

**THE SEARCH FOR MOLECULAR MARKERS, PARENTAL
CHARACTERISATION AND INHERITANCE STUDIES OF
WITCHWEED [*STRIGA ASIATICA* (L.) KUNTZE] RESISTANCE IN
SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH]**

by

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is ranked the third most important cereal crop in Zimbabwe, after maize and wheat. The major biotic constraint to sorghum production by resource poor farmers (RPFs) is attack by the parasitic weed *Striga asiatica* (L) Kuntze, or witchweed. *Striga asiatica* resistant sorghum cultivars could be a major component of integrated witchweed management, if resistance was available in adapted and productive germplasm. The objectives of this work were to; characterize available sorghum cultivars for resistance to witchweeds, study the inheritance of low *S. asiatica* seed germination stimulant production and identify molecular markers that are linked to the genes for *S. asiatica* resistance.

Crosses were made between witchweed resistant (SAR 16, SAR 19 and SAR 29) and susceptible (SV-1) cultivars in a half-diallel arrangement. The F₁s were selfed to generate F₂ generation progeny. Parental lines and F₂ progeny were screened for *S. asiatica* resistance using the pot culture and agar gel techniques. Combining ability analysis for witchweed counts and path coefficient analysis of sorghum grain yield and its components were conducted for parent materials that were grown under *S. asiatica* infestation in pots. The inheritance of low *S. asiatica* germination stimulant production was evaluated using seedlings of F₂ progeny that were screened in water agar and using petri dishes. Parental and F₂ genotypes were transferred from petri dishes into pots filled with clay. Deoxyribonucleic acid (DNA) was then extracted from the potted sorghum seedlings after two weeks for molecular marker analysis using random amplified polymorphic DNA (RAPD) and microsatellite or simple sequence repeat (SSR) markers. A total of 440 RAPD, 24 sorghum SSRs and six maize SSRs were used to screen SV-1 and SAR 29 for polymorphisms. Linkage analysis was conducted using the software Mapmaker/exp 3.0b.

Cultivars SV-1 and SAR 16 were susceptible, while SARs 19 and 29 were resistant to witchweeds. Combining ability analysis revealed that GCA components of genotypic variance were significant. Additive genetic factors were therefore important in determining the response of a cultivar to witchweed infestation. Cultivars SAR 19 and SAR 29 were good general combiners for low *S. asiatica* counts. These resistant cultivars reduced the number of parasite counts in their F₂ progeny, though this was more conspicuous for SAR 19 whose negative GCA effects were significantly different from zero. Cultivars SV-1 and SAR 16 had positive and highly significant GCA effects. These cultivars therefore increased parasite counts among progeny from crosses that involved them. Grain yield components that were important for the cultivars tested were head weight, 100 seed weight, plant height and days to 50 % flowering. However, the direct and indirect contribution of each of these parameters to yield was influenced by the type of cultivar (resistant or susceptible) and whether there was witchweed infestation or not. In general, head weight was the most important sorghum grain yield determinant, having moderate to high direct contributions. Direct effects of *S. asiatica* counts on sorghum grain yield were low. *Striga asiatica* indirectly caused yield reduction by affecting sorghum grain yield components, mostly head weight.

A single recessive gene controlled low *S. asiatica* seed germination stimulant production in sorghum genotypes SAR 19 and SAR 29. A total of 199 markers (187 RAPDs; 10 sorghum

SSRs and 2 maize SSRs) were polymorphic between cultivars SAR 29 and SV-1. Molecular markers that are linked to the gene(s) for low *S. asiatica* seed germination stimulant production could not be identified. Instead, a molecular marker linkage map was constructed and it consisted of 45 markers that were distributed over 13 linkage groups (LGs). The LGs consisted of 2 to 8 markers that were identified at a LOD grouping threshold of 4.0. The map spanned a total distance of 494.5 cM.

Cultivars SAR 19 and SAR 29 are good sources of genes for resistance to witchweeds since they had negative GCA effects, which enabled them to reduce witchweed counts in progeny derived from them. Specifically, these resistant cultivars are a good source of the low *S. asiatica* seed germination stimulant trait. However, sustainable use of *S. asiatica* resistance can be achieved through pyramiding different mechanisms of resistance. This should be combined with the use of an integrated *Striga* management package involving host-plant resistance and other appropriate technologies, to provide a long-term and effective way of combating witchweeds. Field screening for witchweed resistance requires independent and concurrent selection for low *Striga* counts and high yield under *S. asiatica* infested conditions. Improvement in sorghum grain yield can be primarily based upon selection for improved head weight, though 100 seed weight, plant height and days to 50 % flowering should also form part of the selection criteria. The molecular linkage map that was constructed in this investigation can be useful for practical plant breeding purposes, since the polymorphisms that were identified are within the cultivated gene pool of sorghum.

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LIST OF ABBREVIATIONS

ACC:	1-aminocyclopropane-1-carboxylic acid
ACCO:	ACC oxidase
AFLP:	Amplified fragment length polymorphism
ALS:	Actolactate synthase
ASNPC:	Area under <i>Striga</i> numbers progress curve
bp:	Base pairs
BSA:	Bulked segregant analysis
CIMMYT:	International Maize and Wheat Improvement Centre
cM:	Centimorgan
CTA:	Technical Centre for Agricultural and Rural Co-operation
ddH ₂ O:	Double deionised water
DNA:	Deoxyribonucleic acid
DSE:	Days to <i>Striga asiatica</i> emergence
EAGT:	Extended agar gel technique
FAO:	Food and Agriculture Organisation
F2:	Second filial generation
GCA:	General combining ability
GM:	Genetically modified
GMB:	Grain marketing board
HRS:	Henderson Research Station
HR:	Hypersensitive response
IR:	Incompatible response
ICRISAT:	International Crops Research Institute for the Semi-Arid Tropics
IITA:	International Institute for Tropical Agriculture
lhf:	Low haustorial initiation factor
kb:	kilobases
lgs:	Low germination stimulant production
LOD score:	Likelihood of odds ratio score
MAS:	Marker-assisted selection

MGD:	Maximum germination distance
mRNA:	Messenger RNA
NR:	Natural region
NILs:	Near isogenic lines
2,6-DMBQ:	2,6-dimethoxy-parabenzoquinone
OPV:	Open pollinated variety
PCR:	Polymerase chain reaction
PRT:	Paper roll technique
QTL:	Quantitative trait loci
RAPD:	Randomly amplified polymorphic DNA
RFLP:	Restriction fragment length polymorphism
RILs:	Recombinant inbred lines
RPF:	Resource poor farmer
SADC:	Southern African Development Community
SAR:	<i>Striga asiatica</i> resistant
SCA:	Specific combining ability
SCAR:	Sequence characterized amplified region
SE:	Standard error
SH:	Smallholder
SSR:	Simple sequence repeat
UV:	Ultraviolet
YMV:	Yam mosaic virus

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CHAPTER 1

INTRODUCTION AND JUSTIFICATION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops grown for food and beverages by resource poor farmers (RPFs) in Zimbabwe. Some of the local names for this small grain crop are *mapfunde* (Shona language) and *amabele* (Ndebele language). It is ranked as the third most important cereal crop in Zimbabwe, after maize and wheat (FAO, 1996). White sorghum is ground into flour and used mostly for making both “Sadza” and porridge. Its grain is used for making a rice-like product which is cooked in a mixture with groundnuts, cowpeas or bambara nuts to improve the flavour and nutritional value (Mushonga, Gono and Sithole, 1992). Both smallholder (SH) farmers and breweries use red sorghum malt to make opaque beer and non-alcoholic beverages. Sorghum is also used in livestock and poultry feeds. Sweet stalk sorghum is extensively grown in most SH farming areas. The peeled stalks can be chewed fresh or dried and stored for chewing later for the sucrose when the crop is out of season. Sorghum is cultivated in areas considered to be too dry and hot for maize production because it is tolerant to drought and heat stress.

The major biological constraint to increased sorghum production in the SH sector in Africa is attack by *Striga* (*Striga* species) (DeVries and Toenniessen, 2001), or witchweeds. Witchweeds are very devastating obligate root parasites of cereal crops of the family *Gramineae* that includes sorghum, millets [*Pennisetum americanum* (L.) Leek and *Eleusine coracana* (L.) Gaertn], maize (*Zea mays* L.), rice (*Oryza sativa* L.) and sugar

cane (*Saccharum* species) (Stroud, 1993). *Striga asiatica* (L.) Kuntze and *S. forbesii* Benth. are major constraints to sorghum and maize production in natural regions (NRs) III, IV and V of Zimbabwe (Mabasa, 1993; 1994; 1996). *Striga asiatica* is the most common parasitic weed in Zimbabwe (Mabasa, 1996). Natural Regions III, IV and V cover about 19 million hectares or about 50 % of Zimbabwe (Statistical Yearbook of Zimbabwe, 1989). Excluding bird predation, *S. asiatica* may be the most important sorghum yield loss factor in Africa (Hess and Ejeta, 1992). Crop yield losses may be up to 100 % when a susceptible cultivar is grown under high levels of infestation (Obilana and Ramaiah, 1992; Haussmann, Hess, Welz and Geiger, 2000b). However, actual yield losses are difficult to determine in the SH sector due to the complex occurrence of other pests and diseases (Riches, de Milliano, Obilana and House, 1986). The socioeconomic implications of *Striga* infestation include field abandonment and changes in cropping systems (Stroud, 1993; Obilana and Ramaiah, 1992). Several cases of farm abandonment or change in cropping patterns have been reported in Southern Africa (Obilana, Knepper and Musselman, 1987). It is therefore imperative that *Striga* populations be controlled so that they remain below the economic threshold level.

The *Striga* problem has reached epidemic proportions in the SH sector (communal, resettlement and small-scale commercial farming areas). Available evidence indicates that the problem is actually worsening by the day. In a survey, about 52 % of the farmers interviewed reported that *Striga* infestation was increasing (Mabasa, 1994). Currently recommended control measures require costly additional inputs, which are beyond the means of RPFs. Resistant cultivars may provide the most economically promising *Striga* control measure since such cultivars can be grown without any additional costly inputs by

the RPF (Doggett, 1988; Mabasa, 1996). However, the witchweed problem cannot be solved by a single control method. Host-plant resistance has to become part of an integrated control strategy involving appropriate technologies (Obilana and Ramaiah, 1992; Ejeta, Babiker, Belete, Bramel, Ellicott, Greiner, Housley, Kapran, Mohamed, Shaner and Toure, 2001). Since a resistant genotype can support a small number of witchweed plants when grown under infestation, other control strategies will assist in further reducing the number of parasites supported by a host genotype. Used alone, host resistance can also breakdown easily if selection pressure on the parasite population is intense.

It is unfortunate that there are no elite witchweed resistant sorghum or other cereal crop cultivars recommended that are recommended for use by SH farmers in Zimbabwe. Lack of progress in the development of these cultivars has been attributed to a number of reasons. Firstly, biotypes, morphotypes and physiological strains of *Striga* have been identified (Parker and Reed, 1979; Aigbokhan, Berner, Musselman and Mignouna, 2000), in addition to the intra- and inter-specificity of witchweed resistance. As a result sorghum genotypes rarely show resistance across *Striga* species and resistance may not be upheld for one *Striga* species in different locations and in different seasons (Ejeta *et al.*, 1991; Obilana, de Milliano and Mbwaga, 1991). Secondly, there is a lack of both resistance genes in crop germplasm (Ejeta, Butler, Hess and Vogler, 1991) and of rapid, effective and reliable techniques for use in screening for *Striga* resistance (Ejeta *et al.*, 1991; Omany, Haussmann, Hess, Welz and Geiger, 2001). Thirdly, there is limited knowledge of the genetics of *Striga* resistance in the host crops on one hand, and that of virulence in the parasite on the other (Haussmann, Hess, Omany, Reddy, Seetharama, Mukuru, Kayentao,

Welz and Geiger, 2001). Fourthly, there is a lack of research support as well as lack of a functional and rational approach to selection strategy (Ejeta *et al.*, 2001). The individualistic efforts of Plant Breeders, Molecular Biologists, Weed Scientists, Agronomists and Plant Pathologists cannot be as good as adopting a multidisciplinary approach.

Precise and reliable screening techniques are prerequisites for success when breeding for resistance to any biotic or abiotic stress factor. Selection for resistance to witchweeds is normally done under field or greenhouse conditions. Complex interactions between host, parasite and the environment influence germination, attachment and growth of the parasite on host roots. Host resistance is therefore not just a result of the interaction between the host and *Striga*, but also of their independent interactions with environmental factors such as soil type, fertility and rainfall (Ramaiah and Parker, 1982). Field screening for *Striga* resistance is therefore difficult given the many confounding factors that are involved. It is also difficult to establish a uniform level of *Striga* infestation at an appropriate intensity level for reliable and reproducible results (Ejeta *et al.*, 1991).

Evaluation of segregating populations resulting from deliberate crosses in a breeding programme is difficult. This is because emphasis is placed on single plant selection in segregating generations, particularly for mechanisms with a high heritability, and which are simply inherited. It is unfortunate that during selection for field resistance to *Striga*, it is quite difficult to distinguish between infested and uninfested plants when parasitism occurs below the ground. It is also recognized that much of the damage to the host plant occurs before the witchweed plant emerges above ground level (Obilana and Ramaiah,

1992). Thus a witchweed plant emerging on a host plant often suggests its susceptibility though the freedom of a host plant from *Striga* may not necessarily be an indication of its resistance. As a result, screening for field resistance to *Striga* has been slow and largely inefficient. While laboratory techniques are efficient in screening for individual resistance mechanisms, one cannot do away with field screening, which takes into account all the resistance mechanisms. Field screening is therefore the ultimate test to identify witchweed resistant and high yielding genotypes for some targeted environments.

Evaluation of host plant resistance to *Striga* without regard to the basis of resistance has been slow and inefficient (Ejeta *et al.*, 1993). This may be because field resistance to *Striga* (*Striga* spp) is the eventual expression of a combination of resistance mechanisms and is, therefore, inherited as a quantitative trait (Ejeta, Mohamed, Rich, Melake-Berhan, Housley and Hess, 2000). Effective use of genotypes in breeding programmes requires that the resistance mechanisms underlying the resistance of those genotypes be identified, and the inheritance pattern clearly explained. It was therefore important to characterise available *Striga* resistant materials to enhance their effective use in breeding programmes in Zimbabwe and the Southern African sub-region. The best characterised of *Striga* resistance mechanisms is low germination stimulant (*lgs*) production. Production of *Striga* germination stimulants by different genotypes is relatively simple to assay using the agar gel technique (AGT) (Hess, Ejeta and Butler, 1992). In addition to explaining the genetics of *lgs* production in cultivars SAR 19 and SAR 29, it was also important to identify genetic markers that are linked to the same mechanism.

The current practice to make genetic gains for different traits in sorghum involves selecting desirable genotypes in segregating generations following hybridization. Hence it is advantageous to test parental genotypes systematically in order to identify average performance for traits of interest. A diallel mating system is useful for this purpose because progeny performance can be statistically separated into components relating to general combining ability (GCA) and specific combining ability (SCA). Sorghum grain yield and field resistance to *S. asiatica* are quantitative characters that are largely influenced by the environment, and they therefore have a low heritability. As a result, the response to direct selection for both traits may be unpredictable, unless there is good control of environmental variation. Since there is an interest to select for both grain yield and witchweed resistance under infested conditions, there was also a need to examine the relationships between these two traits and other sorghum grain yield components. Path coefficient analysis provides a method of separating direct and indirect effects and measuring the relative importance of the causal factors that contribute to certain traits. All these efforts naturally lead to the expeditious development of sorghum cultivars with durable resistance to witchweeds.

