:

$$S = \frac{2 \text{ x NAB}}{\text{NA} + \text{NB}}$$

in which N_{AB} is the number of bands shared by the individuals A and B, and N_A and N_B are the number of bands in individuals A and B, respectively. The similarity measure can also be called band sharing. Distance can be calculated as $D = 1\text{-}S$ (Swofford and Oslen, 1990). The chance of finding two individuals with the same fragment pattern can be calculated as the mean similarity (*S*) to the power of the mean number of bands (*N*) (Nybom and Hall, 1991).

A cluster analysis was performed to confirm distinction of strains on the basis of their banding profiles. Bands were recorded as present (1) or absent (0) and scores assembled in a data matrix. Pairwise comparisons, similarity matrices and the UPGMA cluster analysis were performed using the Genstat 5 software package Genstat 1987.

5.3 Results

The DNA amplification fingerprints (DAF) in *Striga asiatica* strains were reliable and reproducible. DAF profiles obtained by PCR of independently extracted genomic DNA from 10 plants collected from the same region of Zimbabwe were uniform and similar for all primary bands and most of the secondary bands, although the intensity of some bands between lanes differed.

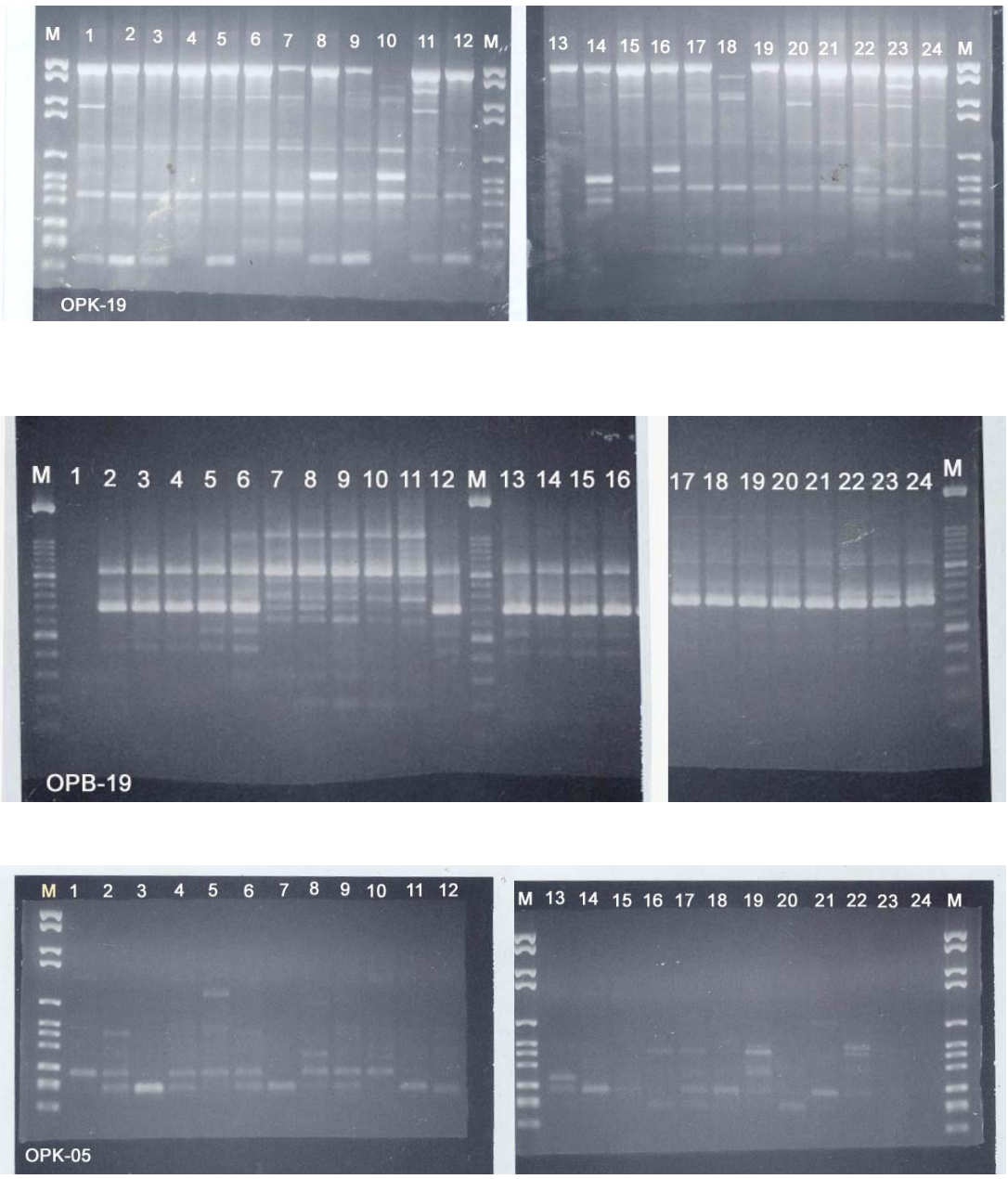

Figure 7. RAPD fragment pattern of several *S. asiatica* populations using the primers OPK-19, OPB-19 and OPK- 05. From lane 1 to lane 24 are the

biotypes A to X (Table 1) respectively. Lanes marked M are the molecular weight markers.

Figure 7 shows the DNA polymorphism among the 24 strains that were amplified using primer OPB-19. Strains from Chinyika had a unique band of about 1500 bp but lacked the 700 bp band present in all the other strains. The strains collected from maize in Tsholotsho had a single band of about 700 bp whilst those collected from the same area but from pearl millet host had two bands of about the same size (700 bp and 750 bp). The strain from Masvingo area was distiguished by two bands, 1000 bp and 700 bp bands.

Table 4. Number of scored and polymorphic RAPD bands produced by *Striga asiatica* using primers from Operon Technologies (Alameda, CA).

Primer	Sequence	Scored bands	Polymorphic bands	Percent polymorphism
OPG-19	5'-GTCAGGGCAA-3'	10	5	50
OPK-19	5'-CACAGGCGGA-3'	9	7	71
OPB-19	5'-ACCCCCGAAG-3'	7	7	100
OPK-05	5'-TCTGTCGAGG-3'	8	8	100
OPJ-18	5'-TGGTCGCAGA-3'	4	4	100
Total		38	31	

Table 5: Sizes (base pairs) of well resolved clear bands amplified by RAPD-PCR in *S asiatica* populations.

Band N°	Primer	Molecular weight	Band Number	Primer	Molecular weight
1	OPK05	660	17	OPB19	1100
2	OPK05	453	18	OPB19	850
3	OPK05	394	19	OPB19	800
4	OPK05	298	20	OPB19	750
5	OPK05	220	21	OPB19	600
6	OPK19	2176	22	OPB19	200
7	OPK19	1760	23	OPG19	1033
8	OPK19	1240	24	OPG19	660
9	OPK19	660	25	OPG19	653
10	OPK19	517	26	OPG19	517
11	OPK19	453	27	OPG19	453
12	OPK19	400	28	OPG19	298
13	OPK19	394	29	OPJ18	1766
14	OPK19	234	30	OPJ18	700
15	OPK19	160	31	OPJ18	298
16	OPB19	1500	32	OPJ18	220

The five RAPD primers generated a total of 38 reliable fragments from the 24 S. asiatica collections. The approximate size of the bands ranged from 160 to 2200 bp (Table 5). The total number of amplified fragments per primer varied from 4 (OPJ-18) to 10 (OPG-19).