The development of reliable screening techniques to identify resistant genotypes has been slow, though much desirable. Molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs) and isozymes can provide a powerful approach in screening germplasm for traits of interest (Tanksley, 1983). The utility of DNA markers in resistance breeding depends on the existence of tight linkage between these markers and the resistance genes of interest. In marker-assisted breeding programmes, such linkage allows the breeder to

select for resistance by indirectly selecting for the DNA marker instead of evaluating the materials directly for resistance traits (Melchinger, 1990). Marker-assisted selection must be adopted to facilitate the breeding of *S. asiatica* resistant sorghums for a number of reasons (Hausmann *et al.*, 2001). Firstly, resistance tests are difficult, complex, expensive or unreliable. Secondly, breeding materials are often advanced in off-season nurseries without *Striga* infestation. Linked markers would facilitate selection for resistance in the absence of the parasite. Thirdly, *Striga* occurrence is frequently erratic since “non-*Striga* years” are often experienced. Fourthly, some *Striga* resistance genes are recessive, thus restricting the effectiveness of classical backcross schemes. When selecting for a recessive resistance gene, selfing has to be done in each backcross generation so that the trait can be expressed prior to selection. This lengthens the period of time within which the trait can be backcrossed into desirable genomic backgrounds. Use of MAS in breeding for witchweed resistance should therefore reduce the time that it takes to develop a resistant cultivar and make it available on the market.

Identification of markers linked to different resistance mechanisms can enhance the development of durable host-resistance. This can be achieved by stacking major genes for different resistance mechanisms into improved sorghum lines by following inheritance of markers that tag them. This assemblage or pyramiding of genes for *Striga* resistance based on different mechanisms would also enhance stability of performance under changing environmental conditions. Preliminary evidence from some experimental sorghum lines that contain multiple mechanisms of *Striga* resistance suggest that there is stronger field resistance expression in those lines than in single mechanism resistance sources (Ejeta *et al.*, 2001).

Identified markers can also potentially be linked to specific physiological strains of *S. asiatica*. It would then be easier for breeders to "pyramid" resistance genes for a broad-spectrum and more durable resistance to witchweeds. As more markers tightly linked to different witchweed resistance mechanisms and/or physiological strains of *S. asiatica* are identified, then the responsible genes could also be introduced, via transformation, into maize where few resistant sources have been identified (Kling, Fajemisin, Badu-Apraku, Diallo, Menkir, and Melake-Berhan, 2000; Menkir, Kling, Badu-Apraku, The, and Ibikunle, 2002). The whole range of resistance mechanisms exhibited by sorghum could then be gradually introduced into maize. This could boost cereal productivity in the Southern African Development Community (SADC) region in the long run. Comparative genome mapping studies have shown that there is extensive commonality of gene content (synteny) between sorghum and maize (Whitkus, Doebley, and Lee, 1992). Sorghum can therefore serve as a model crop for maize since its genome is smaller.

Among the molecular marker techniques, RFLP markers are some of the most robust techniques. However, RFLPs have failed to detect enough polymorphisms in intraspecific crosses of crops with low genetic diversity (Menendez, Hall, and Gepts, 1997). Alternative molecular markers that show high levels of polymorphism among closely related genotypes include microsatellites and RAPDs. Molecular analysis of intraspecific crosses of cowpea revealed that RAPDs produced a higher level of polymorphism compared to RFLPs (Menendez *et al.*, 1997). One-fourth of the RFLP probes were polymorphic versus one-half of the RAPD primers. This may be because RAPD markers are capable of detecting polymorphisms in both single copy and repetitive DNA, whereas RFLP probes

are selected from single copy sequences (Weising, Nybom, Wolff and Meyer, 1995). Analysis of RFLPs is also time consuming and expensive. The nature of this study required large numbers of easily scored genetic markers, which could be generated easily using RAPD and SSR primers. In addition, the segregating sorghum population that was used in this study was derived from an intraspecific cross, resulting in low levels of polymorphisms.

1.1 Objectives of the project:

1.1.1 Overall objectives

The overall objective of the project was to facilitate breeding of *S. asiatica* resistant cultivars through identification of molecular markers, characterization of available parent materials and genetic analysis of witchweed resistance in sorghum.

1.1.2 Specific objectives

- (i) To assess the response of parent sorghum cultivars to *S. asiatica* infestation using the pot culture and agar gel techniques.
- (ii) To investigate the relationships between sorghum yield components and *S. asiatica* parameters through path coefficient analysis.
- (iii) To determine the type and relative magnitude of combining ability influencing the response of sorghum cultivars to *S. asiatica* infestation.
- (iv) To determine the inheritance of low *S. asiatica* seed germination stimulant production, which is one of the recognized mechanisms of *S. asiatica* resistance in sorghum.
- (v) To identify molecular markers that are linked to the genes for low *S. asiatica* seed germination stimulant production using bulked segregant analysis (BSA).

CHAPTER 2

LITERATURE REVIEW

2.1 Sorghum production in the smallholder sector

Sorghum is one of the most drought tolerant small cereal grain crops that is grown by RPFs particularly in Natural Regions (NRs) III, IV and V of Zimbabwe. In Zimbabwe, NRs are demarcated mainly on the basis of total annual rainfall amounts, with NR I being the wettest and NR V, the driest ranging from above 1000 mm per annum to less than 500 mm per annum respectively (Vincent and Thomas, 1961). Sorghum possesses drought resistance mechanisms such as a deep, extensive and fibrous root system, the ability to stop all growth and metabolism in times of severe dryness (dormancy, abiosis), an efficient stomatal apparatus, etc (Doggett, 1988; Ashley, 1993). The demands on the soil are slight, as sorghum is unusually efficient at absorbing mineral nutrients from the soil. It grows in a pH range of 5.0-8.5, withstands salt and alkali soils well, and it also grows on badly drained soils (Rehm and Espig, 1991). While sorghum can be grown on all soil types, higher yields are obtained on sandy loam or heavier textured soils that are deep and well drained.

Traditionally, farmers regarded sorghum as a crop grown to ensure that they could still produce food even under conditions of drought. In a survey, COOPIBO (1995) showed that the importance of small cereal grains, which are sorghum, pearl millet [*Pennisetum*

americanum (L.) Leek] and finger millet [*Eleusine coracana* (L.) Gaertn], increased as one moved from NRs III to V of the Wedza-Buhera-Chiredzi transect in Zimbabwe. The reason for this is because maize, although much preferred by many households, is more adversely affected by reduced moisture than sorghum or millet. Sorghum is therefore one of the major sources of energy in the diets of millions of people in semi-arid regions of Zimbabwe. The bulk of the crop is grown in Matabeleland North and South provinces and they produce about 50 % of the crop. Masvingo province produces about 30 % of the crop. The three Mashonaland provinces account for only about 10 % of the crop (Mushonga *et al.*, 1992)

Large-scale commercial farmers produced about 80 % of the sorghum crop in Zimbabwe prior to 1980 (Mushonga *et al.*, 1992). However, the situation has drastically changed now and SH farmers supply about 90 % of the crop. The area planted to sorghum increased tremendously after 1980 in the SH sector from about 30 000 ha per year to about 200 000 ha per year by 1986 (Mushonga *et al.*, 1992). This area under sorghum production almost remained at the same level up to 1997 (Central Statistics Office, Harare, Zimbabwe, - personal communication). Failure to go beyond this hectarage could be due to problems that are mostly related to crop husbandry and marketing of the sorghum grain. Mechanised cultivation and harvesting characterises sorghum farming in the commercial sector, and much of this goes for brewing and stockfeed (Rowland, 1993). By contrast, smallholder farmers depend on white-grained sorghum for food and they predominantly employ cultural practices when growing the crop (Mushonga *et al.*, 1992).

2.1.1 Constraints to sorghum production in the smallholder farming sector

The sorghum crop is given lower priority in terms of material and labour inputs than maize. Sorghum yields are therefore low in general since the crop is grown with minimal inputs (Mushonga *et al.*, 1992). Crude protein digestibility of sorghum is severely reduced by high percentages of prolamines and tannins, necessitating additional processing of the grain in the home. Fermenting the grain prior to consumption can counteract prolamines and tannins are removed during the process of dehulling (Doggett, 1988). Dehulling of sorghum and processing it into flour is labour intensive. This deters some farmers from growing sorghum as a food crop. Other factors that contribute to low production levels for sorghum might include; limited consumer preference, limited processing of either sorghum grain or flour to high value products and few markets. The latter factors contribute to the low market value of sorghum.

Generally, sorghum is planted after the maize crop, and planting can be done as late as January. Delayed planting decreases grain yield largely due to a decrease in number of grains per head (Mushonga *et al.*, 1992). Poor crop establishment has also been reported. This results from the use of too little seed (usually of poor quality) and planting by broadcasting (Mushonga *et al.*, 1992). Broadcasting seed results in uneven depths of planting and consequently, germination is not uniform. It has been noted that control of plant population and spatial arrangement are vital for producing good and stable yields in sorghum and millets (Rowland, 1993). Weed control is also often done late or not at all. Sorghum is particularly sensitive to weed competition during emergence and establishment because it grows slowly (Mushonga *et al.*, 1992). The benefits of row

planting and ox-weeding, cannot be overstressed since they can allow weeding to be carried out earlier and repeated more often than with hand hoeing (Rowland, 1993).

Reductions in soil fertility levels throughout Africa have also affected sorghum yields, which are generally low (DeVries and Toenniesen, 2001). This is compounded by the fact that most SH farmers in Zimbabwe (Mushonga *et al.*, 1992), and other parts of Africa (DeVries and Toenniesen, 2001), do not apply fertilizer to sorghum. This is one of the major causes of low yields since the soils are inherently low in fertility. The rate of adoption of elite and recommended cultivars is low. Thus, many farmers grow unimproved, tall and late maturing varieties that have low harvest indices. These varieties are adapted to low levels of management and have a very low yield potential. It has been suggested that higher adoption rates can be attained by use of more farmer focused, participatory methods of improvement, which identify constraints and varietal preferences prioritized by farmers (DeVries and Toenniesen, 2001). It is pleasing that a number of improved white-grained cultivars have now been developed in Zimbabwe, for example SV-1, SV-2, SV-3 and Macia. However, there is a need to improve seed production and distribution of sorghum seed in Zimbabwe, as well as in most parts of Africa.

The tasks of bird-scaring and harvesting require a good deal of labour. Farmers prefer maize because of its low demands in both respects, and because its husks give it protection against weather, bird and mould damage (Rowland, 1993). Hence time of harvesting maize is more flexible. Sorghum must be harvested at the right time to avoid losses due to birds, pests and diseases. Quelea (*Quelea quelea*) birds are particularly damaging when the grain is at the soft dough stage and doves move in when the crop is at the hard dough

stage (Mushonga *et al.*, 1992). To reduce bird damage, sorghum should not be grown in isolated fields where bird infestation can be quite high. Although sorghum is well adapted to hot, dry conditions, the crop suffers from a number of pests and diseases that limit its production especially when their effects are compounded with drought, and infestation by parasitic weeds. The three major leaf diseases in Zimbabwe are downy mildew (*Perenosclerospora sorghi*), leaf blight (*Helminthosporium sorghi*) and sooty stripe (*Ramulispora sorghi*). It has been reported that SH farmers do not practice any disease control measure (Mushonga *et al.*, 1992).

2.2 The *Striga* problem

The *Striga* problem is intimately associated with population growth (Berner and Kling, 1995). Traditional African cropping systems included prolonged fallow, rotation and intercropping, which were common practices that kept witchweed infestations at tolerable levels (Berner and Kling, 1995; Doggett, 1984). As population pressure and demand for food increased, land use intensified and hence prolonged fallow and rotations could no longer be practiced. With increased continuous monocropping of cereals and little or no fallow, populations of these parasites gradually increased and became threats to food production. This has been exacerbated by the increased use of high-yielding cultivars in place of landraces, some of which had good levels of resistance or tolerance against witchweeds (Obilana *et al.*, 1991). Presumably, the situation could also have been worsened by the widespread cultivation of maize in *Striga*-endemic agroecological zones that are not suited to it, NRs IV and V. This could have resulted in continued growth of the *S. asiatica* soil seed bank due to continued parasitisation of maize.

Striga species belong to the family Scrophulariaceae whose members are either holoparasitic (without chlorophyll) or hemiparasitic (with chlorophyll). Witchweeds combine life styles of both, with a holoparasitic seedling and a green, chlorophyll-containing emergent plant (Mohamed, Musselman and Riches, 2001). *Striga asiatica* is problematic both as an obligate parasite and as a weed (Obilana and Ramaiah, 1992). It inflicts tremendous damage to the host plants before it emerges from the soil. Being a weed, it emerges late in the season and escapes the early weeding operations. Hence witchweeds have the advantage of staying out of site (and frequently out of mind) while feeding on the host. *Striga* is a Latin name which means “hag” or “witch”. The name was given following the occult behaviour of the parasite that causes serious injury to the host as an underground attachment before it emerges. In this way, the hosts are “bewitched” because the farmer is unaware of the parasite until it comes up (Mohamed *et al.*, 2001b).

Presently uninfested areas are likely to become infested if massive efforts are not undertaken to control the parasite. *Striga* plants are adding seeds to the soil seed bank seasonally. The soil seed bank is extremely important for persistence of *Striga* as a weed. Each *S. asiatica* plant produces 40 000 to 500 000 seeds while that of *S. forbesii* produces about 25 000 seeds (Obilana and Ramaiah, 1992). The seeds are so small that they can be dispersed efficiently in many ways. Man disperses the seeds as they use the witchweed plant for medicinal purposes, as done in some societies. The minute witchweed seeds can also be transported by machinery and through movement of infested seed of the host-crop. Animals disperse the seeds by means of droppings, and water through field erosion (Obilana and Ramaiah, 1992).

In general, low soil fertility, nitrogen deficiency, well-drained soils, and water stress accentuate the severity of *Striga* damage to the host (Stroud, 1993; Mohamed *et al.*, 2001b). Obilana and Ramaiah (1992) noted that the success of *Striga* as a parasite is somehow related to the farming systems in semi-arid areas where its hosts are grown. The environment is dry, semi-arid and harsh, while the farmers lack resources. Weed surveys in communal area farms revealed that *S. asiatica* and other *Striga* species occur in all the eight provinces of Zimbabwe (Chivinge, 1988). In another survey (Mabasa, 1994), 79.13 % of the farmers reported that *Striga* was present in their fields. They recognize the *Striga* problem but they have no simple means to solve it. Mabasa (1993) noted that *Striga* is a significant production constraint in three of the five NRs of Zimbabwe (NRs III, IV and V). Nearly 75 % of the communal areas are located in NRs IV and V (Mabasa, 1993). Efforts to combat the *Striga* problem in Zimbabwe should therefore be intensified.

2.3 Current control methods and their problems

Stroud (1993) describes control options to solve the *Striga* problem including crop rotation, fertilizer or manure application plus hand weeding, trap crops, catch crops, use of herbicides, germination stimulants and biological control agents. These methods have their advantages and disadvantages, and in general are not able to effect total control. Fallowing or crop rotation has to be continued for many years because seeds of *Striga* remain viable for up to 20 years in the soil (Doggett, 1988). In addition, any wild hosts and volunteer plants have to be weeded out for these two methods to be effective.

The application of fertilizer or manure raises soil fertility and promotes crop growth, resulting in suppression of *Striga* (Stroud, 1993). This practice has problems in that the

whole field must be treated and availability of the fertilizer or manure is critical. Resource-poor farmers cannot afford to purchase fertilizer and have too few or no livestock for sufficient manure production. Trap crops stimulate *Striga* seed germination but they are not parasitized by *Striga*. They can cause suicidal germination of witchweed seeds when used since the emerged parasite will not be able to attach to non-host roots (Doggett, 1988). Effectiveness is achieved when these crops are grown several times, which results in the gradual depletion of the *Striga* seed bank. There are problems, however, because there are heavy demands for land to produce preferred crops that are normally cereals. "Catch" crops can be planted to stimulate *Striga* seed germination and they are parasitised but the crop is destroyed before *Striga* sets seed. This poses problems because in semi-arid areas the season length is very short such that it will not be possible to plant another crop after the "catch" crop has been removed. This practice also has to be repeated several times to deplete the witchweed seed bank in the soil (Doggett, 1988), which is also not practical in areas where the demand for land is high.

Herbicides can be used but they are not normally affordable to the RPF. It is also recognized that sorghum is mostly grown as a subsistence food crop by RPFs. The use of herbicides under these conditions cannot therefore be economical for SH farmers, even if they afforded them. Commercial farmers who grow sorghum for the brewing industry can afford to use herbicides, since they get good returns for the crop. However, continued use of chemicals has environmental implications that have to be considered. These include pollution of the soil and water sources, potential danger posed to farmers who apply the herbicide and the harm caused to other non-targeted organisms.

Research by CIMMYT and other collaborators has resulted in the development of a unique approach of controlling *Striga* species in maize. The single recessive gene *XA-17* confers resistance to acetolactate synthase (ALS)-inhibiting herbicides like nicosulfuron and imazapyr in maize (Hausmann *et al.*, 2000b; Kanampiu, Ransom, Friesen and Gressel, 2002). Seed treatment of herbicide-resistant maize with imazapyr has been shown to be an effective and inexpensive way of controlling *Striga*, with immediate benefits to farmers (Kanampiu, Ransom and Gressel, 2000; Kanampiu *et al.*, 2002). Imazapyr is expected to kill *Striga* on attachment to host roots. This technology, coupled with pulling rare *Striga* escapes, can deplete the *Striga* seed bank, reduce infestation of rotation crops, delay the evolution of resistant populations, and can be used as a stop gap measure until genetic crop resistance becomes available. However, CIMMYT is still working on commercializing the technology and ensuring that herbicide-treated maize is made available to farmers (Kanampiu *et al.*, 2002).

The efficacy of controlling *Striga* using maize seed coated with the herbicide imazapyr is also being investigated in Malawi (Kabambe, 2001) and Zimbabwe (Jasi and Mabasa, 2001). Preliminary investigations have shown the potential of this technology in reducing *S. asiatica* populations and also substantially increasing the yield of maize (Kabambe, 2001; Jasi and Mabasa, 2001). It is also possible to transfer the *XA-17* maize gene into sorghum through genetic engineering. However, this is only recommended for geographic regions where the crop does not have feral or weedy relatives, that is, Asia, and not in Africa (Hausmann *et al.*, 2000). The cultivation and use of such a genetically modified (GM) crop is subject to acceptance of the technology. While others embrace the technology, others maintain that there are numerous uncertain risks that are posed to

human health and the environment (Stewart and Wheaton, 2003). Smallholder farmers may also have insufficient resources to purchase improved seed and the herbicide. The use of herbicide tolerant crops is also faced with problems of the evolution of herbicide resistance in witchweeds. However, it has been suggested that a strict regime of rouging by hand pulling before seed-set will be needed to preclude the rapid build-up of resistance in the parasite (Kanampiu *et al.*, 2000).

Striga control methodologies can be grouped into three major categories with different effects on a *Striga* population (Obilana and Ramaiah 1992; Haussmann *et al.*, 2000b). These are: (i) reduction of seed numbers in the soil; (ii) prevention of new seed production; and (iii) prevention of movement of seeds from infested to noninfested areas. An effective control strategy should integrate at least one control principle from each of the three major categories (Obilana and Ramaiah, 1992). *Striga* management practices in the SH sector are not in harmony with these principles. Good crop husbandry practices such as timely planting, weeding or hand-pulling of the parasite, application of inorganic fertilizers and manure, rotations etc, cannot be practiced efficiently because of the inherent environmental and socioeconomic conditions. Persistent droughts worsen the problem.

2.4 Use of resistant cultivars to control *Striga* species

Resistance against *Striga* is defined as the capacity of a plant to support fewer emerged *Striga* plants and to yield more grain than a susceptible plant when grown under witchweed infestation (Ejeta *et al.*, 1991). In contrast, tolerant genotypes are those which germinate and support as many *Striga* plants as do susceptible genotypes without showing a concomitant reduction in yield (Ejeta *et al.*, 1991). Immune genotypes do not allow

parasitic attachment of witchweeds on their roots. Cereals that are immune to *Striga* have not yet been identified. Tolerant cultivars are not desirable because their cultivation results in continued growth of the *Striga* seed bank. *Striga* resistant cultivars can offer an economically feasible and culturally sustainable technology under SH farmer conditions (Ejeta *et al.*, 1991; Mabasa, 1996). This ensues from the recognition that crop resistance is environmentally benign, requires no additional inputs and it is potentially durable. *Striga* can then be controlled by an integrated approach involving resistant cultivars and cultural practices such as use of *Striga*-free planting material, weeding, cereal-legume and cereal-cotton rotation, intercropping and other input-based farming practices. Such an approach should offer an effective and broad scale method of combating *Striga* and also prevent build-up of new strains of *Striga* to levels that can overcome the resistance of a new variety. The beneficial effects of a herbicide and/or supplementary nitrogen on resistant or tolerant sorghum genotypes in *S. hermonthica* infested fields has already been demonstrated (Ejeta *et al.*, 1993).

2.4.1 Sources of *S. asiatica* resistance among cereals

Variation in the reaction of cereals to *Striga* has been observed. Sorghum is the only cereal that exhibits multiple levels of resistance because this crop originated from Africa and it co-evolved with *Striga* parasites for at least an extra 1000 years compared to maize (Obilana and Ramaiah, 1992; Lane, Moore, Child, Bailey and Obilana, 1995). In sorghum, resistance results from one or a combination of the several recognized mechanisms that influence the development of parasitism (Ejeta *et al.*, 1993; Ejeta *et al.*, 2000; Mohamed, Rich, Housley, and Ejeta, 2001a). A host plant showing more than one of these mechanisms is more desirable as it is most likely to offer a broad-based and durable control of *Striga*.

Multiple mechanisms of *Striga* resistance have not been identified in maize and this might be expected for a crop that was not exposed to *Striga* long enough during its evolutionary course since it was domesticated in the Western Hemisphere under *Striga* free conditions (Ramaiah, 1987; Bennetzen *et al.*, 2000). International Institute of Tropical Agriculture (IITA) researchers started selection of maize genotypes that support a reduced number of *Striga* plants in the early 1990s (Menkir, *et al.*, 2002). Considerable progress has been made as excellent sources of resistance were obtained from *Zea diploperenis*, African landraces and elite tropical germplasm. Intensive screening of these sources of germplasm yielded open pollinated varieties, inbred lines and hybrids with high levels of resistance to *S. hermonthica* (Menkir *et al.*, 2002).

Kling, Fajemisin, Badu-Apraku, Diallo, Menkir, and Melake-Berhan (2000) reported that a high level of resistance in a fixed line can be used readily in any breeding programme. Such genotypes can be used as parents in hybrids or synthetics, or to introgress resistance into locally adapted germplasm. It is also interesting to note that maize varieties developed for *S. hermonthica* resistance in West Africa have been found to provide useful levels of resistance in many lowland environments throughout sub-Saharan Africa, under both *S. hermonthica* and *S. asiatica* infestation (Kling *et al.*, 2000). For instance, researchers in Malawi found good levels of resistance against *S. asiatica* in maize genotypes that had been identified by IITA to be resistant to *S. hermonthica* in West Africa (Kabambe, DeVries, Kling, Ngwira, and Nhlane, 2000). The important thing to be noted here is that resistance for a particular variety will be upheld if it is grown within its region of adaptation. The resistance sources that have been identified for pearl millet (Wilson, Hess

and Hanna, 2000) and rice (Johnson, Riches, Jones and Kent, 2000) could also be used variously in breeding programmes in Southern Africa.

2.5 Mechanisms of resistance to *Striga* species

Striga is heavily dependent on the host for its survival. Its life cycle is thus closely coordinated with that of the host plant. *Striga* seeds have very specific requirements for after-ripening, conditioning, and stimulation by chemical compounds exuded by host and non-host plants before they can germinate. Subsequent development events of haustorial formation, attachment and penetration as well as further growth and development of the parasite also require signal or resource commitment from the host plant. Interruption or disruption of one of these signals or resources results in failure of parasitism by the pest. *Striga*-resistance mechanisms have thus been defined on the basis of host-dependent developmental processes and the essential signals exchanged between *Striga* and its hosts (Ejeta, Mohamed, Rich, Melake-Berhan, Housley and Hess, 2000). Part of the complex trait of *Striga* resistance, as measured in the field, has therefore now been broken down into simpler components that are based on a better definition of the specific signals exchanged at each stage of the life cycle. These advances have been made possible by the development of simple, rapid, reliable and reproducible bioassays (Hess, Ejeta and Butler, 1992; Mohamed *et al.*, 2001a) that can screen for specific resistance mechanisms. These laboratory procedures have an added advantage in that they can be used to screen for individual progeny in a segregating population.

Distinct defense responses to *Striga* parasitism have been identified using the agar gel, extended agar gel and the paper roll assays (Hess *et al.*, 1992; Mohamed *et al.*, 2001a).

These responses point to the existence of at least four separate mechanisms of *Striga* resistance in sorghum involving: 1) little or no *Striga* seed germination stimulant (*lgs*) or presence of germination inhibitors; 2) low production of the haustorial initiation factor (*lhf*), 3) a hypersensitive response (*HR*) characterised by a distinct necrotic area on the host root at the attachment site; 4) an incompatible response (*IR*) where parasite development is arrested with no apparent necrosis on the host root, but the attached *Striga* seedlings appear withered or stunted (Mohamed *et al.*, 2001a). The actual host defenses that discourage parasitic growth and establishment on genotypes with an *IR* are not known (Mohamed *et al.*, 2001a). The seedlings are often stunted or withered and turn purple. Incompatible response was shown to be heritable and to segregate independently from *lgs* (Grenier, Rich, Mohamed, Ellicott, Shaner, and Ejeta, 2001). While the first two mechanisms concern host-parasitic interactions during the early infection process, the latter two are associated with attachment and penetration.

Resistance involving the ability of host genotypes to tolerate the *Striga* “toxin” has been reported (Ejeta *et al.*, 2000). The toxin presumably results in pronounced host damage that may be expressed as an array of symptoms including stunting, chlorosis, and wilting. Avoidance and tolerance have also been considered to be additional *Striga*-resistance mechanisms (Ejeta *et al.*, 2000). Inhibition of germ tube exoenzymes by root exudates; phytoalexin synthesis; and antibiosis, that is, reduced *Striga* development through unfavourable phytohormone supply by the host, have also been reported as resistance mechanisms (Hausmann *et al.*, 2000). The root growth pattern of certain sorghum genotypes has been implicated as an avoidance mechanism. A sorghum genotype with a significantly lower root-length-density in the upper 15 to 20 cm of the soil profile was

found to support less *Striga* under field conditions (Ejeta *et al.*, 2000). More focus will be placed on *lgs* and *lhf*. These two mechanisms are incited during the early infection process and can be sequentially screened for by using the agar gel and extended agar gel assays.

2.5.1 Germination stimulant production

Freshly produced, but mature, *Striga* seeds are effectively dormant. They require a period of after-ripening under dry conditions to lower seed moisture content and break their dormancy (Mohamed, Ejeta, Butler and Housley, 1998). *Striga* seeds will only germinate after the after-ripened seeds are conditioned by exposure to warm moist conditions for several days, followed by chemical stimulation (Doggett, 1988). Stimulation of the seeds to germinate initiates the potential host-parasite relationship. It also introduces the first opportunity for host resistance against *Striga*. Roots of many host and non-host plants exude a variety of compounds that are effective as germination stimulants, but they are often present at very low levels so that their isolation and identification has been difficult (Ejeta *et al.*, 1993). However, strigol (from cotton, maize, sorghum and millet roots), sorgoleone and sorgolactone (from sorghum roots) are some of the compounds that have been identified (Ejeta *et al.*, 1993). Sorgolactones are the most common and important in terms of controlling *Striga* germination by sorghum in the field (Siame, Weerasuriya, Wood, Ejeta, and Butler, 1993; Ejeta *et al.*, 2000). Strigol and sorgoleone are also produced by sorghum and stimulate *Striga* seeds to a smaller extent relative to sorgolactone (Siame *et al.*, 1993). A fourth water-soluble compound with a quantitative biosynthetic pathway has also been isolated from sorghum but has not yet been identified (Hess *et al.*, 1992; Vogler, Ejeta and Butler, 1996).

Striga germination is a multifactorial phenomenon modulated by an orderly sequence of events that reflects adaptations to the environment and to the parasitic habit (Babiker, Sauerbon, Bangerth, Wegmann, Geiger, Sugimoto and Inanaga, 2001). The diverse nature of germination stimulants suggests that they may elicit the production of a common molecule, or act as a signaling mechanism that triggers germination. Babiker, Butler, Ejeta, and Woodson (1994) observed that *Striga* seed germination was positively correlated with ethylene production. They observed that *Striga* seed germination and ethylene production also increased with strigol concentration. It is now known that conditioning removes restrictions on metabolism and ethylene biosynthesis (Babiker *et al.*, 1994; Babiker *et al.*, 2001). Germination stimulants therefore promote metabolic activity and ethylene biosynthesis. The enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC oxidase (ACCO) catalyse essential steps in ethylene biosynthesis. The stimulant, in addition to enhancement of metabolism, induces ACC synthase. The CO₂ produced as a result of enhanced metabolism promotes ACCO activity (Babiker *et al.*, 2001).

Sorghum genotypes differ by as much as a billion fold in the amount of stimulant they produce (Ejeta *et al.*, 1993). This variation is responsible in part for the resistance against *Striga* found in some sorghum cultivars (Hess *et al.*, 1992). A host plant that produces low amounts of stimulants will cause fewer *Striga* seeds to germinate, and thus will be subject to less infestation. Conversely, high stimulant producers will cause a greater number of *Striga* seeds to germinate resulting in serious infestation in the absence of other resistance mechanisms (Doggett, 1988). While not all *Striga*-resistant sorghum genotypes are low producers of the stimulant exudates, invariably all susceptible sorghum genotypes appear to be high stimulant producers (Ejeta *et al.*, 2000). Hess *et al.* (1992) reported that low

stimulant production is sufficient to confer field resistance to *Striga*, independent of other mechanisms. However, coefficients of correlation between maximum germination distance (MGD) and *Striga* resistance under field conditions are generally positive but vary among genetic materials and test locations (Hausmann *et al.*, 2000b). Furthermore, it was also illustrated that the few *Striga* parasites that emerge on resistant cultivars can cause massive yield reductions (Gurney, Press and Scholes, 1999). These are some of the reasons why multiple mechanisms of resistance are a necessity in a single cultivar, to cater for the shortfalls of other mechanisms. Thus, in vitro germination distance cannot be used as an indirect selection trait for total field resistance to *Striga*, since the relationship between the two is dependent on genotype and test locations.

Several researchers have done studies on the inheritance of low stimulant production. Ramaiah, Chidley and House (1990) screened F1, F2 and backcross generation progeny from several crosses against *S. asiatica*. A single recessive gene was found to control *lgs* in sorghum genotypes Framida, 555 and SRN4846. Vogler, Ejeta and Butler (1996) investigated the inheritance of low *S. asiatica* seed germination stimulant production for sorghum cultivar SRN39 using the agar gel assay. Segregation ratios of F1, F2 and backcross generation progeny indicated that *lgs* was inherited as a single recessive gene. Segregation of the *lgs* trait in a recombinant inbred population also confirmed that a single gene controls *lgs* in the genotype SRN39 (Greiner *et al.*, 2001). Other genetic studies have indicated that different sets of alleles or genes are responsible for *lgs* production (Hausmann *et al.*, 2000b), and that a major gene and some modifiers govern low stimulant production in some genomic backgrounds (Hausmann *et al.*, 2000a). The fact that there is a very wide variation in the capacity of different genotypes to produce

germination stimulants (Ejeta *et al.*, 1993) makes it important to study the inheritance of this trait in genotypes that one would want to use as resistant sources in breeding programmes. This is even more important for genotypes where the genes of interest have to be tagged with DNA markers. For instance, it would not have been appropriate to begin searching for a marker for a trait whose inheritance pattern was not investigated in the SAR cultivars concerned. The failure to get a marker would have had too many possible explanations.