Genetic Distances (%) (Simple matching Coefficient)

Striga asiatica strains

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0																							
16.12903	0																						
16.12903	6.451613	0																					
22.58064	19.35484	19.35484	0																				
48.3871	64.51613	64.51613	58.06452	0																			
48.3871	51.6129	51.6129	45.16129	38.70968	0																		
41.93548	38.70968	32.25806	32.25806	51.6129	45.16129	0																	
41.93548	38.70968	38.70968	38.70968	51.6129	38.70968	32.25806	0																
35.48387	32.25806	32.25806	38.70968	51.6129	45.16129	32.25806	19.35484	0															
35.48387	38.70968	38.70968	32.25806	45.16129	45.16129	19.35484	32.25806	32.25806	0														
19.35484	22.58064	16.12903	29.03226	54.83871	48.3871	41.93548	48.3871	35.48387	48.3871	0													
22.58064	19.35484	12.90323	25.80645	51.6129	51.6129	38.70968	38.70968	32.25806	51.6129	22.58064	0												
32.25806	35.48387	35.48387	35.48387	48.3871	54.83871	48.3871	41.93548	35.48387	48.3871	38.70968	22.58064	0											
29.03226	32.25806	32.25806	32.25806	45.16129	45.16129	38.70968	38.70968	45.16129	32.25806	41.93548	32.25806	22.58064	0										
29.03226	32.25806	32.25806	25.80645	51.6129	51.6129	32.25806	38.70968	38.70968	32.25806	35.48387	32.25806	22.58064	12.90323	0									
32.25806	22.58064	22.58064	35.48387	54.83871	54.83871	35.48387	35.48387	35.48387	35.48387	38.70968	29.03226	45.16129	29.03226	29.03226	0								
32.25806	29.03226	35.48387	35.48387	48.3871	35.48387	35.48387	41.93548	29.03226	35.48387	32.25806	35.48387	38.70968	35.48387	29.03226	25.80645	0							
29.03226	38.70968	38.70968	38.70968	45.16129	45.16129	45.16129	38.70968	25.80645	32.25806	35.48387	38.70968	35.48387	38.70968	38.70968	41.93548	22.58064	0						
25.80645	22.58064	29.03226	35.48387	54.83871	48.3871	41.93548	35.48387	35.48387	35.48387	38.70968	41.93548	32.25806	29.03226	29.03226	25.80645	19.35484	22.58064	0					
38.70968	29.03226	22.58064	22.58064	67.74194	41.93548	35.48387	54.83871	41.93548	41.93548	25.80645	35.48387	38.70968	35.48387	29.03226	38.70968	32.25806	41.93548	32.25806	0				
29.03226	25.80645	25.80645	25.80645	51.6129	51.6129	38.70968	38.70968	25.80645	32.25806	35.48387	32.25806	22.58064	25.80645	25.80645	35.48387	29.03226	19.35484	16.12903	22.58064	0			
32.25806	29.03226	35.48387	35.48387	48.3871	54.83871	54.83871	54.83871	35.48387	48.3871	32.25806	29.03226	35.48387	35.48387	48.3871	54.83871	54.83871	54.83871	35.48387	48.3871	22.58064	0		
29.03226	32.25806	25.80645	32.25806	38.70968	51.6129	38.70968	51.6129	32.25806	38.70968	22.58064	25.80645	35.48387	45.16129	38.70968	35.48387	29.03226	19.35484	29.03226	29.03226	19.35484	22.58064	0	
29.03226	25.80645	19.35484	19.35484	51.6129	51.6129	32.25806	45.16129	38.70968	32.25806	29.03226	25.80645	35.48387	38.70968	32.25806	35.48387	35.48387	32.25806	29.03226	22.58064	12.90323	29.03226	12.90323	0

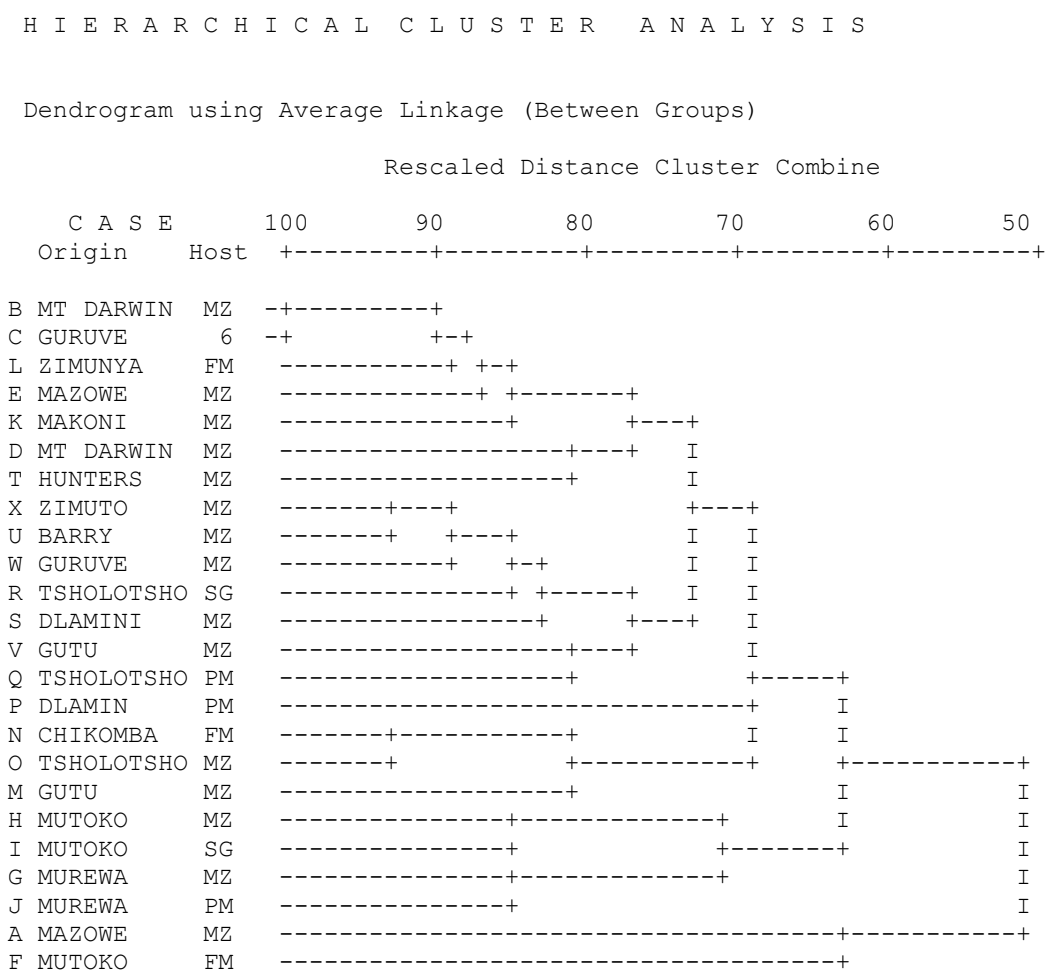

Figure 8. Dendogram showing the phenetic relationships among *S. asiatica* strains from various locations throughout Zimbabwe based on genetic polymorphisms generated by RAPD-PCR.

Agglomeration Schedule

	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
Stage	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	2	6	.935	0	0	5
2	23	24	.871	0	0	4
3	1	20	.871	0	0	14
4	7	23	.839	0	2	10
5	2	22	.839	1	0	6
6	2	12	.817	5	0	9
7	17	18	.806	0	0	18
8	14	15	.806	0	0	18
9	2	11	.798	6	0	15
10	7	19	.796	4	0	11
11	4	7	.782	0	10	16
12	8	21	.774	0	0	16
13	3	10	.774	0	0	15
14	1	9	.774	3	0	20
15	2	3	.732	9	13	17
16	4	8	.726	11	12	17
17	2	4	.694	15	16	19
18	14	17	.677	8	7	22
19	2	5	.671	17	0	20
20	1	2	.668	14	19	22
21	13	16	.613	0	0	23
22	1	14	.613	20	18	23
23	1	13	.497	22	21	0

Table 6: Agglomeration table from cluster analysis.

5.4 Discussion

The DNA amplification fingerprinting technique described detected genetic polymorphisms among *Striga asiatica* populations, indicating that the RAPD technique can be employed to examine the distribution and extend of genetic diversity in this parasitic weed. The RAPD results presented here are comparable with similar studies in Striga with reference to percent

polymorphism and the number of amplified DNA fragments per lane (Aigbokhan, Berner, Musselman and Mignouna 2000). Nevertheless, the results reported in this study indicate that *Striga asiatica* exhibits a very low degree of genetic polymorphism as compared to other *Striga* species like *S. aspera* and *S. hermonthica.*

Based on DNA polymorphism some strains, especially those from Tsholotsho are clearly different from the others. Parker and Reid (1979) raised suspicions about *Striga*-resistant cultivars that lost their resistance when introduced to different geographical environments. This could possibly be explained by the evidence provided in the present study where different strains, based on RAPD profiles were found to be occurring in different geographical areas of Zimbabwe. However, it should be noted that although certain clusters in the phenogram (Figure 8) contained accessions from geographically proximal locations, it is difficult to make generalizations on the regional bias in the relationships. There are also some accessions from distant geographical regions which are clustered together. This could be possibly be a result of *Striga* seed dispersal through crop seed packs as some of the collection sites were farms belonging to seed growers.