2.5.2 Haustorial initiation signal production

A germinating *Striga* seed develops a radicle that does not differentiate further until a second host-derived signal is received. If this signal is not received within four days of germination, the *Striga* seedling dies (Butler, 1995). After receiving the chemical signal, the radicle rapidly differentiates into a specialised attachment structure, the haustorium. Attachment, penetration and establishment of vascular contact with the host are accomplished through this haustorium. The stages of haustorial induction are marked initially by the arrest of normal root elongation, followed by redirection of cellular expansion from longitudinal to radial dimensions in the cells just distal to the root tip (O'Malley and Lynn, 2000). This swelling continues, producing a large bulbous root tip. Haustorial hairs emerge around the periphery of the swollen tip and they become extended in due course, marking completion of development of the haustorium. This organ of attachment forms a morphological and physiological bridge between host and parasite (O'Malley and Lynn, 2000).

The development of a haustorium marks the beginning of parasitic expression for *Striga*. Several phenolic compounds have been shown to function as haustorial initiators for *Striga*. A simple quinone, 2,6-dimethoxy-parabenzoquinone (2,6-DMBQ) has been shown to act as a strong haustorial initiating factor. However, this quinone is not found in root exudates (Ejeta *et al.*, 2000). A quantitative assay for the production of host-derived signals for haustorial formation in sorghum roots has not yet been developed. However, the extended agar gel assay is used to qualitatively separate host genotypes on the basis of their ability to induce haustorial formation.

The germination signal and the haustorial initiation signal are independent in that neither has any activity of the other type and they are completely independently inherited (Ejeta *et al.*, 2000). However, the genetics of the haustorial initiation factor has not been reported. Crop cultivars which produce *Striga* germination stimulants abundantly but which fail to produce the haustorial initiation signal would be uniquely useful. Apart from being resistant to *Striga*, they should also deplete the *Striga* seed population in the soil by promoting suicidal germination (Ejeta *et al.*, 1993).

Absence of a haustorial induction compound in root exudates is unlikely to be a resistance mechanism in sorghum (Haussmann *et al.*, 2000a). Horseradish peroxidase efficiently metabolizes syringic acid to the haustorial inducer, 2,6-DMBQ. All sorghum host plants probably produce 2,6-DMBQ since syringic acid is a ubiquitous metabolite of lignin biosynthesis and also because peroxidase reactions are involved in most pathogenic processes (Haussmann *et al.*, 2000a). This might explain why sorghum genotypes differ relatively little in their capacity to produce the haustorial factor, compared to their wide

differences in capacity to produce the germination stimulant (Butler, 1995). This is also one of the reasons why low *Striga* seed germination stimulant production was chosen for investigation in this study.

2.6 Techniques used to screen for individual *Striga* resistance mechanisms

Laboratory techniques are now available to screen for specific resistance mechanisms. This will facilitate identification of valuable genetic variants, establishment of mode of inheritance, characterization of mechanisms of resistance, identification of reliable molecular markers, introgression of resistance into desired cultivars and pyramiding of multiple mechanisms for durable resistance (Mohamed *et al.*, 2001a). Efforts to develop and deploy high yielding cultivars with durable witchweed resistance may therefore be close to fruition.

2.6.1 Agar gel and extended agar gel techniques

The agar gel technique (AGT) provides a simple means for screening host genotypes for low production of *Striga* seed germination stimulants (Hess *et al.*, 1992). In terms of procedure, preconditioned *Striga* seeds are dispersed in water agar in petri dishes. A germinating sorghum seed is added to each dish, with its root pointing across the centre of the petri dish. The maximum distance between sorghum rootlets and germinated *Striga* seeds is then measured after 3 to 5 days of incubation at 28°C in the dark (Hausmann *et al.*, 2000b). Genotypes with a germination distance below 10 mm are classified as low stimulant types (Hess *et al.*, 1992). The extended agar gel technique (EAGT) involves increasing the assay time for the AGT by 24 hours (Reda *et al.*, 1993). This technique promises to be a useful tool for screening sorghum germplasm for *Striga* seed germination

stimulant production, haustorial initiation factor production and hypersensitive reaction. The assay allows repeated observations of the sorghum-*Striga* associations prior to and after infection. Sorghum genotypes whose roots develop necrotic areas upon infection are also quite clear in the water agar by employing the EAGT.

2.6.2 The paper roll technique

The paper roll technique (PRT) is used to evaluate post-infection *Striga* resistance (Mohamed *et al.*, 2001). Genotypes with a *HR* and *IR* mechanisms are identifiable with this assay. Sorghum seedlings are grown with their roots between rolled layers of germination paper. When seedlings are one week old, papers are unrolled and filter-paper strips containing artificially germinated *Striga* seed are placed on sorghum roots. Papers are then rolled and placed in a glass container that allows light to reach growing sorghum shoots. After an interval of 2 to 3 weeks, papers are unrolled to reveal progressive invasion of the parasite on host roots (Mohamed *et al.*, 2001). Although the PRT is relatively simple and several genotypes can be evaluated repeatedly under the same optimized conditions, it is not as rapid as the agar-based assays for screening a large collection of host genotypes. The assay therefore still needs some modification for it to be employable on a large scale such as in high throughput breeding programmes (Ejeta, 2000). In addition, data on the correlation between results from the PRT and *Striga* resistance under field conditions are not yet available.

2.7 Breeding for durable resistance to *Striga* in sorghum

Progress from past efforts in breeding witchweed resistant crops has been limited. Reasons for slow progress vary from complexity of the trait to lack of research support and lack of

appropriate screening techniques and selection strategies (Ejeta *et al.* 2001). However, resistant sorghum cultivars that have been developed so far have shown good levels of resistance mainly based on low stimulant production (Ejeta, *et al.*, 2000). Nevertheless, crop cultivars with durable resistance to *Striga* have not yet been identified (Ejeta *et al.*, 2001). Over the years, resistance has not been long-term or applicable over a wide geographical area. One of the main causes of failure of resistance is that breeding efforts have not been taking good account of both the interspecific variability among *Striga* species, and intraspecific variation for virulence. Thus, witchweed populations have an extraordinary elasticity and capacity to adapt to new host species through the gradual build-up of new “biological forms” (Koyama, 2000b). The reported variability of witchweeds imply that using single resistance genes to manage infestations is inadequate. Stacking of resistance genes may be essential to manage witchweeds effectively.

Various researchers have suggested a diversity of breeding strategies that could lead to the development of long-term and polygenic resistance to witchweeds (Ejeta *et al.*, 1992; Ejeta and Butler, 1993; Haussman *et al.*, 2000a). If sources of resistance have been identified, they can be improved for agronomic performance. Alternatively, the resistance genes in those sources can be transferred to productive, well-adapted types. More durable and stable, polygenic resistance can be obtained by pyramiding resistance genes. Crop genotypes that possess multiple genes for *Striga* resistance, based on distinct mechanisms, are likely to have genetic resistance that is durable across several environmental conditions as well as across ecological variants of the parasite. It has also been emphasized that breeding programmes should target sources of resistance at different areas and understand the nature of resistance required (Koyama, 2000a). Suggested breeding

methods include: early generation selection for individual resistance mechanisms; use of recurrent selection procedures to develop breeding populations with multiple sources of resistance; lines with different resistance mechanisms are combined to form hybrids or synthetics, to increase durability of resistance; and, use of marker-assisted selection techniques for the development of broad-based, quantitative resistance to witchweeds under field conditions (Haussman *et al.*, 2000b).

Progress has been made in breeding for single *Striga* resistance mechanisms. The *lgs* mechanism has been extensively exploited. Diverse sorghum genotypes with little or no stimulant production capacity have been identified. A number of improved sorghum varieties with *Striga* resistance due to *lgs* have been developed (Ejeta *et al.*, 2001). Screening of landraces and improved sorghum lines using different bioassays has shown that host variants with *lhf*, *HR*, and *IR* are rare. Rather, a greater preponderance of genetic variation for these traits has been found among wild and related species of sorghum (Ejeta *et al.*, 2001). Introgression of these genes for *lhf*, *HR*, and *IR* into elite sorghum cultivars is still to be accomplished.

It is generally not possible to select single plants for witchweed resistance in the field, as there are no appropriate techniques. Selection for resistance is therefore usually deferred until true breeding progenies are available (Haussman *et al.*, 2000a). This means that large numbers of progeny have to be advanced before *Striga* resistance is assessed, a time- and cost-intensive procedure. On the other hand, screening for individual resistance mechanisms in the laboratory could result in a loss of valuable materials possessing resistance mechanisms other than those evaluated. One strategy could be to use laboratory

assays for individual resistance mechanisms as an initial screening of a large number of breeding materials, followed by field screening. This would offer the possibility to identify resistance sources with multiple resistance mechanisms (Hausman *et al.*, 2000a). All prospective resistant cultivars should be evaluated at various locations with different *Striga* populations or host-specific races (Ramaiah, 1997; Koyama, 2000a).

2.8 Genetic Markers

A genetic marker is a variant allele that is used to label a biological process or structure during the course of an experiment (Suzuki, Griffiths, Miller and Lewontin, 1989). Geneticists and breeders use two types of markers to map gene sequences and for marker-assisted selection (MAS). These are morphological and molecular markers. Morphological markers consist of observable phenotypic traits such as plant colour. Molecular markers, however, consist of protein and DNA-based markers. The two most important factors determining the utility of a marker technique are informativeness and ease of genotyping. Informativeness is measured by expected heterozygosity, which is the probability that two alleles taken at random from a population are different. The number of loci that can be simultaneously analysed per experiment (multiplex ratio) provides a good measure of the ease of genotyping (Weising *et al.*, 1995). These two factors differ for different molecular marker technologies.

2.8.1 Morphological markers

Morphological markers are the oldest and most widely used genetic markers. Morphological marker traits include rare and recessive genes such as those for dwarfism, albinism and

altered leaf morphology. The loci often cause discrete and visible changes in morphological characteristics. Their prime advantages are that they provide a simple, rapid and inexpensive way of evaluating cultivars. For instance, in maize, the number of kernel rows is highly polymorphic, relatively highly heritable, and has served as a morphological marker for systematics and evolutionary purposes (Sanchez, Goodman and Rawlings, 1993).

While morphological markers are very effective for many purposes, they do have many setbacks. They are time-consuming and in most cases, insufficient agro-morphological traits are available for use in germplasm characterization. Because the number of markers segregating in one cross is generally limited, a combination of segregation data from multiple crosses will be required to construct one complete linkage map. Scoring of these markers depends on gene expression that may be sensitive to environmental influence, management practices, genetic background, developmental stage and tissue type (Van den Berg, Chaslow and Waugh, 1994). Comparisons of data from different experiments may also have limitations including subjectivity in the analysis of the character. This limits the usefulness of this marker type.

It is a fact that conventional plant breeding, which relies on morphological markers to aid selection, has made very significant contributions to crop improvement, since the majority of cultivars that are currently being grown are its products. These methods, however, have generally been slow in targeting complex and economically important traits like grain yield, grain quality, drought tolerance and field resistance to *Striga* species. This is because polygenic characters are difficult to analyse using morphological markers for the reasons cited above. The situation is compounded by the fact that the environment affects

expression of quantitative traits. Molecular markers are consequently being used to speed-up and increase the efficiency of selection for those traits that are difficult to assay using morphological markers.

2.8.2 Molecular markers

Molecular markers simply detect differences in genetic information carried by two or more individuals. They are either DNA or non-DNA based. Non-DNA molecular markers are mainly isozyme and/or allozyme markers. There are several DNA-based markers such as RFLPs, RAPDs and SSRs. The greater utility of molecular markers compared with morphological markers is due to greater expected heterozygosity and a higher multiplex ratio (Weising *et al.*, 1995). These factors result from several properties that give molecular markers advantages over morphological markers. Molecular markers are insensitive to the influence of the environment, developmental stage, genetic background, alleles at most loci are codominant, do not show epistatic interactions because they are not gene products, genotypes can be determined at whole plant, tissue and cellular levels and there is theoretically no limit to the number of markers that can be monitored in a single population (Tanksley, 1983). Furthermore, allele frequency tends to be much higher at molecular loci compared with morphological markers. Molecular mutants tend to be benign, whereas morphological mutants tend to be associated with undesirable phenotypic effects.

2.8.2.1 Protein markers

Proteins are direct gene products, having been derived from DNA. Protein markers are mostly generated by the electrophoretic separation of proteins, followed by staining of a

distinct protein subclass. The majority of protein markers are represented by allozymes and isozymes (Weising *et al.*, 1995). A few protein marker studies use seed proteins. For instance, more than 20 bands can be found on starch gel electrophoresis of wheat gluten, and barley albumin can be separated into seven fractions (Goodwin and Mercer, 1972). An advantage associated with the analysis of seed storage proteins is that they are predictive of end-use quality (Koeber, Powell and Donini, 2001). Such variation can be useful in characterising different cultivars or for linkage studies.

Allozymes are different forms of an enzyme that share the same catalytic activity but are coded by different alleles on the same locus. Different forms of an enzyme that share the same catalytic activity but are coded for by more than one gene locus are called isozymes (Weising *et al.*, 1995). The term isozymes, however, is used to refer to both classes of enzymes. Mutations in DNA will result in slightly different enzymes (proteins) that may show different mobility when separated by gel electrophoresis. It has been reported that protein-coding genes are fairly polymorphic, that is, they exist in the form of one or more alleles. This property has been found to exist in virtually all animal and plant species that have been studied (Pasteur, Pasteur, Bonhomme, Catalan and Britton-Davidian, 1988). In wheat, the isoenzyme endopeptidase was found to be a tag for eyespot (*Pseudocercospora herpotrichoides*) resistance (Koeber *et al.*, 2001). Allozymes were also successfully used to assess the genetic structure of sorghum landraces from North-western Morocco (Dje, Forcioli, Ater, Lefebve and Vekemans, 1999).

The advantages of protein markers are that they are codominant, reproducible within and between laboratories, relatively inexpensive and they are also simple to generate. They are

codominant in that heterozygotes are distinguishable by possession of both alleles from homozygotes that would have been crossed to constitute them. The limitations of protein markers are that the number of loci resolved and the number of alleles per locus is small, and the number of individuals needed for the complete analysis of a trait is large. Isozymes have limited abundance, there being only about 10 to 20 polymorphic isozyme loci in most plant breeding populations (Melchinger, 1990). Protein-based markers are also influenced by the physiological stage of the plant and by environmental factors, thus limiting their repeatability. For instance, isozymes may be active at different life stages or in different cell compartments (Weising *et al.*, 1995). Their resolution is hence not as good as DNA markers.

2.8.2.2 DNA-based markers

A DNA marker is a DNA sequence variation (polymorphism) that can differentiate between genotypes. First generation DNA markers are RFLPs and RAPDs (Koeber, Powell and Donini, 2001). Other markers have been developed as variations and/or combinations of these basic techniques, using one of the following methods; restriction enzyme digestion, polymerase chain reaction (PCR) and hybridisation with labelled probes (Karp *et al.*, 1997). Such techniques include SCARs (sequence characterised amplified regions) and CAPS (cleaved amplified polymorphic sequence). Second generation DNA-based molecular markers were developed as from the latter half of the 1990s and they were shown to be capable of exploiting variation occurring in the repetitive DNA fraction. These marker systems are (1) amplified fragment length polymorphisms (AFLPs); (2) SSRs or microsatellites and (3) retrotransposon-based markers (Koeber *et al.*, 2001). Third generation marker assays have their primary focus on single nucleotide polymorphisms

(SNPs). These seek to dispense with gel electrophoresis as a core technology, since this is among the most rate limiting of the steps involved in detecting specific DNA fragments (Koebner *et al.*, 2001). Single nucleotide polymorphisms exploit the DNA sequence information that is becoming available for different crop species and seeks to identify a defined position on a chromosome at which the DNA sequence of two individuals differs by a single base.