The DNA polymorphisms detected by the RAPD assay were consistent in at least two tests conducted at different times. Although the inheritance of these bands cannot be confirmed by genetic analysis, the bands scored in this study can be used for future population studies because RAPDs are repeatable between laboratories. However in this study with *S. asiatica* biotypes a

dendogram was generated using RAPD fragments as characters. The dendogram showed four main clusters as opposed to the three groups which were produced by isozymes. Two groups, from Tsholotsho and from Mt Darwin were quite distinct as was with isozymes. There was also a clear correlation between the geographical distance and the genetic distances between the *Striga* populations. The relationships derived from RAPDs were to an extend similar to those generated from isozyme analysis.

Although some relationships between the biotypes could be found Wolf and Peters (1992) states that the general use of RAPD fragment patterns for taxonomic purposes is debatable as one should check whether fragments are identical by either sequencing or, probably easier, by using the fragments as a probe. Weeden (undated) also states that another complication in using the RAPD phenotypes for diversity studies is the continuum of band intensities observed. As shown in figure 7 some RAPD products are intense, well resolved bands that can be used as genetic markers. At the other extreme, however are faint or fuzzy products that are very difficult to score, but, in general also appear to have a genetic component.

In this experiment, total plant DNA was used in trying to detect any genetic differences. Although some differences could be detected it would have been more appropriate to use DNA from highly conserved regions of the parasite to check if the differences were real. dePamphilis, Young and Wolfe (1997) stated that most parasitic plants retain their plastids and plastid genomes. Analysis of this region in the different biotypes should provide a powerful tool for the

interpretation of molecular evolution relative to photosynthetic ability. Photosynthetic ability will also probably explain any differences in virulence between the different biotypes of *S. asiatica.*

CHAPTER 6

Investigating the inter-crop specificity of Striga *asiatica.*

Hypothesis: Distinct populations of S*. asiatica* can only attack specific cereal crops.

6.1 Introduction

There have been several reports on differential damage and distribution of Striga on the cultivated hosts. Lewin (1932) is reported to have noticed differential damage and distribution of *Striga* in maize and some veld hosts such as *Digitaria milanjiana* (Rendle) Stapf. and *Setaria sphacelata* (Schum.) (Musselman, 1987). Lewin's (1932) study also suggested that once *Striga* becomes adapted to maize, it may become increasingly virulent in the succeeding generations. Gurney, Press and Scholes (1999) reported that field and laboratory studies show large differences in the response of cereal species and cultivars to *Striga* which is attributed to one or more sources of variation: (i) genetic differences between host species and cultivars; (ii) genetic differences between populations of *Striga*; (iii) interaction between the environment and both genotypes. Mutikainen, Salonen, Puustinen and

Koskela (2000) reported that in the antagonistic coevolutionary interaction between hosts and parasites, the parasite specializes on the most common host genotypes, which creates an advantage for the rare resistant host genotypes. It is expected therefore that as a consequence of specializing on the most common genotypes, parasites may become adapted to their sympatric host. On average, locally adapted parasites are expected to be more infectious to sympatric hosts than to allopatric hosts or their fitness is expected to be higher on sympatric than allopatric hosts (Lively,1989; Gandon, Capowiez, Dubois, Michalakis and Olivieri, 1996).

Studies made by Wilson (1955) with *S. hermonthica* showed that if sorghum was grown in traditional millet areas, and vice versa, *S. hermonthica* did not appear on the crops. Cross inoculating *Striga* seeds collected from the two hosts showed that two strains existed in the Sudan. In India *Striga asiatica* did not parasitize sorghum growing in the traditionally pearl millet growing regions and the opposite was also true for millets grown in sorghum zones (Musselman, 1987). Work done by Ramaiah (1984) in pots suggested that sorghum cultivars supported Striga from both sorghum and pearl millet, while pearl millet cultivars hosted *Striga* only from pearl millet.

Many breeders have screened for *Striga* resistance and have not been consistent in the populations they use. This has therefore led to inconsistent performance of the so called resistant varieties as they are moved from one area to another. Comparison of results from one researcher to another is also difficult once the populations used for screening are different. In light of the

need to improve the screening techniques and thus breeding programmes for *Striga* resistance in sorghum currently going on in Zimbabwe, there is need to characterize the *Striga* populations occurring in the country.

The present experiment aimed to determine whether the populations of *Striga* in Zimbabwe have any host specialisation as part of their characterization. A pot experiment was used. In this experiment we examined the response of four *Striga* populations to four different host species: *Sorghum bicolor, Zea mays, Pennisetum glaucum* and *Eluesine coracana. Striga asiatica* collected from the above four hosts was used to cross infest the hosts. The cultivars used in the experiment were chosen on the basis of farmer preference. Furthermore the *Striga* populations used for the infestations had been collected from the same cultivars in the farmers' fields. In this study we report how the different *Striga* strains affect biomass accumulation, grain yield, root /shoot ratio and the emergence of *Striga* on maize, pearl millets, finger millets and sorghum.

6.2 Materials and Methods

6.2.1 Experimental design and plant material.

The study was conducted in seven litre pots at Henderson Research Station, near Harare in November 1999 during the rainy season (October to February). A hybrid cultivar of *Zea mays* (SC401), a landrace cultivar for *Eluesine coracana* Gaertn (Pachedu), an open pollinated variety (OPV) of *Pennisetum glaucum* (Mukonde) and an OPV of *Sorghum bicolor* (L) Moench

(DC75) all susceptible to *Striga asiatica* were investigated. Host specialization of *S. asiatica* to four of its hosts was tested in this experiment. Using a reciprocal cross-infection experiment, four host plants were exposed to four populations of *S. asiatica* collected from plants of the same species and varieties. To estimate the virulence of the parasite, host plants were also grown alone, without parasitic infection. Plants were sown in a split-plot arrangement laid in a completely randomised blocks design with the crop species as the main factor (4 levels) and the *Striga* strains as the sub-factor (5 levels, including a control). Twenty experimental plots, each made up of three pots were established and each treatment was replicated four times. At planting the pots were three quarter filled with sand soil and the rest of the soil used to fill up was mixed with about 3000 seeds of *Striga asiatica.*

At planting all plants received the equivalent of 70kg/ha of basal fertilizer (7%:15%:8%, N: P: K) and 35 days after planting (dap) all plants received 60 kg/ha ammonium nitrate and an application of an insecticide (carbarlyl) to control chewing insects; another application was done 65 dap. Maize, sorghum and pearl millets were treated with Dipterex for the control of stalkborers. Plants were weeded for all the other weeds except *Striga asiatica* every time weeds were noticed once weekly.

6.2.2 Measurements

Number of emerged *Striga asiatica* plants per pot was recorded at 67, 74, 81 and 88 dap. Day to flowering for the host crops was recorded. Grain dry weight per pot, head weight, total biomass (above ground) and root weight

were recorded between 123 and 130 dap. Washed roots and biomass above ground were dried at 75 ^{o}C in an oven before weights were recorded.

6.2.3 Data Analysis

The recorded measurements were subjected to ANOVA using the Minitab version 8 statistical package. *Striga* counts were transformed by Log (X+1) to fulfil assumptions of ANOVA before statistical analysis procedure for split-plot were done. Grain weight, stover weight and root weights were not transformed and analysis for these was done individually for each host species as complete randomised block design. The root/shoot weight ratios were calculated and subjected to ANOVA. Mean separation was done using the least significant difference (LSD). The relative decrease of grain and stover weight of the *Striga* infested plots as compared to the infested plots was used to measure variation of virulence in the different *Striga* populations.

6.3 Results

6.3.1 *Striga asiatica* counts.

Analysis of variance (ANOVA) for *Striga* counts was done at peak emergence, which was 88 dap. There were obvious significant differences in the number of emerged *Striga* plants between host species ($P<0.001$) (Append Y). The *Striga* strains showed remarkable differences in germination, within host species ($P<0.002$). This is evidence that there is a degree of specialization in the *Striga* populations occurring in Zimbabwe. There was no significant interaction between host species and *Striga* strain ($P = 0.200$).

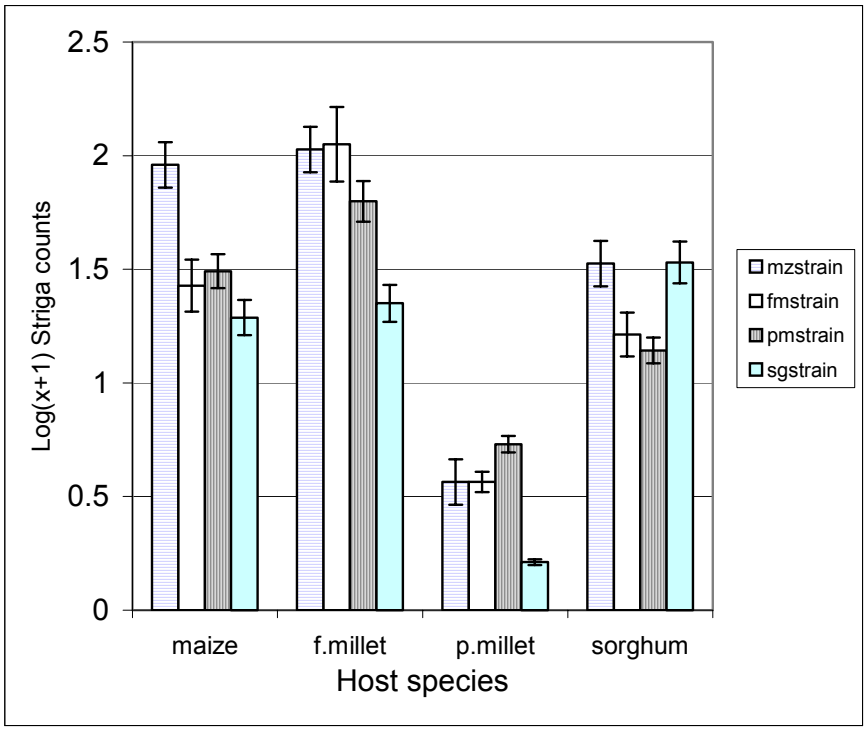

Figure 9: Interaction of *Striga* strain counts and four host species, maize, pearl millet, finger millet and sorghum.

6.3.2 Maize.

The Striga strains were significantly different (P<0.01) on their effect on maize stover weight.

Table 6. Root:shoot ratio, stover and head weight of *Zea mays* measured after treatment with different Striga strains.(Means followed by the same letter are not significantly different)

Striga strain	Stover d. wt(g)	Head d.wt(g)	Root/shoot ratio
Mzstrain	21.00[a]	2.25[a]	1.1400[a]
Fmstrain	26.50[b]	5.25[b]	0.8865[a]
Pmstrain	23.08[b]	4.00[b]	1.0180[a]
Sgstrain	22.00[a]	3.99[b]	1.3860[a]
Control	36.50[c]	11.58[c]	0.5720[b]
CV	16.59%	82.38%	25.00%

Table 7. Root, grain and stover weight of *Eluesine coracana* measured after infesting with four *Striga* populations (Means followed by the same letter are not significantly different).

Striga strain	Head d. wt (g)	Grain d. wt (g)	Root d. wt (g)	Stover d. wt (g)
Mzstrain	10.17[a]	8.00[a]	14.50[b]	14.50[a]
Fmstrain	9.583[a]	5.83[b]	21.67[a]	19.17[a]
Pmstrain	11.92[a]	8.50[a]	22.25[a]	15.50[a]
Sgstrain	10.33[a]	9.25[a]	18.75[ab]	16.33[a]
Control	18.25[b]	14.25[c]	19.17[ab]	21.25[a]
CV	24.83%	27.98%	17.78%	29.57%

All the *Striga* strains drastically reduced grain dry weight when compared to the uninfested control plot. The biggest grain yield reduction was by the finger millet strain. There was no significant difference in virulence of the maize, pearl millet and sorghum strains. The four strains affected the head weight of *Eluesine coracana* in the same way and they all significantly reduced head weight yield as compared to the control. There was however no significant difference between any of the Striga strains and control on the accumulation of above ground dry matter of *Eluesine coracana.*

6.3.3 Sorghum

Table 8. Grain weight of *Sorghum bicolor* under infestation with different strains.

Striga strain	Grain d. wt
Mzstrain	14.83[ac]
Fmstrain	12.92[a]
Pmstrain	13.00[a]
Sgstrain	10.58[b]
Control	18.58[c]
CV	34.40%

All the parameters measured for *Sorghum bicolor* (except grain weight) and *Pennisetum glaucum* did not show any significant differences either between strains or between any strain and the control.

6.4 Discussion

The results to some extend give evidence of host specialisation based on *Striga* counts. The results also indicate between -population variation in the virulence of the parasite. The *Striga* populations collected from *Eluesine coracana* tend to affect the same host much more than the other *Striga* populations do with an exception of maize-*Striga* population, which had the highest counts on all the species. In pearl millets, the pearl-millet- *Striga* population had the highest counts and this is also an indication of some form of specialization in the parasite. The pearl millet-*Striga* population was collected in Tsholotsho, a mainly dry area of the country where pearl millet is the major crop. It is only in pearl millet where the maize-*Striga* population has lower counts than the other populations. Maize is rarely grown in this area and this could explain why the maize-*Striga* population did not attack pearl millet that much. In sorghum the maize-*Striga* and the sorghum-*Striga* populations had the highest counts and were not significantly different suggesting that the maize-*Striga* population is well adapted to the sorghum host.

Although the results of specialization to sympatric host did not apply to all the four host-parasite interactions studied, it can be conclusively claimed that there is some form of host specialization in the *striga* populations occurring in Zimbabwe.

The virulence of a parasite is typically defined in terms of its effect on host mortality. By this definition, some *Striga* strains have been shown to be extremely virulent, whereas others are much less so, even though they might be quite closely related to forms that are highly virulent (as shown by RAPDS and isozymes profiles).

The different populations of *Striga* have been shown to have differential effects on the different host species. This is probably due to genetic variation, which can be supported with results obtained with RAPD-PCR and isozymes. In addition to genetic variation, Mutikainen *et al.* (2000) reported that this variation could be due to maternal effects, in the form of seed size. However Mutikainen *et al* (2000) reported that seed size and virulence did not correspond perfectly as his results showed that the population with the lightest seeds was the least virulent. Seed size was however not measured in this study and thus cannot be used to explain any of the outcomes.

Although the results above give evidence for differential virulence in *Striga*, Bonhoeffer *et al*. (1996) pointed out that virulence cannot be understood in isolation, but instead must be considered in relation to other aspects of a parasite's life history, such as its rate of transmission. For example, if one strain of parasite produces more infectious propagules than another strain, then the former may have both greater virulence and increased transmissibility relative to the latter. In that case, selection may favour high or intermediate levels of

virulence, whereas virulence would be minimized by selection if it were uncoupled to the rate of propagule production (May and Anderson, 1983).

Implications of host specialization to cereal growers

Since the results here show some degree of host specificity, it is possible for the cereal growers with *Striga* infested fields to use crop rotations as a means of controlling the parasite. In the south-west part of Zimbabwe for example where there are strains that specialize on pearl-millet and not finger-millet, farmers could be encouraged to rotate pearl-millets and finger millets. This farming practice will not only check *Striga* build up but will offer other beneficial rotation effects like reduced disease incidences. It should be noted however that the farmers cannot use finger millet as a trap crop as the most common mode of host specialization is through the recognition of stimulant by the specific strains.