The PCR is an incredibly powerful molecular biology tool enabling large quantities of DNA to be amplified from very small starting quantities. It is a procedure of enzymatic amplification of specific segments of DNA using specific primers (Saiki, Gelfand, Stoffel, Scharf, Higuchi, Horn, Mullis and Erlich 1988). PCR-based DNA markers have a number of advantages over markers that are based on restriction enzymes and hybridization with labeled probes. Procedures based on PCR only require small amounts of DNA (5 to 100 ng), relative to the larger quantities required for RFLPs (5 to 10 µg) (Weising *et al.*, 1995). PCR-based methods also involve fewer steps, making them faster and technically simple.

2.8.2.2.1 Restriction fragment length polymorphisms (RFLP)

The first DNA profiling technique to be widely applied in the study of plant variation was the RFLP assay (Morrell, 1995). Restriction fragment length polymorphism refers to different lengths of fragments resulting from restriction endonuclease digestion of DNA as detected by a labeled probe. Genomic DNA is initially digested with restriction enzymes. Each different restriction enzyme recognizes a specific and characteristic nucleotide sequence on the DNA molecule, called a restriction site (Weising *et al.*, 1995). A single nucleotide alteration can create or destroy a restriction site and thus, point mutations can

cause variation in the number of sites and, therefore, fragment lengths. In addition, insertions or deletions between two restriction sites can cause changes in the lengths of the fragments. Thus there is variation between individuals in the positions of cutting sites and the lengths of DNA between them, resulting in RFLP (Toure, Hausmann, Jones, Thomas, and Ougham, 2000).

To make the polymorphism visible, restricted DNA fragments are separated according to their size by agarose gel electrophoresis. The double stranded DNA is first denatured prior to transferring it from the gel to a nylon or nitrocellulose filter membrane by Southern transfer (Southern, 1975). A probe will match the whole or part of one of the fragments on the membrane. A probe is a short DNA fragment (typically ~800 bp in length). It could be a cloned expressed sequence, an unknown fragment of genomic DNA, or part of the sequence of a cloned gene. If it is labeled with a radioactive or chemical tag, the probe will hybridize and detect any fragment with which it shares sequence complementarity (Karp and Edwards 1997). Bands will appear where the probe has hybridized to different fragments. Analysis of these alleles (bands) in segregating populations will determine their linkage to characters of economic importance and they can also be used for linkage map construction (Staub *et al.*, 1996).

Specific enzyme/probe combinations will give highly reproducible results for a given individual, even when the assay is carried out in different laboratories. Restriction fragment length polymorphisms are codominant markers in that the different allelic variant bands are visible in the heterozygotes, enabling all the three genotypic classes (two homozygotes and a heterozygote), in the case of a single gene pair, to be distinguished.

This is very useful when dealing with segregating populations. Though low levels of polymorphisms have been reported, RFLP remains a powerful technology in the genetic analysis of maize where it shows extensive levels of polymorphism, even in comparisons between well-adapted genotypes (Koeber, *et al.*, 2001). However, Southern blot hybridisation is laborious, time consuming, and involves high costs per assay (Karp *et al.*, 1997). This may make it unsuitable for plant breeding projects with high sample throughput requirements. Radioactive isotopes such as ^{32}P , which is often used to label probes, require expertise to handle in the laboratory since the material is extremely dangerous. Thus RFLP has been rapidly superseded by second generation techniques based on the PCR.

2.8.2.2.2 Genetic Mapping with RAPD markers

Random amplified polymorphic DNA markers are generated by PCR amplification of random genomic DNA segments with single primers of arbitrary sequence (Williams, Kubelik, Livak, Rafalski and Tingey, 1990). The primers are usually 10 nucleotides long. Several discrete DNA products are amplified from regions of the genome that contain two short segments with some homology to the primer. These target sequences must be present on opposite DNA strands and sufficiently close (200 to 2000 base pairs) to allow DNA amplification to occur. The amplified fragments are then separated by gel electrophoresis and visualised under ultraviolet (UV) light after staining with ethidium bromide. Polymorphisms between individuals result from sequence differences in one or both primer binding sites, and are visible as the presence or absence of a band (Rafalski and Tingey, 1993). The RAPD procedure is popular because of its simplicity and ease of use in a modestly equipped laboratory. Because RAPD data can be gathered within short periods

of time, this technique is suitable for large sample throughput systems required for plant breeding programmes, population genetics and studies of biodiversity (Waugh and Powell, 1992).

The Mendelian inheritance of RAPD fragments makes them valuable tools for linkage studies (Echt, Erdahl and McCoy, 1991). It is generally believed that the majority of RAPD bands represent unique loci with two alleles, where the presence of a specific band represents the dominant allele (A) and the absence of the same band represents the recessive allele (a) (Hallden, Hansen, Nilsson and Hjerdin, 1996). RAPD markers are therefore dominant in general since the homozygous dominant (AA) and heterozygote (Aa) genotypes are indistinguishable. Such dominant RAPD phenotypes give less information than codominant markers in linkage analysis of F₂ and other segregating populations (Williams *et al.*, 1992).

Bands of differing intensities can also be scored to reveal more RAPD phenotypes leading to the identification of codominant RAPD fragments (Echt *et al.*, 1991). However, scoring of RAPD bands on the basis of their intensity might be misleading in some cases. It has been reported that the presence of a band of identical molecular weight in RAPD gels does not prove beyond doubt that the individuals have the same band (Karp and Edwards, 1997; Weising *et al.*, 1997). The situation is further complicated if single RAPD bands consist of several co-migrating RAPD bands of different identities (Karp *et al.*, 1997). Inclusion of parental samples in all agarose gels when conducting genetic studies with segregating populations will therefore provide a quick reference point.

Unless the most consistent of reaction conditions are strictly adhered to, RAPD profiles may not be reproducible within laboratories, and between laboratories when different PCR machines or sources of polymerase and associated buffers are used (Karp *et al.*, 1997). Hallden *et al* (1996) defined reproducibility as the phenomenon that different band patterns are produced when the same genotype is repeatedly assayed under the same conditions. Different thermal cyclers may have different temperature versus time ramping rates. It has been reported that differences in ramp rate from the annealing step to the extension step can significantly affect RAPD results (Hoffman and Bregitzer, 1996). Different PCR tubes will have different diameters and this may influence the results because of different surface-to-volume ratios leading to different heating and cooling rates of the reaction tube contents (Hoffman and Bregitzer, 1996). Kelly (1995) reported that the source of variation may be imprecise matches between the short oligonucleotide primers and template DNA at the low annealing temperatures (35-40⁰C) typical for these reactions. To address this problem, it was suggested to convert informative RAPD fragments into SCARs. The longer primers (~24 bp) and elevated annealing temperatures (50- 65⁰C) that SCARs use make this technique highly reproducible (Kelly, 1995).

Other studies seem to indicate that RAPDs can indeed be reproducible. Weeden, Timmerman, Hemmat, Kneen, and Lodhi (1992) investigated the reproducibility of RAPDs using different concentrations of reaction components. The most critical reaction component in these studies was found to be the quality of DNA, which has to be of high purity and quality for reproducibility. However, DNA concentration could be varied from 3-30 ng per 25 µl reaction without seriously affecting the RAPD pattern. Primer and magnesium chloride concentrations could also be varied two- to five-fold without

affecting the output of the amplification reaction. Reports by Morrell *et al* (1995) indicated that reproducible RAPDs could be produced by careful selection of primers and optimization of PCR conditions for the target species. It should only be those intensely staining bands that should then be scored so as to ensure that reproducible bands can be obtained.

Reproducible RAPD polymorphisms were also found among a set of closely related six-rowed malting barley cultivars (Hoffman and Bregitzer, 1996). Results of primers that generated differences among closely related malting cultivars were reproduced in two laboratories. It is interesting to note that the second laboratory used a different make of thermal cycler with different temperature versus time ramping rates and tube diameters. Reproducibility of these results was attributed to use of the Stoffel fragment and elevated polymerase and primer concentrations in the PCR reaction mixtures. The Stoffel fragment is a modified form of recombinant *Taq* DNA polymerase (AmpliTaq) in which the 289 N-terminal amino acids have been deleted so that it lacks 5' to 3' exonuclease activity (Hoffman and Bregitzer, 1996). The Stoffel fragment is two times more thermostable than AmpliTaQ and it has optimal activity over a broader range of magnesium ion concentrations. The Stoffel fragment also discriminates against misextension and has increased stringency at lower ionic strengths (Hoffman and Bregitzer, 1996).

2.8.2.2.3 Genetic mapping with Microsatellite repeat polymorphisms

The genomes of higher organisms are interspersed with highly variable regions comprised of repeats of short simple sequences. This repetitive DNA may comprise up to 90 % of total DNA of certain plant genomes (Weising *et al.*, 1995). It has been estimated that on

average a repeat longer than 20 base pairs (bp) in length occurs every 33 kilobases (kb) in plant nuclear genomes (Powell, Machray and Provan, 1996). These repeats are called microsatellites, SSRs, short tandem repeats (STRs), variable numbers of tandem repeats (VNTRs) or simple sequence length polymorphisms (SSLPs). They have basic repeat units of around two to eight base pairs in length. Di-, tri-, and tetranucleotide repeats are the most common, for example (TG)_n, (AAT)_n and (GATA)_n, respectively (Peakall, Gilmore, Keys, Morgante and Rafalski, 1998).

Microsatellite polymorphisms appear because of variation in the number of tandem repeats in a given repeat motif. These differences are thought to be caused by errors in DNA replication, where the DNA polymerase "slips" when copying the repeat region, changing the number of repeats (Jarne and Lagoda, 1996). These markers are hypervariable, that is, they often produce multilocus patterns. The hypervariability of SSR loci is a result of unusually high mutation rates for these nucleotide sequences (Peakall *et al.*, 1998). Microsatellites are thus powerful tools for genome mapping, population genetic studies, determination of hybridity, cultivar identification, forensic identification, paternity analysis and for use as diagnostic markers for important traits in plant breeding (Karp and Edwards, 1997; Jarne and Lagoda, 1996; Peakall *et al.*, 1998; Powell *et al.*, 1996).

Microsatellite primers have been assessed for cross-species amplification by many researchers. Studies have indicated that SSR primers may amplify the same SSR region in closely related taxa, though the number of loci tested so far is low (Peakall *et al.*, 1998). For example, White and Powell (1997) surveyed the Meliaceae, using primers designed

for *Swietenia humulis*. They were able to amplify DNA from seven of the 11 microsatellite loci in other *Swietenia* species, six loci in other genera of the same tribe, and four to six loci in species of the same family. Steinkellner, Lexer, Turetschek and Glossl (1997) also described the conservation of microsatellite loci between *Quercus* species (Fagaceae). Peakall *et al* (1998) demonstrated that Soybean (*Glycine max*) primers amplified SSRs within the subgenus *Glycine* with a success rate of 65 %, though a lower rate of amplification (3-13 %) was observed outside the genus. A discouraging result was obtained for sorghum. Screening of 67 maize and ten seashore paspalum primer pairs on sorghum lines indicated that only 2-3 % were suitable for use as primers (Brown *et al.*, 1996). It appears that more loci still need to be investigated in this crop. Successful cross-species amplification of SSRs would mean that development of suitable SSR primers may not be necessary in closely related taxa (Peakall *et al.*, 1998).

The greatest advantage of microsatellite analysis is the large number of polymorphisms that the method reveals. One locus in soybean is reported to have 26 alleles (Cregan, Bhagwat, Akkaya and Rongwen, 1994). In sorghum, one highly informative trinucleotide repeat locus (*Sb6-325*) was found to have 16 possible alleles in the size interval of 106-151 bp (Dje *et al.*, 1999). Since such a large number of alleles can be identified per single locus, microsatellites are co-dominant markers, allowing heterozygotes to be readily identified. Microsatellites are expensive to generate in the first place. Nevertheless, their subsequent use has cost advantages, as the quality of DNA to be used does not have to be high, and the possibility of multiplexing several assays in a single experiment is possible through the use of automated sequencers (Koebner *et al.*, 2001). Once the sequences of

primers specific for a given microsatellite marker are published, this technology then becomes available to the whole scientific community through databases without any need to distribute clones or other materials (Rafalski and Tingey, 1993). Because of their many advantages, SSRs are the marker techniques that are currently generating real interest among breeders. The major focus of cereal genetics is then to replace the RFLP map with a SSR-based genetic map (Koebner *et al.*, 2001).

2.9 Marker-assisted selection in plant breeding

Conventional plant breeding has depended upon phenotypic differences between individuals to aid selection. This works well when the phenotype (morphological marker) truly represents the genetic effect. This is not always the case because of environmental effects and epistatic interactions that mask the true genetic effects. Molecular marker techniques are providing new opportunities for breeders to select desirable individuals on the basis of their genotype, or combinations of selection for both genotype and phenotype. This should bring about an improvement in breeding precision and efficiency, consequently expediting the development of elite varieties with higher yield potential.

For the variation (polymorphism) that genetic markers detect to be useful, it has to be associated with a character of economic importance such as disease resistance. The concept of molecular marker-assisted selection (MAS) dictates that selection for one or more desirable genes is practiced by selection for a marker, or two flanking markers, tightly linked to the genes of interest (Melchinger, 1990). Kelly (1995) reported that selection based on the marker would be more efficient provided that there was tight linkage (< 5 cM) between the marker and trait of interest, and assuming selection for the marker was more convenient

(faster, cheaper, reproducible, expressed earlier). Such a short recombination distance will increase selection efficiency since chances of a recombination that would separate a marker and trait of interest will be minimal.

Breeders use MAS particularly when an important trait that is difficult to assess is tightly linked to a trait that is easily measured. Both morphological and molecular markers are used for this purpose. For instance, a gene for resistance to brown plant-hopper (BPH) is closely linked to a gene specifying purple coleoptile color in some traditional rice varieties grown in Northeast India (Zheng, Huang, Bennett and Khush, 1995). When a resistant plant with purple coleoptile is crossed with a susceptible plant with a green coleoptile, 95 % of F₂ plants showing purple coleoptile are also resistant to BPH. In this case, coleoptile color is a morphological marker that is used to assist the selection for BPH resistance (Zheng, *et al.*, 1995). Unfortunately, the BPH resistance linkage relationship is specific for particular rice varieties from Northeast India. There are numerous examples in literature where linkages were detected between molecular markers and traits (both qualitative and quantitative) of economic importance in crop plants (Michelmore *et al.*, 1991; Barua Chalmers, Hackett, Thomas, Powell and Waugh, 1993; Bennetzen, Gong, Xu, Newton, and de Oliveira, 2000; Grattapaglia, Bertolucci, Penchel and Sederoff, 1996; Haussmann, Hess, Omany, Reddy, Seetharama, Mukuru, Kayentao, Welz and Geiger, 2001).

2.9.1 Molecular markers for quick identification of *S. asiatica* resistant varieties

Marker assisted selection is appropriate for studies in *S. asiatica* because of the complexity of the host-parasite-environment relationship prevalent in pot or field-testing for resistance. Interaction of the above factors determines establishment of successful

parasitism. This is worsened by the occurrence of physiological strains of *S. asiatica*. In some parental backgrounds, the genetics of witchweed resistance has been found to be complex both with regard to number of genes and environmental influence (Ejeta *et al.*, 1991). Resistance tests are thus difficult, expensive and sometimes unreliable. The effectiveness of backcross breeding schemes is restricted in cases where resistance genes are recessive in nature (Hausmann *et al.*, 2001). In this study, RAPDs and microsatellites were utilized to study and identify segregating alleles linked to *S. asiatica* resistance in sorghum.