CHAPTER 7

Evaluating resistance of 15 sorghum genotypes to physiological variants of *Striga asiatica* (L.)Kuntze

7.1 Introduction

One of the main approaches to decrease yield loss caused by *S. asiatica* infestation is breeding for resistance. Sorghum is one cereal crop which confers some level of resistance to the witchweed. The development of resistant/tolerant lines of susceptible crops constitutes an important, practical and reliable approach to solving the *Striga* problem. The main effort using this approach has been in sorghum. Resistance based on low stimulant production in sorghum was found to be controlled by a single recessive gene. However, due to genetic plasticity of the parasite, resistance or tolerance in some recommended varieties has been overcome. This could be as a result of different strains of *Striga* occurring in different geographical areas thus the inconsistency of performance of resistant varieties as they are moved from one area to another. Ramaiah (1987) reported that progress in selection is slowed down when there are virulent physiological strains in Striga. This means that the process of developing resistant cultivars is never-ending, when new virulent strains keep appearing in *Striga.*

Development of reliable screening techniques to identify *Striga resistant* varieties is very important for breeding programmes. *Striga* emergence is significantly affected by non-host factors like soil type, fertility, rainfall and many other factors. Ramaiah (1986) has confirmed that direct field evaluation is a less successful

approach for developing resistant lines than a combination of laboratory testing and field assessment. The laboratory test is based on detecting low stimulant producers using the double pot technique. Identification of resistant varieties using the double pot technique has led to the development of resistant lines with more acceptable agronomic traits, yield and grain quality (Kim, 1991). However Lagoke, Parkinson and Agunbiade (1993) noted that no immune variety of sorghum has been developed.

The inconsistency of host resistance to *Striga* infestations in different geographical areas has made many investigators reluctant to put forward comparable claims which may later prove unfounded (Parker and Reid, 1979). This may be because investigators do not consider the possible existence and effects of physiological variants among the species of witchweed when screening for resistance. Work done by Bebawi (1981) on sorghum resistance to *Striga hermonthica* showed that there was evidence for the existence of intraspecific variants of *S. hermonthica* with differing virulence. Although the work was on an obligate outcrossing species of Striga the same could be found in *S. asiatica*, a strongly inbreeding species. Although Mabasa (1996) reported that the sorghum varieties SAR 29 , SAR 16 and SAR 33 were resistant to *Striga* infestation, his work shows that there were inconsistencies in performance between two sites. This could have been a result of different strains of the weed occurring between the two sites. This difference in virulence between sites means that should a relatively resistant crop variety be developed its resistance may not hold in different geographical areas.

Because the occurrence of physiological strains of the parasite further complicates the breeding programmes, identification of resistant cultivars in the laboratory under controlled conditions and using known populations of the parasite offers a good scope of understanding host-parasite interactions.

The sorghum genotypes developed at U.Z, those in the breeding programmes of local seed companies and those which have been described as resistant in Zimbabwe were screened against the identified different strains of *S. asiatica*. The test materials included three *S. asiatica* resistant (SAR) genotypes : SAR 16, SAR 19, SAR 29 and crosses of these with local landraces. These lines have been reported to have dual resistance to *S. asiatica* (white flowered mutant and red flowered mutant) (Obilana and Ramaiah 1992). Resistance to the red flowered mutant is thought to be stable from studies done in Botswana, Tanzania and Zimbabwe (Mabasa, 1996). Root exudate technique was used to screen the genotypes.

The objective of this experiment was therefore to investigate the possible existence of intraspecific physiological variants of S*. asiatica* collected from different farming zones of Zimbabwe and at the same time to identify sorghum varieties with stable resistance across these strains.

Objective: To investigate intra-crop specificity of Striga strains collected from Zimbabwe.

Hypothesis: There is intra-crop specificity of *Striga* strains found in Zimbabwe.

7.2 Materials and Methods

Low stimulant production as a mechanism of resistance was investigated in this experiment. Experimental procedures were adopted with some modifications from Bebawi (1981). Sorghum seeds of 15 genotypes (some classified as resistant and some unclassified) were surface sterilised with 75% ethanol. The seeds were pre-germinated on filter paper and were placed on moist glass wool in small plastic cups perforated at the bottom and incubated at 27 ^{o}C for 5 days. Root exudates were collected through suction starting from the sixth day. There were three replicate cups for each sorghum variety. Discs of pre-conditioned *Striga* seeds (10 populations collected from different geographical regions and hosts) were dabbed on dry filter paper to remove excess moisture and placed in groups of six in a petri dish which contained glassfibre filter paper moistened with sorghum root exudate. The dishes were then closed, wrapped in polyethylene bags and incubated at 27^{0}C for 24 hours before counting the germinated seeds of *Striga.* The percent germination for the witchweed strains by each sorghum variety was recorded. The stability of each genotype across the 10 strains was evaluated by regressing the varietal mean versus the environmental mean (mean germination % of a strain across all the sorghum host genotypes) (Eberhat and Russell, 1966). A stable resistant variety/cross is defined as one with a mean value (X= mean number of germinated Striga plants) and regression coefficient (b) close to zero.

7.3 Results

Basing on the germination percentages, the *Striga asiatica* populations were significantly different ($P<0.01$) suggesting that these were physiological variants. The performance of the genotypes was also significantly different ($P<0.01$). As shown in Appendix E there was a very strong interaction ($P< 0.001$) between the *striga* populations and the different varieties of sorghum. The genotypes SAR29xSAR19, SAR16xSAR19 and SV1xSAR19 produced the lowest germination percentage of witchweed and their performance was relatively stable across the different strains of *Striga*. SAR29 produced a low germination percentage of the witchweed but its performance was not stable across strains as can been shown by the gradient of its regression line (Fig.). Its stimulant produced germination percentages ranging from 24 - 73 across the *Striga* strains. SV1xSAR16 was an intermediate stimulant producer with stable performance across witchweed populations. The high stimulant producers, DC75, IS26955 and SAR16xTwelane did not show any differences across the strains. The *Striga* populations Dlam-PM, Gutu-Mz and Dlam-SG were the most sensitive as they were stimulated by more than 40 percent of the genotypes (Table.9.).

Table 9. Percent of Genotypes that stimulate germination of the different *S. asiatica* populations within groups as defined by DMRT (5%).

	***Striga asiatica* strains**									
Genotype groups*	Chin-FM	Chiu-MZ	Dlam-PM	Barr-SG	Chin-MZ	Chiu-SG	Gutu-MZ	Maka-SYN	Kato-MZ	Dlam-SG
A (Highly affected)	26.7	26.7	46.7	33.3	26.7	26.7	46.7	26.7	40.0	40.0
B	20.0	26.7	40.0	26.7	20.0	26.7	33.3	33.3	13.3	53.3
C	20.0	26.7	26.7	20.0	20.0	26.7	20.0	20.0	13.3	46.6
D	20.0	13.3	26.7	26.7	26.7	20.0	20.0	13.3	20.0	26.6
E	26.7	13.3	20.0	20.0	26.7	20.0	33.3	6.7	20.0	33.3
F (Least affected)	26.7	13.3	6.7	20.0	13.3	20.0	0.0	26.7	26.7	0.0

* Sorghum genotypes grouped by Duncan's Multiple Range Test with respect to ability to stimulate a Striga population.

**Codes for the *Striga* strains derived from area of collection and host collected from (MZ=maize, SG=sorghum, PM=pearl millet, FM=finger millet).