Striga asiatica resistance genes have recently been mapped independently by a number of researchers in rice and sorghum (Bennetzen *et al.*, 2000) and in sorghum (Ejeta, 2000; Hausmann *et al.*, 2000b) using RAPD and SSR markers. Genes, for quantitative resistance to *S. hermonthica* have also been identified and mapped in sorghum (Hausmann *et al.*, 2001). Using area under *Striga* number progress curve (ASNPC) as the index of resistance for two recombinant inbred populations, five to 11 QTL were identified to condition resistance to *S. hermonthica* for the two populations. Numbers and positions of QTL for ASNPC varied with environment, indicating that partly different chromosomal regions affect the reaction to witchweeds in different environments. The results apparently point out to the need to identify QTL for different environments and mapping population samples before applying MAS in a breeding programme.

CHAPTER 3

RESISTANCE TO WITCHWEED [*STRIGA ASIATICA* (L.) KUNTZE] IN SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH]: PARENT CHARACTERIZATION, COMBINING ABILITY AND PATH COEFFICIENT ANALYSES

3.1 Introduction

Sorghum production environments are characterised by several biotic and abiotic stresses. One of the major problems of sorghum production in the smallholder production sector in Zimbabwe is attack by the parasitic weed, witchweed [*Striga asiatica* (L.) Kuntze]. *Striga asiatica* thrives best under conditions of low soil fertility in semi-arid areas (Stroud, 1993). These are the areas where sorghum and other small cereal grains predominate as major crops because of their drought tolerance. In cases of severe witchweed-infestation, total crop failures can occur and this leads to land abandonment if there are no alternative non-host crops. Obilana and Ramaiah (1992) reported that 30 to 40 % of the total farmlands devoted to sorghum or maize cultivation have been abandoned, in some Western and Southern African countries, due to *Striga* species infestation.

It is important to search for sources of resistance and to characterize identified crop germplasm when breeding for any trait of interest. When the response of available germplasm to witchweeds is known, decisions can then be made on whether to improve those sources of resistance for agronomic performance or to transfer their resistance genes into productive, well adapted genotypes, or to follow both approaches (Hausmann *et al.*,

2000a). Screening for witchweed resistance in pots has the advantage of providing control over environmental conditions. The major disadvantage, however, is having a largely artificial root environment (Haussmann *et al.*, 2000a). As a result, the root volume will not be properly represented because of the absence of interplant competition. In practice, there may be a doubling of root volume as a result of interplant competition (de Milliano, - personal communication).

The diallel crossing system is a genetic model that allows a penetrating analysis into components of genetic variance. Diallel crosses involve crossing a set of parents in all possible combinations or in a specifically defined combination (Griffing, 1956). In a complete diallel, all possible combinations between parents and their reciprocals are made. With p parents, the total number of families or populations would be p^2 . If neither parents nor reciprocals are included in the analysis of progeny from crosses among p parents, the result is a half diallel and the number of families produced is determined by the following formula; $p(p-1)/2$ (Griffing, 1956). The concept of combining ability is useful to study and compare the performance of lines in hybrid combinations. The main interest is on estimating the genetic value of parents from the performance of its offspring. Diallel analysis is therefore regarded as a special type of progeny testing (Hallauer and Miranda, 1981). General combining ability (GCA) is used to designate the average performance of a line in hybrid combination. Specific combining ability (SCA) is used to designate the cases in which certain hybrid combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved. The two types of combining abilities are a reflection of different types of interactions between alleles at the

loci. While GCA is a reflection of a parent's additive genetic value, SCA gives a reflection of dominant or epistatic interactions between alleles (Hallauer and Miranda, 1981).

Studies of associations between pairs of different characters and yield are of interest to plant breeders because they indicate the correlated responses that may occur when single-trait-selection or index selection is practised (Eckebil, Ross, Gardner and Maranville 1977). However, a study of correlations *per se* does not reveal the direct and indirect contributions of individual traits to yield. Path coefficient analysis (Wright, 1921) has been found to be useful in establishing direct and indirect causes of associations between variables. It leads to a detailed examination of the specific forces acting to produce a given correlation and enables quantification of the relative importance of each causal factor. A path coefficient is simply a standardised partial regression coefficient (Wright, 1921). A careful formulation of selection indices for both witchweed resistance and yielding ability should enhance development of *S. asiatica* resistant cultivars in breeding programmes.

3.1.1 Study objectives

- (i) To assess the response of five sorghum cultivars to *S. asiatica* infestation by screening them using the pot culture technique.
- (ii) To investigate the relationships between sorghum yield factors and *S. asiatica* components through path coefficient analysis.
- (iii) To identify the predominant type of gene action at the loci for *S. asiatica* resistance through combining ability analysis.

- (iv) To consider the implications of genetic effect estimates of each parent for future breeding efforts aimed at incorporating *S. asiatica* resistance in sorghum.

3.1.2 Hypotheses tested

- (i) There are no differences in the response of sorghum cultivars to witchweed infestation.
- (ii) There are no relationships, which can be detected by path coefficient analysis, between sorghum grain yield components and *S. asiatica* parameters.
- (iii) Both GCA and SCA components of genetic variance are important in determining the performance of a population under witchweed infestation.
- (iv) The parent genotypes that were used in this study do not transmit a detectable genetic value that could determine the resistance of their offspring to witchweed infestation.

3.2 Materials and methods

Pot experiments were conducted at Henderson Research Station (HRS) (17°30'S and 31° E; 1500 metres above sea level), 25 km from Harare. The sorghum cultivars that were used in this study consisted of SAR 16, SAR 19, SAR 29, SV-1 and DC 75. The three *Striga asiatica* resistant (SAR) cultivars (SAR 16, SAR 19 and SAR 29) were sourced from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). They are open pollinated varieties (OPVs) that were shown to have resistance to white-flowered *S. asiatica* in India (Obilana *et al.*, 1991). A significant observation was made on SAR 19. It exhibited multispecies resistance, being resistant to red-flowered *S. asiatica* and *S.*

forbesii in Botswana and Zimbabwe and tolerant to *S. hermonthica* in Tanzania (Obilana *et al.*, 1991). Such resistance is very useful for the control of a highly specialised pest such as *Striga*.

Cultivar DC 75 is a red-seeded hybrid cultivar that is used in the brewing industry. This cultivar has been previously classified as tolerant to *S. asietica* (Mabasa, 1996) and it was used as a control on account of its ability to induce large numbers of parasites to germinate. Cultivar SV-1 is a drought tolerant OPV that is recommended for production in marginal rainfall areas of Zimbabwe. It has also previously been classified to be susceptible to *S. asiatica* (Mabasa, 1996). A four-parent half diallel cross (Griffing, 1956) between the SAR cultivars and SV-1 was initiated during the 1995/96 rainy season. A half diallel mating design was chosen because there have not been any reports of maternal effects in the inheritance of *Striga* resistance. The F1 generation seeds were selfed during the 1996/97 rainy season to give six F2 populations which were used in this study. The following crosses were made; Cross 1 (SAR 16 X SAR 19), Cross 2 (SAR 16 X SAR 29), Cross 3 (SAR 16 X SV-1), Cross 4 (SAR 19 X SAR 29), Cross 5 (SAR 19 X SV-1) and Cross 6 (SAR 29 X SV-1).

Plastic pots measuring 22.2 cm in diameter and 22.3 cm deep were utilised for pot experimentation. The experiment was established on 18 October during the 1998/99 rainy season. Parental stocks were evaluated in two separate experiments, for resistance under *S. asiatica* infestation, and for yield without infestation. Second filial generation progeny were only evaluated under *S. asiatica* infestation. About 0.02 g of *S. asiatica* seeds and 2 g of compound D fertilizer (7% N; 14% P₂O₅; 7% K₂O) were thoroughly mixed with the top

5 cm of soil prior to planting as described by Mabasa (1996). Five sorghum seeds were subsequently planted per pot and thinning was done 14 days after crop emergence to leave one plant per pot. The experiments were laid out in randomised complete block designs with four replications for parent genotypes and each F2 population. A replication for each F2 population consisted of a sample of five pots.

Variables that were recorded from each pot for sorghum were; grain yield (g/pot), 100 seed weight (g/pot), head weight (g/pot), head length (cm), head width (cm), leaf length (cm), leaf width (cm), stover dry weight (g/pot) and days to 50 % flowering. Witchweed components were days to first *S. asiatica* emergence (DSE) and *S. asiatica* counts per pot. Where *S. asiatica* did not emerge, days to physiological maturity of sorghum, estimated to be 160 days for this experiment, was recorded as DSE. Days to physiological maturity were considered to be the latest stage at which *S. asiatica* could have inflicted detectable damage on the host crop if it had emerged.

Witchweed counts at maximum emergence for each parent and F2 population were used for statistical analyses (Vasudeva Rao, 1987; Mabasa, 1996). Homogeneity of variance was tested for all the six populations to determine if the distributions of DSE and *S. asiatica* counts per plant were uniform as described by Gomez and Gomez (1984). Crosses 2, 3, and 5 had similar distributions for both parameters and they were thus combined and frequency distributions were plotted for 60 progeny for each parameter. The observed variation for witchweed counts among the F2 progeny was partitioned into GCA and SCA components following Griffing's Model I, Method 4 (Griffing, 1956). Method 4 was used since parent genotypes and reciprocal F2 progeny were not included in the analyses. The

genetic model for combining ability analysis using method 4 is shown in Appendix 1. Model 1 assumes that the parent genotypes that were tested in this experiment constitute the experimental material about which inferences can be made. Table 1 shows an outline of the analysis of variance for combining ability estimates. The calculation of variances and estimation of GCA and SCA effects is shown in Appendix 1.

Table 1: Outline of the analysis of variance table for combining ability effects using Method 4, Model 1 (Griffing, 1956)

Source	D.F. [‡]	Sum of Squares*	Expected mean Square
GCA	p-1	S_g	$\sigma^2 + (p-2)/(p-1) \sum g_i^2$
SCA	p(p-3)/2	S_s	$\sigma^2 + 2/p(p-3) \sum S_{ij}^2$
Error	m	S_e	Me'

*Where: $S_g = SS_{GCA} = \sum X_{i..}^2 / (p-2) - 4X_{..}^2 / p(p-2)$
 $S_s = SS_{SCA} = \sum X_{ij}^2 - \sum X_{i..}^2 / (p-2) + 2X_{..}^2 / (p-1)(p-2)$

[‡] D.F = degrees of freedom

p = number of cultivars evaluated

m = error degrees of freedom

Me' = error mean square for testing GCA and SCA

$Me' = Me/r$, Where;

Me = error mean square from anova table for variable concerned

r = number of replications from which mean of variable was calculated

Sorghum parameters that had moderate to high correlations with grain yield and *S. asiatica* counts were used in path coefficient analysis of sorghum grain yield and its components. Head length and head width were not included because they are some of the components that explain head weight. Days to *S. asiatica* emergence were left out of the analyses because they were explained by *S. asiatica* counts. Basically, when witchweeds emerged late, then there were fewer *S. asiatica* parasites at the end of the growing season.

On the other hand, early parasite emergence meant that there was a very large number of parasites by the end of the season. Statistical analyses were based on individual plot data for all the variables.

The importance of each causal factor was determined by path analysis of phenotypic correlations involving data for the two resistant and susceptible cultivars under infested and uninfested conditions. Principal and minors matrix determinants and path coefficients were computed following procedures outlined by Wright (1921). While susceptible and resistant uninfested cultivars had four variable models each for sorghum grain yield, susceptible and resistant cultivars had six and five variable models, respectively, under *S. asiatica* infestation. The path coefficients were then used to solve the simultaneous equations associated with each model as suggested by Dewey and Lu (1959). The nature of the causal systems is depicted in Figs. 3-6. In the path diagrams, the double-arrowed lines indicate mutual associations as measured by the phenotypic correlation coefficients, (r_{ij}), and the single-arrowed lines represent direct influence as measured by path coefficients, P_{ij} . The direct effect of one variable upon another is the influence it has when other variables are held constant. The indirect effect, $r_{ij}P_{ij}$, is the effect one variable has on a trait of interest through its influence on another variable that is also associated with the trait of interest. The “X” variable consists of all residual factors that influenced sorghum grain yield and is independent of the remaining variables (Mduruma, Nchimbi-Msolla, Reuben, and Misangu 1998).

The following simultaneous equations were solved to obtain path coefficients for *S. asiatica* susceptible and resistant cultivars under infested and uninfested conditions. These

equations express the basic relationship between correlations and path coefficients (Dewey and Lu, 1959).

Uninfested, resistant and susceptible cultivars

1. $r_{14} = P_{14} + r_{12}P_{24} + r_{13}P_{34}$
2. $r_{24} = r_{12}P_{14} + P_{24} + r_{23}P_{34}$
3. $r_{34} = r_{13}P_{14} + r_{23}P_{24} + P_{34}$
4. $1 = P^2X_4 + P^2_{14} + P^2_{24} + P^2_{34} + 2P_{14}r_{12}P_{24} + 2P_{14}r_{13}P_{34} + 2P_{24}r_{23}P_{34}$

Infested, resistant cultivars

1. $r_{15} = P_{15} + r_{12}P_{25} + r_{13}P_{35} + r_{14}P_{45}$
2. $r_{25} = r_{12}P_{15} + P_{25} + r_{23}P_{35} + r_{24}P_{45}$
3. $r_{35} = r_{13}P_{15} + r_{23}P_{25} + P_{35} + r_{34}P_{45}$
4. $r_{45} = r_{14}P_{15} + r_{24}P_{25} + r_{34}P_{35} + P_{45}$
5. $1 = P^2X_5 + P^2_{15} + P^2_{25} + P^2_{35} + P^2_{45} + 2P_{15}r_{12}P_{25} + 2P_{15}r_{13}P_{35} + 2P_{15}r_{14}P_{45} + 2P_{25}r_{23}P_{35} + 2P_{25}r_{24}P_{45} + 2P_{35}r_{34}P_{45}$

Infested, susceptible cultivars

1. $r_{16} = P_{16} + r_{12}P_{26} + r_{13}P_{36} + r_{14}P_{46} + r_{15}P_{56}$
2. $r_{26} = r_{12}P_{16} + P_{26} + r_{23}P_{36} + r_{24}P_{46} + r_{25}P_{56}$
3. $r_{36} = r_{13}P_{16} + r_{23}P_{26} + P_{36} + r_{34}P_{46} + r_{35}P_{56}$
4. $r_{46} = r_{14}P_{16} + r_{24}P_{26} + r_{34}P_{36} + P_{46} + r_{45}P_{56}$
5. $r_{56} = r_{15}P_{16} + r_{25}P_{26} + r_{35}P_{36} + r_{45}P_{46} + P_{56}$
6. $1 = P^2X_6 + P^2_{16} + P^2_{26} + P^2_{36} + P^2_{46} + P^2_{56} + 2P_{16}r_{12}P_{26} + 2P_{16}r_{13}P_{36} + 2P_{16}r_{14}P_{46} + 2P_{16}r_{15}P_{56} + 2P_{26}r_{23}P_{36} + 2P_{26}r_{24}P_{46} + 2P_{26}r_{25}P_{56} + 2P_{36}r_{34}P_{46} + 2P_{36}r_{35}P_{56} + 2P_{46}r_{45}P_{56}$

3.3 Results

3.3.1 Response of sorghum cultivars to *S. asiatica* infestation

3.3.1.1 *Striga asiatica* emergence

Table 2 shows mean DSE and *S. asiatica* counts of five cultivars, while Table 3 shows means of the same variables for six F2 populations, all evaluated at HRS during the 1998/99 season. Days to emergence for *S. asiatica* in the parent stocks ranged from 62 to 146 from sorghum planting. Cultivars SAR 19 and SAR 29 significantly ($P < 0.05$) delayed the emergence of witchweeds by at least 74 days, compared to DC 75, SAR 16 and SV-1 whose emergence was after 66, 62, and 69 days, respectively, from sorghum planting (Table 2).