Table 10. Germination percentages of 10 seed populations of *Striga* across 15 sorghum varieties**.**

Sorghum varieties	***Striga strains***										
	Chin-FM	Chiu-MZ	Dlam-PM	Barr-SG	Chin-MZ	Chiu-SG	Gutu-MZ	Maka-SYN	Kato-MZ	Dlam-SG	**Means %**
SAR19	27.0	32.7	57.7	25.3	29.7	27.7	64.3	22.0	29.0	70.3	**38.6**
SAR29	24.3	25.3	56.7	31.3	20.7	24.0	68.0	23.0	30.3	73.0	**37.7**
SAR33	40.0	44.0	72.7	38.0	40.3	41.3	78.0	40.0	38.7	80.7	**51.4**
SAR16	50.3	48.7	81.0	40.3	49.0	43.3	74.0	52.3	47.0	78.7	**56.8**
SAR16xSAR19	12.3	16.3	48.0	12.3	14.7	15.0	50.3	15.0	15.3	54.3	**25.4**
SAR29xSAR19	9.3	9.3	45.0	12.0	14.3	12.0	49.3	9.7	72.0	50.3	**22.3**
SAR16xSAR29	79.3	77.3	80.3	79.0	77.3	76.7	84.0	75.3	72.7	83.3	**78.5**
DC75	89.3	88.0	87.3	81.0	86.0	85.3	88.0	76.3	76.3	72.7	**83.0**
SV1	78.3	68.3	78.3	67.7	71.7	70.3	53.0	68.7	59.7	71.0	**68.7**
SV1xSAR16	43.3	82.3	38.3	48.3	39.7	44.3	80.7	63.3	50.7	60.7	**55.2**
SV1xSAR19	17.0	11.7	23.0	31.0	37.7	48.7	46.0	52.0	48.0	52.3	**36.7**
SAR16xTwelane	89.0	89.7	90.0	80.7	80.7	86.0	86.7	86.3	76.3	86.0	**85.1**
IS26955	71.0	78.0	78.0	75.7	88.0	79.7	82.0	77.3	79.3	83.3	**79.2**
IS26955xSAR19	15.3	12.7	51.0	13.0	15.0	19.0	46.7	24.7	31.3	56.0	**28.5**
Isifumbate	67.0	70.0	79.0	69.0	66.7	72.0	88.0	67.0	68.0	77.0	**72.4**
Means %	**47.5**	**50.3**	**64.4**	**47.0**	**48.8**	**49.9**	**69.3**	**50.2**	**49.0**	**70.0**	**54.6**
LSD (P = 0.05)	for intreraction = 10.9										

There was high significcant differences in germination percentages between the sorghum varieties (P<0.01) and also between the Striga strains (P<0.05).

Table 11. Regression of *Striga asiatica* germination percentage(SGP) induced by sorghum genotypes' stimulant on the mean of *S. asiatica* populations across genotypes.

Genotype		Stability Parameter	
	X	**b**	**S^2**
SAR 19	38.6	1.91	3.51
SAR 29	37.7	2.10	5.06
SAR 33	51.4	1.92	1.90
SAR 16	56.8	1.54	4.71
SAR 16 x SAR 29	25.4	0.29	2.24
SAR 29 x SAR 19	22.3	1.47	20.09
SV 1 x SAR 19	36.7	0.44	15.51
SAR16xTwelane	85.1	0.17	4.44
SV 1 x SAR 16	55.2	0.57	16.13
SAR 19 x IS26955	28.5	1.68	6.54
DC 75	83.0	0.071	6.32
SV 1	68.7	0.013	8.85
Isifumbate	72.4	3.29	3.29
SAR 16 X SAR 19	78.5	1.12	10.88
IS26955	79.2	0.175	4.54

7.4 Discussions.

The findings in the in this experiment show evidence for the existence of variability in the ability of varieties to stimulate germination of different *Striga* populations. This may imply that the chemistry of the root exudates of different varieties is distinctly different. The implication of this on variety development is that a witchweed resistant variety should only be recommended for cultivation after thoroughly testing it across all possible physiological strains and in different agro ecological zones. Those varieties which have stable resistance should be made available to farmers whose fields are infested with *S. asiatica.* Basing on this study the two genotypes, SAR16xSAR19 and SV1xSAR19 would be the most suitable to develop into cultivars as they are stable across strains. Such genotypes' resistance is unlikely to break down when exposed to new and more virulent strains of *Striga.*

Chapter 8

8.1 General Discussion and Conclusions

The major findings of this study are that there are physiological strains of the parasitic weed *S. asiatica,* which are adapted to specific hosts within and inter species. Another finding was that molecular techniques such as RAPDs and isozymes can be effectively used to differentiate strains of the parasitic weed, S asiatica however there is need to determine the relationship between molecular differences and differential virulence.

The present investigation reveals that there is genetic divergence between populations of *Striga* collected from different geographical areas and different hosts. It is possible that the genetic differences are a result of or are the causal agent for host specialization. The host specialization could have evolved recently due to ecological pressures resulting from cultivation practices. In Tsholotsho for example, where the farmers predominantly grow pearl-millet, the Striga from this area has specialized on the crop and produces distinct RAPD and Isozyme banding profiles. On the other hand *Striga* strains from other areas like Chiundura where crops like maize and finger millets are grown either in rotations or as intercrops did not show any strict specialization. The *Striga* strains from this area would attack maize, sorghum and millets in the same manner without showing any greater virulence on one crop. However, because there is evidence for host specialization it is possible that physiological specialization is maintained by

natural selection in host-specialised populations in the face of substantial gene flow between strains adapted for different crops.

Another observation made from this study is that the genetic distances from RAPD data corresponds well with geographic distances from which the *striga* were collected. Those with the least similarity also were the furthest apart (i.e.) those from Tsholotsho and those from Mt Darwin. This means that the genetic differences might not be a result of host specialization alone, but also due to environment x genotype interactions. On the other hand the correspondence of genetic distance and geographic distance would be the expected outcome in a non-crop plant like Striga whose dispersal is not through commerce but rather very slow random migration. Another possible source of the genetic differences in the *Striga* strains is natural cross pollination with related species. This could possibly happen in populations from Kwekwe where both *S. asiatica* and *S. forbesii* are found in the same fields.

8.2 RAPD and isozyme techniques in phylogenic studies.

Although the RAPD and isozyme techniques have been used before for phylogenetic studies, this study showed that the two techniques do not always give identical results. The isozyme technique did give some polymorphism but only gave three groups of biotypes. Isozyme variation was not detected between some strains which showed clear distinctness in their virulence both in the intra-crop specificity and inter- crop specificity experiments. However a greater number of genetic polymorphism was detected using RAPD-PCR and,

consequently the technique was better able to discriminate between biotypes. These results demonstrate what has been long suspected: isozyme analysis does not adequately reflect the true level of genetic variation in *Striga asiatica.* The same observation has been made (Kim, 1991).

However, although RAPD-PCR gave much more polymorphism in *Striga*, the technique also has its shortfalls. The major complication in using the RAPD phenotypes were the continuum of band intensities observed. Some RAPD products were intense, well resolved bands while at the other extreme there were faint or fuzzy products that were difficult to score. Hence, numerical genetic diversity estimates based on RAPD data did not only contain a certain error component intrinsic to the technique itself, but also a "clarity" factor that may depend on the eyesight of the person scoring the gel. Also as discussed earlier similar-sized fragments could be scored as homologous when in fact they might be having different nucleotide sequences.

8.3 Implications of the existence of *Striga asiatica* strains

The results have shown the existence of *Striga asiatica* strains occurring in different geographical regions of Zimbabwe. The occurrence of pathogenic variation in virulence of *S. asiatica* in Zimbabwe suggests that breeding for resistance to the parasite may not be as straightforward as previously believed. It is therefore highly advisable to intensify research in such areas as physiologic specialization and inheritance of resistance. Should a resistant variety be developed it will have to be extensively tested using as many populations of *Striga* as possible. This will ensure that the variety is stable and

has durable resistance which will not easily breakdown once the variety is moved to another area or when a more virulent strain surfaces. In the germplasm screening for resistance to S. asiatica at Henderson Research Station, *Striga* seeds collected from Mt Darwin and Chiundura represented by collection 'B' and 'T' are often used for artificial inoculations (Tagurika, pers. comms.). However the present study showed that there are some varieties like, SV1xSAR19 and SAR16xSAR19 which have relatively stable resistance across the different biotypes of *S. asiatica*.

The present study was a preliminary investigation into the S. asiatica populations occurring in Zimbabwe. Though the sample studied was small, distinct groups were discernible.

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APPENDICES

APPENDIX A_ Staining solutions for isozymes**.**

POLYACRYLAMIDE GEL ELECTROPHORESIS OF ENZYMES FROM STRIGA

Staining

Table 4. Stain solutions

Solution 1	Ingredient	Concentration (mg/ml)	Usual volume (ml)
BB	Fast Blue BB salt (SC-F0250) 2	100	30
Cis-AA	cis-Aconitic acid (SC-A3412) (Titrate to pH 7.0 with 5N NaOH)	17.2	25
GBC	Fast garnet GBC salt (SC-F0875) (filtered)	50	40
F-6-P	D-fructose-6-phosphate, disodium salt (SC-F3627)	50	20
G-6-PDH	NADP-dependent glucose-6-phos--phate dehydrogenase (SC-G6378)	50 U/ml	100
G-6-PDH	NAD dependent glucose-6-phosphate dehydrogenase (SC-G5885)	50 U/ml	40
HEX	Hexokinase (SC-H4502) (1 ml/vial - keep in freezer	156.26 U/ml	16
IDH	Isocitric dehydrogenase (SC-I5882) suspended in 75% glycerol (1 ml/vial keep in freezer)	67 U/ml	15
MgCl2	Magnesium chloride (SC-M0250)	100	100
MA	DL-Malic acid (SC-M0875) (neutra--lised with NaOH to pH 8.0)	50	100
MTT	MTT, tetrazolium thiazolyl blue (SC-M2128)	10	60
a-NA	a-Naphthyl acetate (SC-N8505) (in 1 part water: 1 part acetone	20	50
b-NA	b-Naphthyl acetate (SC-N6875) (in acetone)	20	50
NAD	b-Nicotinamide adenine dinucleotide (SC-N7004)	20	100
NADP	b-Nicotinamide adenine dinucleotide	10	
	Enough for 1-2 weeks phosphate (SC-N0505)		
NBT	Nitro blue tetrazolium (SC-N6876)	10	100

PMS	Phenazine methosulfate (SC-P9625)	5	100
6-PGA	6-Phosphogluconic acid, trisodium salt (SC-P7877)	20	50
NAP	a-Naphthyl acid phosphate, sodium salt (SC-N7000)	100	50
ISCA	DL-Isocitric acid (SC-I1252)	100	50
GA	L-Glutamic acid (SC-G1251)	100	50
FIX1	Fixative solution (acetic acid, glacial: methanol : water; 1:5:5 by volume)	--	--
FIX2	Fixative solution (glycerol:acetic acid, glacial:water:ethanol; 1:2:4:5 by volume)	--	--

1 Aqueous solutions unless otherwise noted. G-6-PDH, HEX, and IDH are frozen in disposable capsules of 1.5 ml. Other solutions are refrigerated and kept 1-2 weeks; NADP and F-6P solutions are made fresh each week.
2 SC = Sigma Catalogue Number

APPENDIX B. Stain buffers for isozymes

Table 5. Stain buffers 1

Assay	Buffer	pH	Chemicals
ACO, ADH, AK, DIA, HEX, IDH, PHI/PGD, TPI	Assay	8.0	0.05 M Trizma base (6.05 g/l) Titrate to pH 8.0 with HCl
ACP	Assay	5.0	0.1 M Sodium acetate.3H2O (13.6 g/l) Titrate to pH 5.0 with 1 N HCl (29.2 ml)
AMP	Stock	3.7	0.2 M Trizma base (24.2 g/l) 0.2 M Maleic acid (23.2 g/l)
	Assay	6.2	4 (stock):3 (water):3 (0.2 M NaOH)
ENP	Stock	3.7	Same as AMP
	Assay	5.6	5 (stock):3 (water):2 (0.2 M NaOH)
EST	Stock A	4.46	0.2 M Sodium phosphate, monobasic (27.8 g/l)
	Stock B	9.1	0.38 M Sodium phosphate, dibasic (53.6 g/l)
	Assay	6.0	5 (stock A):1 (stock B):4 (water)
GDH, PGM, ME	Assay	8.5	0.1 M Trizma base (12.1 g/l) Titrate to pH 8.5 with HCl
GLU	Assay	6.5	0.05 M Potassium phosphate (mono) (6.8 g/l) Titrate to pH 6.5 with 5 N NaOH
MDH SAD	Assay	9.1	0.1 M Trizma base (12.1 g/l) Titrate to pH 9.1 with HCl

1 Can be kept at room temperature for several weeks.

APPENDIX C Analysis of Variance for %Germination of *Striga*

SOURCE	DF	SS	MS	F	P
Reps	2	113.2	56.6	1.29	0.276
Varieties	14	206759.0	14768.5	337.37	0.000
Error (a)	28	1877.4	67.0	1.53	0.046
Strains	9	35189.9	3910.0	89.32	0.000
Varieties x Strains	126	42730.1	339.1	7.75	0.000
Error (b)	270	11819.5	43.8		
TOTAL	449	298489.0			

APPENDIX D Analysis of Variance for maize stover

SOURCE	DF	SS	MS	F	P
Rep	3	38.71	12.90	0.69	0.573
Strains	4	606.72	151.68	8.15	0.002
Error	12	223.23	18.60		
TOTAL	19	868.67			

APPENDIX E Analysis of Variance for maize root

SOURCE	DF	SS	MS	F	P
Reps	3	363.62	121.21	6.27	0.008
Strains	4	119.45	29.86	1.54	0.252
Error	12	232.02	19.33		
TOTAL	19	715.08			

APPENDIX F Analysis of Variance for maize root/shoot ratio.

SOURCE	DF	SS	MS	F	P
Reps	3	1.164	0.388	6.30	0.008
Strains	4	1.481	0.370	6.01	0.007
Error	12	0.739	0.062		
TOTAL	19	3.384			

APPENDIX G Analysis of Variance for finger millet stover weight

SOURCE	DF	SS	MS	F	P
Reps	3	2.06	0.69	0.03	0.994
Strains	4	124.35	31.09	1.18	0.368
Error	12	315.90	26.32		
TOTAL	19	442.31			

APPENDIX H Analysis of Variance for finger millet root weight

SOURCE	DF	SS	MS	F	P
Reps	3	132.76	44.25	3.77	0.041
Strains	4	150.63	37.66	3.21	0.052
Error	12	140.75	11.73		
TOTAL	19	424.14			

APPENDIX I Analysis of Variance for finger millet root/shoot ratio

SOURCE	DF	SS	MS	F	P
Reps	3	0.698	0.233	1.77	0.206
Strains	4	0.897	0.224	1.71	0.213
Error	12	1.577	0.131		
TOTAL	19	3.172			

APPENDIX J Analysis of Variance for pearl millet stover weight

SOURCE	DF	SS	MS	F	P
Reps	3	219.16	73.05	2.11	0.153
Strains	4	133.83	33.46	0.96	0.462
Error	12	416.12	34.68		
TOTAL	19	769.11			

APPENDIX K Analysis of Variance for pearl millet root weight

SOURCE	DF	SS	MS	F	P
Reps	3	178.86	59.62	3.16	0.064
Strains	4	27.41	6.85	0.36	0.830
Error	12	226.43	18.87		
TOTAL	19	432.70			

APPENDIX L Analysis of Variance for pearl millet root/shoot ratio

SOURCE	DF	SS	MS	F	P
Reps	3	0.378	0.126	1.73	0.214
Strains	4	0.264	0.066	0.91	0.490
Error	12	0.874	0.073		
TOTAL	19	1.517			

APPENDIX M Analysis of Variance for sorghum stover weight

SOURCE	DF	SS	MS	F	P
Reps	3	578.59	192.86	10.95	0.001
Strains	4	7.24	1.81	0.10	0.979
Error	12	211.39	17.62		
TOTAL	19	797.22			

APPENDIX N Analysis of Variance for sorghum root weight

SOURCE	DF	SS	MS	F	P
Reps	3	115.932	38.644	4.94	0.018
Strains	4	33.441	8.360	1.07	0.414
Error	12	93.934	7.828		
TOTAL	19	243.306			

APPENDIX O Analysis of Variance for sorghum root/shoot ratio

SOURCE	DF	SS	MS	F	P
Reps	3	0.321	0.107	3.14	0.065
Strains	4	0.110	0.027	0.80	0.547
Error	12	0.410	0.034		
TOTAL	19	0.841			

APPENDIX P Analysis of Variance for maize head weight

SOURCE	DF	SS	MS	F	P
Reps	3	15.91	5.30	0.27	0.847
Strains	4	209.34	52.34	2.64	0.086
Error	12	237.53	19.79		
TOTAL	19	462.78			