Table 2: Mean *Striga asiatica* counts pot^{-1} and days to *S. asiatica* emergence of five cultivars at Henderson Research Station, 1998/99 season

	Days to <i>Striga</i> emergence	<i>S. asiatica</i> counts		Days to max. [‡] <i>S. asiatica</i> count
		Non-transformed	Transformed ^a	
DC 75	66.00b [‡]	28.75 a	1.473	112
SAR 19	146.00a	1.00 c	0.175	126
SV-1	69.25b	10.25 b	0.993	119
SAR 16	62.00b	9.25 b	0.936	119
SAR 29	140.00a	0.75 c	0.151	101
P	0.0005		0.000	
LSD (0.05)	39.379		0.383	
CV %	26.45		33.37	

^a Data subjected to $\log_{10}(X+1)$ transformation

[‡] Means followed by the same letter do not differ significantly ($P \leq 0.05$) from each other.

[‡] Days to maximum *S. asiatica* count

Witchweeds began to emerge between 60 to 115 days for the six F2 populations and there were no significant differences ($P < 0.05$) for DSE between these populations (Table 3). The segregants from crosses 2, 3 and 5 (refer to 3.2, above) had *S. asiatica* emergence

occurring between 34-160 days from sorghum planting, after combining data from the three populations (Fig 1). From the frequency distributions that were plotted, class one (0-33 DSE) had a frequency of zero. The mean ($\pm$ SE) number of DSE for the other classes that had F2 populations with emerged *S. asiatica* parasites were: 34-66 (62.1 ± 0.7); 67-99 (73.1 ± 1.5); 100-132 (103.5); 133-165 (160). There were only two plants having between 100 and 132 days and all the progeny in class five did not have *S. asiatica* emergence such that DSE coincided with days to physiological maturity. The mean number of sorghum plants per class was 12 ± 5.3 .

Table 3: Mean *S. asiatica* counts plot⁻¹ and days to *Striga* emergence for F2 progeny at Henderson Research Station, 1998/99 season

Crosses		DSE	<i>Striga</i> counts	
Combination	No. [¶]		Non-transformed	Transformed [¥]
SAR16 X SAR19	(1)	91.90	4.55 c	0.66
SAR16 X SAR29	(2)	68.85	16.25 ab	1.21
SAR16 X SV-1	(3)	66.65	23.90 a	1.38
SAR19 X SAR29	(4)	114.70	1.10 d	0.29
SAR19 X SV-1	(5)	91.60	4.80 c	0.68
SAR29 X SV-1	(6)	60.50	8.70 bc	0.93
P		0.056		0.00
LSD (0.05)		37.64 ns		0.33
CV %		34.64		28.74

[¶] Numbers in parentheses show the numbers that represent different cross combinations.

[¥] Data subjected to $\log_{10}(X+1)$ transformation

ns - non-significant

3.3.1.2 *Striga asiatica* counts

Cultivar SAR 29 reached maximum *S. asiatica* count after 101 days, SAR 19 after 126 days, DC 75 after 112 days and both SV-1 and SAR 16 after 119 days from planting (Table 2). Cultivars SAR 19 and SAR 29 supported significantly ($P < 0.01$) lower numbers

of *S. asiatica* plants than cultivars DC 75, SV-1 and SAR 16. On the other hand, SV-1 and SAR 16 had significantly ($P < 0.01$) lower *Striga* counts than DC 75, which had the highest mean count of 28.75 (Table 2). The frequency distribution of *S. asiatica* counts for combined F2 progeny of crosses 2, 3 and 5 is shown in Fig 2. Most of the segregants from the combined population of crosses 2, 3 and 5 were resistant and this resulted in highly significant ($P < 0.01$) skewing (3.09) and kurtosis (10.36) values.

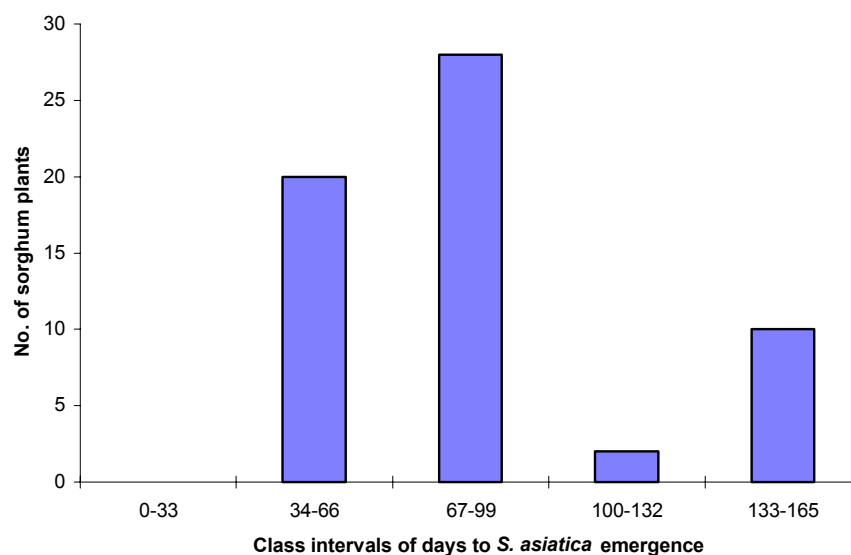


Figure 1: Frequency distributions of days to *S. asiatica* emergence for combined F2 progeny of Crosses 2(SAR 16 X SAR 29), 3 (SAR 16 X SV-1) and 5 (SAR 19 X SV-1).

The class interval was seven *S. asiatica* plants. Class one had zero *S. asiatica* plants and the last class, class 17, had 119 parasites. Standard errors of means were only calculated for classes with more than three progenies. The mean ($\pm$ SE) witchweed counts for the nine classes with *S. asiatica* plants were: 0-7 (2.6 ± 0.4); 8-14 (10.9 ± 0.7); 15-21 (17.1 ± 0.6); 22-24 (24); 29-35 (32.5); 36-42 (39); 43-49 (47.5); 64-70 (68.5); 113-119 (116). The mean number of sorghum plants per class was 3.5 ± 1.8 . Progeny derived from Cross 4 supported

a significantly ($P < 0.05$) lower number of *S. asiatica* plants compared to the other populations (Table 3). Progeny derived from Cross 3 had significantly higher *S. asiatica* counts than those from Crosses 1, 2, 5 and 6, which tended to have intermediate counts. It is notable that intermediate values were obtained from progeny of crosses involving either SAR 16 or SV-1 on one hand, and either SAR 19 or SAR 29 with SV-1 on the other.

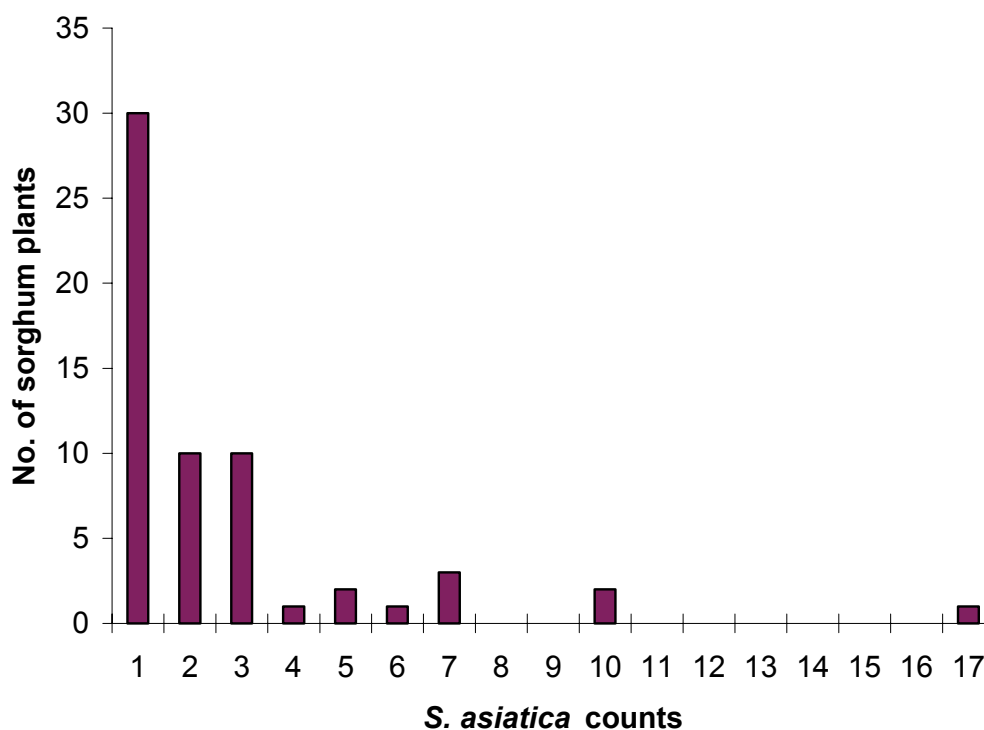


Figure 2: Frequency distribution of *S. asiatica* counts for combined F2 progeny of Crosses 2, 3 and 5.

3.3.1.3 Combining ability analysis for *S. asiatica* counts

Since there were no significant differences in DSE (Table 3) and sorghum grain yield among the F2 populations (Appendix 3, Table 3.1), combining ability analysis was only conducted for *S. asiatica* counts. The mean squares for GCA components were highly

significant ($P < 0.01$) (Table 4). Thus additive genetic factors were operative predominantly for witchweed counts.

Table 4: ANOVA table for *S. asiatica* counts at maximum emergence for F2 progeny according to Griffing's, Model 1, Method 4

Source	D.F.	SS	MS
Crosses	5	3.98	0.796**
GCA	3	0.77	0.257**
SCA	2	0.02	0.012ns
GCA/SCA			38.5
CV(%)			28.74

*,**Significant ($P < 0.05$) and ($P < 0.01$) respectively: ns - non-significant

The GCA:SCA ratio was very large, 38.5 (Table 4), further revealing the greater influence of additive genes than non additive effects. The relative contribution that individual parents made to their progeny in determining their response to witchweed infestation was evaluated by comparing GCA effects (Table 5).

Table 5: Combining ability effects for *S. asiatica* counts at maximum emergence for four parental genotypes

Parents	SAR16	SAR 19	SV-1	SAR 29	GCA Effects
SAR 16		-0.063	-0.025	0.086	0.334**
SAR 19			0.372	-0.025	-0.473**
SV-1				-0.063	0.211*
SAR 29					-0.074ns
GCA var.	0.107	0.220	0.040	0.001	
SCA var.	0.00	0.065	0.065	0.00	

SE($g_i - g_j$)=0.011; LSD(0.01)=0.257; LSD(0.05)=0.188

SE($s_{ij} - s_{kl}$)=0.011; LSD(0.01)=0.445; LSD(0.05)=0.326

***Significant ($P < 0.05$) and ($P < 0.01$), respectively (Significantly different from zero)

ns - non-significant

Cultivars SAR 16 and SV-1 had positive and significant GCA effects, while those for SAR 19 and SAR 29 were negative. While the negative GCA effects of SAR 29 on *S. asiatica* counts were not significantly different from zero, those for SAR 19 were highly significant. Progenies derived from crosses between SAR 19 and cultivars SV-1 and SAR 16 consequently had lower *S. asiatica* counts than those between SAR 29 and the same cultivars (Table 3). Overall, cultivars SAR 19 and SAR 29 reduced the number of parasite counts in their F2 progeny. Conversely, the significant and positive GCA effects of cultivars SAR 16 and SV-1 caused their F2 progeny to have an increased number of *S. asiatica* parasites.

3.3.4 Sorghum grain yield.

Under noninfested conditions, cultivar DC 75 had significantly ($P < 0.05$) higher grain yield than the other cultivars (Table 6). Cultivar SAR 29 yielded significantly lower ($P < 0.05$) grain than the other four cultivars. It was followed by SAR 19, which itself had a significantly lower yield than SV-1 and SAR 16.

Table 6: Sorghum mean grain yields (g/pot) for infested and noninfested cultivars at Henderson Research Station, 1998/99 rainy season

	Noninfested (g ⁻¹)		Infested (g ⁻¹)	
DC 75	17.50 a	(4.20)	9.75	(2.93)
SAR 19	7.25 c	(2.77)	3.50	(1.98)
SV-1	12.75 ab	(3.63)	2.33	(1.57)
SAR 16	8.50 bc	(2.97)	2.86	(1.75)
SAR 29	3.35 d	(1.88)	2.36	(1.68)
P		0.00		0.08
LSD (0.05)		0.67		1.04 ns
CV %		15.88		33.93

Numbers in parenthesis are square root ($x+0.5$) transformed data
ns - non-significant

There were no significant yield differences between all the cultivars under *Striga*-infested conditions (Table 6). Infestation with *S. asiatica* induced significant ($P<0.05$) yield losses for cultivars DC 75, SV-1, SAR 16 and SAR 19 (Table 7). Cultivar SAR 29 was the only genotype that did not have a significant ($P<0.01$) yield reduction between infested and noninfested pots. Cultivar SV-1 had the greatest yield reduction of 81.7%, while SAR 29 had the lowest yield reduction of 29.4% between infested and noninfested conditions (Table 7).

Table 7: Sorghum mean grain yields (g/pot) for infested and noninfested cultivars at Henderson Research Station, 1998/99 rainy season

	Original data				Transformed ^a		
	^φ Noninf.	^f Inf.	^γ #nce	[‡] %Loss	Noninf.	Inf.	#nce
DC 75	17.50	9.75	7.50	42.9	4.20	2.93	1.27**
SAR 19	7.25	3.50	3.75	52.0	2.77	1.98	0.79*
SV-1	2.75	2.33	0.42	81.7	3.63	1.57	2.06**
SAR 16	8.50	2.86	5.65	66.4	2.97	1.75	1.22**
SAR 29	3.35	2.36	0.98	29.4	1.88	1.68	0.23ns

^aData subjected to square root transformation

Transformed: CV (%)23.21, LSD (0.01):0.824, LSD (0.05):0.608

^φNoninfested ; ^fInfested; [‡] Percentage grain yield loss between infested and uninfested;

^γDifference between infested and uninfested

***Significant ($P<0.05$) and ($P<0.01$), respectively

Overall, cultivars SV-1 and SAR 16 incurred high and significant yield losses of 81.7 % and 66.4 % respectively. In addition, these cultivars promoted an early emergence of *S. asiatica* and supported intermediate numbers of the parasitic weed. These observations suggested that both cultivars were susceptible to *S. asiatica* (Table 8). Cultivar DC 75 was also susceptible. This is because this cultivar caused drastic increases in the number of parasites, there was very early emergence of witchweeds at 66 days after crop emergence, and also because of the moderate yield losses of 43.5 % (Table 8). Cultivars SAR 19

and SAR 29 supported the lowest *S. asiatica* counts and delayed emergence of *S. asiatica* by at least 10 weeks. They were therefore considered to be resistant (Table 8).