APPENDIX Q Analysis of Variance for maize grain weight

SOURCE	DF	SS	MS	F	P
Reps	3	9.65	3.22	0.25	0.861
Strains	4	95.24	23.81	1.84	0.185
Error	12	155.02	12.92		
TOTAL	19	259.91			

APPENDIX R Analysis of Variance for finger millet head weight

SOURCE	DF	SS	MS	F	P
Reps	3	16.457	5.486	0.61	0.620
Strains	4	204.132	51.033	5.70	0.008
Error	12	107.459	8.955		
TOTAL	19	328.048			

APPENDIX S Analysis of Variance for pearl millet head weight

SOURCE	DF	SS	MS	F	P
Reps	3	29.394	9.798	2.28	0.132
Strains	4	15.256	3.814	0.89	0.501
Error	12	51.634	4.303		
TOTAL	19	96.284			

APPENDIX T Analysis of Variance for pearl millet grain weight

SOURCE	DF	SS	MS	F	P
Reps	3	15.446	5.149	1.95	0.175
Strains	4	4.978	1.244	1.47	0.756
Error	12	31.644	2.637		
TOTAL	19	52.067			

APPENDIX U Analysis of Variance for sorghum head weight

SOURCE	DF	SS	MS	F	P
Reps	3	853.87	284.62	8.05	0.003
Strains	4	129.83	32.46	0.92	0.485
Error	12	424.49	35.37		
TOTAL	19	1408.20			

APPENDIX V Analysis of Variance for sorghum grain weight

SOURCE	DF	SS	MS	F	P
Reps	3	487.69	162.56	6.64	0.007
Strains	4	100.59	25.15	1.03	0.432
Error	12	293.77	24.48		
TOTAL	19	882.05			

APPENDIX W Analysis of Variance for finger millet grain weight

SOURCE	DF	SS	MS	F	P
Reps	3	11.754	3.918	0.55	0.660
Strains	4	114.527	28.632	4.00	0.028
Error	12	85.976	7.165		
TOTAL	19	212.256			

APPENDIX X ANOVA for counts of emerged striga plants at 7 WACE

SOURCE	DF	SS	MS	F	P
Reps	3	2617.0	872.3	0.95	0.427
Host	3	46664.4	15554.8	16.94	0.000
Error (a)	9	1529.7	1699.7		0.002
Strain	3	16556.7	5518.9	6.01	0.200
Host x strain	9	12063.9	1340.4	1.46	
Error (b)	36	33052.7	918.1		
TOTAL	63	126252.2			

APPENDIX Y Regression ANOVAS for Sorghum Stability Analyses

The regression equation is
sar19 = - 65.7 + 1.91 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-65.668	6.945	-9.46	0.000
All	1.9080	0.1255	15.20	0.000

s = 3.510 R-sq = 96.7% R-sq(adj) = 96.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	2847.0	2847.0	231.14	0.000
Error	8	98.5	12.3		
Total	9	2945.5			

The regression equation is
SAR 29 = - 76.9 + 2.10 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-76.90	10.01	-7.68	0.000
All	2.0972	0.1809	11.59	0.000

s = 5.058 R-sq = 94.4% R-sq(adj) = 93.7%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	3439.6	3439.6	134.43	0.000
Error	8	204.7	25.6		
Total	9	3644.2			

The regression equation is
sar33 = - 53.2 + 1.92 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-53.249	3.751	-14.20	0.000
All	1.91502	0.06778	28.25	0.000

s = 1.895 R-sq = 99.0% R-sq(adj) = 98.9%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	2867.8	2867.8	798.21	0.000
Error	8	28.7	3.6		
Total	9	2896.6			

The regression equation is
sar16 = - 27.2 + 1.54 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-27.214	9.311	-2.92	0.019
All	1.5373	0.1682	9.14	0.000

s = 4.705 R-sq = 91.3% R-sq(adj) = 90.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	1848.1	1848.1	83.49	0.000
Error	8	177.1	22.1		
Total	9	2025.2			

The regression equation is
sar16x19 = - 39.9 + 1.12 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-39.95	21.53	-1.85	0.101
All	1.1242	0.3891	2.89	0.020

s = 10.88 R-sq = 51.1% R-sq(adj) = 44.9%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	988.3	988.3	8.35	0.020
Error	8	947.3	118.4		
Total	9	1935.6			

The regression equation is
sar29x19 = - 51.7 + 1.47 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-51.73	39.76	-1.30	0.229
All	1.4656	0.7185	2.04	0.076

s = 20.09 R-sq = 34.2% R-sq(adj) = 26.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	1679.8	1679.8	4.16	0.076
Error	8	3230.0	403.8		
Total	9	4909.8			

The regression equation is
sar29x16 = 62.4 + 0.295 All

Predictor	Coef	Stdev	t-ratio	p
Constant	62.431	4.430	14.09	0.000
All	0.29476	0.08006	3.68	0.006

s = 2.239 R-sq = 62.9% R-sq(adj) = 58.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	67.942	67.942	13.56	0.006
Error	8	40.096	5.012		
Total	9	108.038			

The regression equation is
dc75 = 86.9 - 0.071 All

Predictor	Coef	Stdev	t-ratio	p
Constant	86.93	12.50	6.96	0.000
All	-0.0713	0.2258	-0.32	0.760

s = 6.315 R-sq = 1.2% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	3.98	3.98	0.10	0.760
Error	8	319.00	39.88		
Total	9	322.98			

The regression equation is
sv1 = 70.2 - 0.013 All

Predictor	Coef	Stdev	t-ratio	p
Constant	70.21	17.50	4.01	0.004
All	-0.0131	0.3163	-0.04	0.968

s = 8.845 R-sq = 0.0% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.13	0.13	0.00	0.968
Error	8	625.90	78.24		
Total	9	626.04			

The regression equation is
sv1xsa16 = 24.2 + 0.566 All

Predictor	Coef	Stdev	t-ratio	p
Constant	24.23	31.93	0.76	0.470
All	0.5663	0.5769	0.98	0.355

s = 16.13 R-sq = 10.7% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	250.8	250.8	0.96	0.355
Error	8	2082.4	260.3		
Total	9	2333.2			

The regression equation is
sv1xsa19 = 13.0 + 0.435 All

Predictor	Coef	Stdev	t-ratio	p
Constant	12.99	30.69	0.42	0.683
All	0.4347	0.5546	0.78	0.456

s = 15.51 R-sq = 7.1% R-sq(adj) = 0.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	147.8	147.8	0.61	0.456
Error	8	1923.9	240.5		
Total	9	2071.7			

The regression equation is
16xtwela = 75.6 + 0.175 All

Predictor	Coef	Stdev	t-ratio	p
Constant	75.562	8.790	8.60	0.000
All	0.1752	0.1588	1.10	0.302

s = 4.442 R-sq = 13.2% R-sq(adj) = 2.4%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	24.00	24.00	1.22	0.302
Error	8	157.82	19.73		
Total	9	181.83			

The regression equation is
is26955 = 69.7 + 0.175 All

Predictor	Coef	Stdev	t-ratio	p
Constant	69.682	8.981	7.76	0.000
All	0.1748	0.1623	1.08	0.313

s = 4.538 R-sq = 12.7% R-sq(adj) = 1.8%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	23.90	23.90	1.16	0.313
Error	8	164.77	20.60		
Total	9	188.67			

The regression equation is
19xis269 = - 63.4 + 1.68 All

Predictor	Coef	Stdev	t-ratio	p
Constant	-63.35	12.95	-4.89	0.000
All	1.6808	0.2341	7.18	0.000

s = 6.546 R-sq = 86.6% R-sq(adj) = 84.9%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	2209.2	2209.2	51.55	0.000
Error	8	342.8	42.9		
Total	9	2552.1			

The regression equation is
isifumba = 35.9 + 0.668 All

Predictor	Coef	Stdev	t-ratio	p
Constant	35.853	6.516	5.50	0.000
All	0.6684	0.1177	5.68	0.000

s = 3.293 R-sq = 80.1% R-sq(adj) = 77.6%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	349.36	349.36	32.22	0.000
Error	8	86.73	10.84		
Total	9	436.10			