Table 8: Summary of the response of five sorghum cultivars to *S. asiatica* screening using the pot culture and agar gel techniques

Cultivar	Yield Loss (%)	Days to <i>Striga</i> Emergence	<i>Striga</i> counts	Classification
SAR 19	52*	146 (late)	1.0 (low)	Resistant
SAR 29	29ns	140 (late)	0.8 (low)	Resistant
SAR 16	66*	62 (early)	9.3 (int. [‡])	Susceptible
SV-1	82**	69 (early)	10.3 (int.)	Susceptible
DC 75	43**	66 (early)	28.8 (high)	Susceptible

*,** -Significant (P<0.05) and (P<0.01) respectively

ns - non-significant

[‡] int. - intermediate *S. asiatica* counts

3.3.2 Path coefficient analysis

3.3.2.1 Uninfested, susceptible cultivars

Head weight and 100 seed weight were significantly (P<0.01) and positively correlated, $r=0.941$ and $r=0.951$, respectively, with sorghum grain yield (Table 9 and Appendix 4, Matrix 3). Head weight had a strong and positive indirect effect (0.651) upon grain yield through weight of 100 seeds. On the other hand, a moderate positive influence (0.502) was registered indirectly by 100 seed weight upon grain yield through head weight. Head weight and 100 seed weight had low but negative indirect effects on sorghum grain yield through days to 50 % flowering (-0.236) and (-0.256), respectively, mainly because days to 50 % flowering had a negative direct influence (-0.331) on yield (Table 9 and Fig. 3).

The result is that head weight had a moderate (0.536) and 100 seed weight a large (0.695) direct positive effect on sorghum grain yield.

Table 9: Path coefficients of phenotypic correlations of selected variables to sorghum grain yield for two *S. asiatica* susceptible cultivars without infestation

Variable ¹	Indirect effect via			Total correlation
	Head weight	Days to 50% sorghum flowering	100 seed weight (g)	
Head weight	(0.536)	-0.236	0.651	0.951**
Days to 50% sorghum flowering	0.382	(-0.331)	0.537	0.588ns
100 seed weight	0.502	-0.256	(0.695)	0.941**

1. Residual effect: 0.175

2. Figures in parenthesis (diagonal) are direct effects; other figures are indirect effects.

* - Significant at 5%; ** - Significant at 1%; ns - non-significant

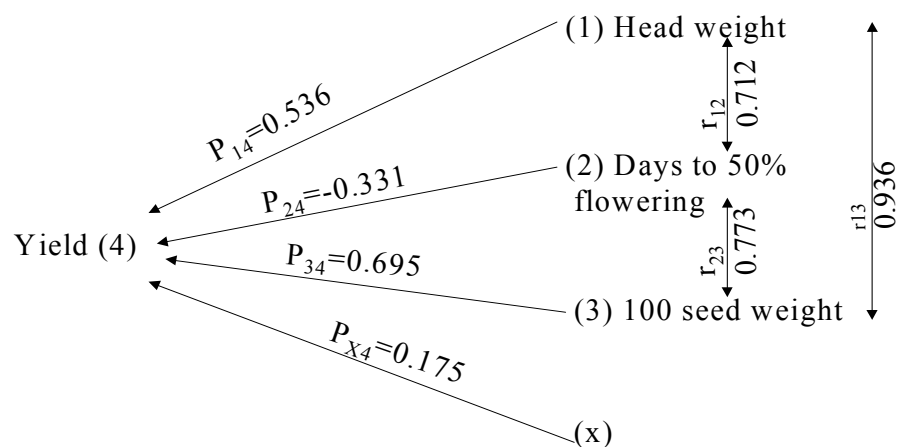


Figure 3: A path diagram and coefficients of factors affecting sorghum grain yield for two *S. asiatica* susceptible cultivars without infestation

3.3.2.2 Uninfested, resistant cultivars

Head weight had a large and positive direct effect (0.945) on sorghum grain yield (Table 10 and Fig.4). This was consistent with the highly significant ($P < 0.01$) correlation ($r = 0.974$) between head weight and sorghum grain yield.

Table 10: Path coefficients of phenotypic correlations of selected variables to sorghum grain yield for two *S. asiatica* resistant cultivars without *S. asiatica* infestation

Variable ¹	Indirect effect via			
	Head weight	Plant height	100 Seed weight (g)	Total correlation
Head weight	(0.945)	0.051	-0.023	0.974 ^{**}
Plant height	0.733	(0.066)	-0.022	0.777 [*]
100				
Seed weight	0.537	0.037	(-0.038)	0.536ns

1. Residual effect: 0.222

2. Figures in parenthesis (diagonal) are direct effects; other figures are indirect effects.

* - Significant at 5%; ** - Significant at 1%; ns - non-significant

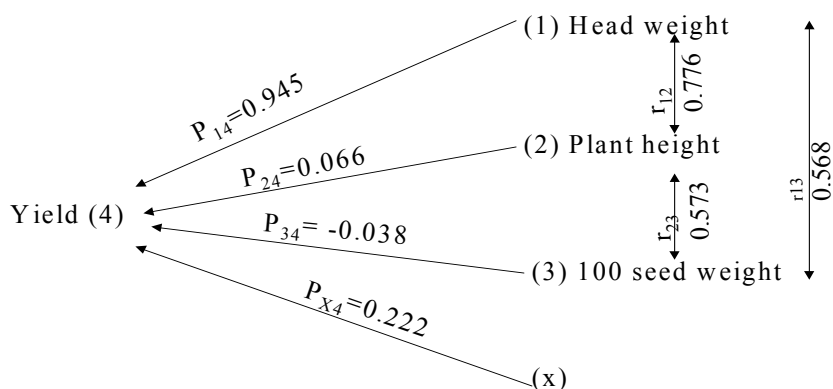


Figure 4: A path diagram and coefficients of factors affecting sorghum grain yield for two *S. asiatica* resistant cultivars without *S. asiatica* infestation

The high ($r=0.777$) and moderate ($r=0.536$) correlations between plant height and 100 seed weight, respectively, with yield (Table 10 and Appendix 4, Matrix 2) can be explained by their positive indirect effects on yield through head weight. Head weight and plant height had negative indirect effects on yield through 100 seed weight because the latter character had a negative direct influence on yield. This negative influence may be due to the small seed sizes that were characteristic of SAR 19 and SAR 29. The grain sizes of these cultivars can potentially be improved by crossing them with large seeded types that have a higher yield potential. There is scope for increase in grain size because this trait was found to be highly heritable and its gene action is almost entirely additive (Doggett, 1988). It is interesting to note that contrary to the resistant cultivars, 100 seed weight of uninfested susceptible cultivars (SV-1 and SAR 16) had a large and direct positive effect on sorghum grain yield. This shows that genetic variability for this trait is readily available.

3.3.2.3 Infested, susceptible cultivars.

There was a positive and significant ($P<0.01$) correlation ($r=0.951$) between head weight and sorghum grain yield (Table 11 and Appendix 4, Matrix 3). This was predominantly due to its large and positive direct effect (1.337) on grain yield (Fig.5). This association can also be explained through the large and positive indirect effects of days to 50 % flowering (0.952), leaf width (0.863) and 100 seed weight (1.252) on sorghum grain yield through head weight. Weight of 100 seeds registered a very low and negative direct influence (-0.066) on grain yield, contrary to uninfested conditions. Seed sizes for SV-1 and SAR 16 may have been reduced due to witchweed infestation. *Striga asiatica* counts were highly and significantly ($P<0.05$) negatively correlated to sorghum grain yield ($r=-$

0.750), 100 seed weight ($r=-0.730$), days to 50 % flowering ($r=-0.709$), leaf width ($r=-0.619$) and head weight ($r=-0.806$).

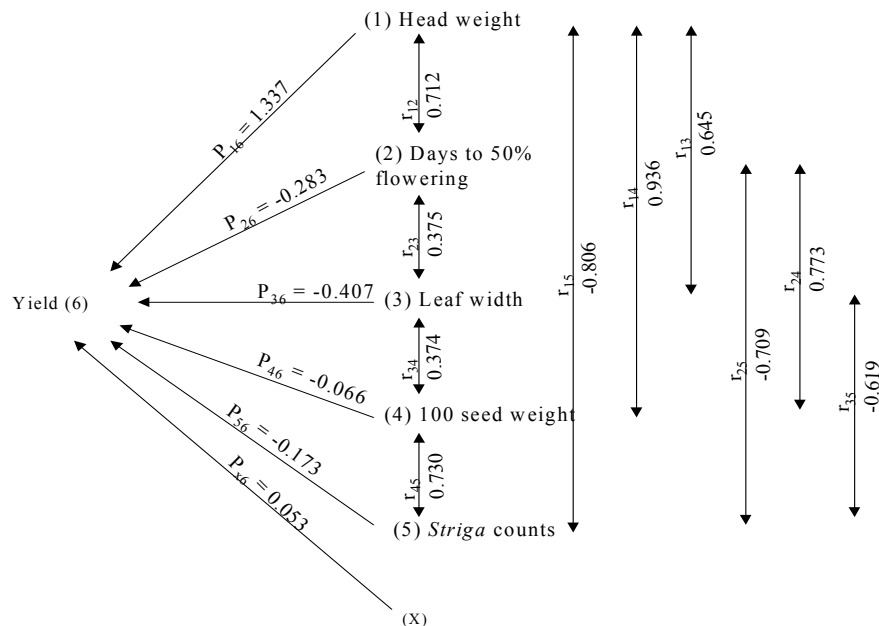


Figure 5: A path diagram and coefficients of factors affecting sorghum grain yield for two *S. asiatica* susceptible cultivars under infested conditions.

However, the direct effect of *S. asiatica* counts on grain yield was low (-0.173). Its major impact was through a large and negative indirect effect through head weight (-1.078). Head weight was therefore the most important yield determinant both directly and indirectly through other variables.

3.3.2.4 Infested, resistant cultivars

Head weight had a large and positive direct effect (1.050) on sorghum grain yield (Table 12 and Fig. 6). This positive direct effect predominantly explains the highly significant ($P<0.01$) association ($r=0.974$) between head weight and grain yield. The positive indirect

effects of plant height (0.815) and 100 seed weight (0.596) on grain yield through head weight explained the positive correlations of these variables with grain yield. Direct and indirect effects of *S. asiatica* counts on grain yield were very low and positive, except for the negative indirect effects through weight of 100 seeds.

Table 11: Path coefficients of phenotypic correlations of selected variables to sorghum grain yield for two *S. asiatica* susceptible cultivars under *S. asiatica* infestation

Indirect effect via						
Variable ¹	Head weight (g)	Days to 50% sorghum flowering	Leaf width (cm)	100 Seed weight (g)	<i>Striga</i> count (pot ⁻¹)	Total correlation
Head weight (g)	(1.337)	-0.202	-0.262	-0.062	0.139	0.951 ^{**}
Days to 50% sorghum flowering	0.952	(-0.283)	-0.153	-0.051	0.122	0.588ns
Leaf width (cm)	0.863	-0.106	(-0.407)	-0.025	0.107	0.432ns
100 Seed weight (g)	1.252	-0.219	-0.152	(-0.066)	0.126	0.941 ^{**}
<i>Striga</i> counts pot ⁻¹	-1.078	0.201	0.252	0.048	(-0.173)	-0.750 [*]

1. Residual effect: 0.053

2. Figures in parenthesis (diagonal) are direct effects; other figures are indirect effects.

* - Significant at 5%; ** - Significant at 1%; ns - non-significant

Table 12: Path coefficients of phenotypic correlations of selected variables to sorghum grain yield for two *S. asiatica* resistant cultivars under *S. asiatica* infestation

Indirect effect via					
Variable ¹	Head weight (g)	Plant height (cm)	100 seed weight (g)	<i>Striga</i> counts pot ⁻¹	Total correlation
Head weight (g)	(1.050)	0.011	-0.096	0.009	0.974**
Plant height (cm)	0.815	(0.015)	-0.097	0.044	0.777*
100 Seed weight (g)	0.596	0.009	(-0.170)	0.101	0.536ns
<i>Striga</i> count pot ⁻¹	0.064	0.005	-0.120	(0.143)	0.092ns

1. Residual effect: 0.208

2. Figures in parenthesis (diagonal) are direct effects; other figures are indirect effects.

* - Significant at 5%; ** - Significant at 1%; ns- non-significant

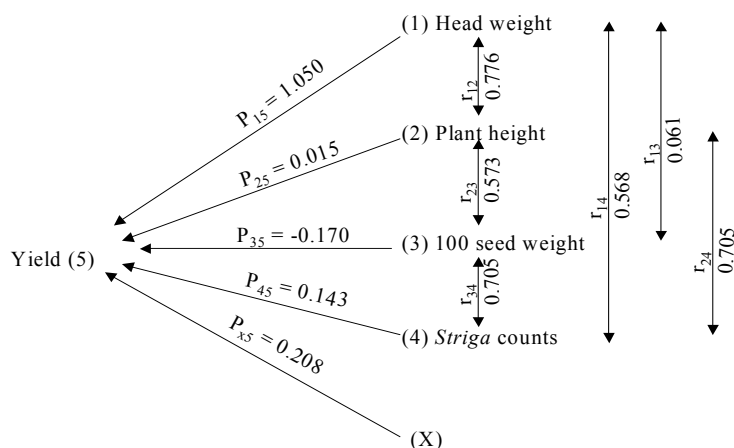


Figure 6: A path diagram and coefficients of factors affecting sorghum grain yield for two *S. asiatica* resistant cultivars under infested conditions.

3.4 Discussion

3.4.1 Response of sorghum cultivars to *S. asiatica* infestation in pots

Cultivars SV-1 and SAR 16 were susceptible because *S. asiatica* emerged early in their pots, they had intermediate *Striga* counts and suffered high and significant yield losses due to infestation (Table 2). These findings are contrary to earlier reports in which SAR 16 has been reported to be resistant to *S. asiatica* (Obilana and Ramaiah, 1992; Mabasa, 1996). The observed response of SAR 16 in this experiment may be due to the fact that the cultivar was exposed to different physiological strains of *S. asiatica* compared to those it was exposed to in previous investigations. It has been reported that *S. asiatica* is a highly variable parasite (Ejeta *et al.*, 1992; Aigbokhan *et al.*, 2000) and *S. asiatica* collections from different parts of Zimbabwe showed considerable genetic variability and virulence (Musimwa *et al.*, 2001). Ramaiah (1987) suggested that breeding materials should be evaluated against different *S. asiatica* morphotypes, host specific races in various locations and/or under differing environmental conditions in order to obtain stable, polygenic resistance. However, no deliberate attempts were made to screen sorghum cultivars using *S. asiatica* collections from different areas of Zimbabwe and/or from different cereal host crops in this investigation. Efforts should be made to identify and name specific physiological strains, biotypes or morphotypes of *S. asiatica*. This could be achieved by developing host plant differential sorghum lines (Hess, 2000). A regional approach where sorghum cultivars for use as differentials are drawn from the Eastern and Southern African sub-region would be recommended. Promising witchweed resistant sorghum cultivars would then have to be tested against the major pathotypes through screening in pots. The

next step would be multilocal evaluation of the same genotypes under witchweed ‘hot spots’, prior to their release and distribution as new cultivars.

Cultivar DC 75 was also susceptible to witchweed infestation. This cultivar caused a very early emergence of witchweeds at 66 days after planting, giving the parasites ample time to adversely affect the cultivar before maturity. Cultivar DC 75 also supported significantly large numbers of witchweeds, in addition to the large and highly significant losses in yield when grown under witchweed infestation (Table 6). However, this cultivar has previously been classified as tolerant of *S. asiatica* (Mabasa, 1996). The reason for that deduction appears to be that the host genotypes that were tested in those experiments were not evaluated under non-infested conditions. The potential yield of the cultivars under non-infested conditions, and therefore the magnitude of the yield losses caused by infestation, was not ascertained. As a result, the higher yields of DC 75 relative to the other cultivars under infested conditions lead to classification of this cultivar as being tolerant. However, it is recommended that this cultivar, including the others that were evaluated in this study, be evaluated in multilocal trials under both infested and non-infested conditions. This will confirm their response to witchweed infestation, and establish the magnitude of yield loss under infested conditions. The results of such replicated testing, over time and space, are expected to give a better indication of the tested genotypes in breeding for witchweed resistance.

Cultivars SAR 19 and SAR 29 were resistant to witchweeds. Their low grain yield potential should be improved through crossing them with high yielding cultivars or germplasm lines and selecting for both yield and resistance under witchweed infestation.

The delay of witchweed emergence on SAR 29 may partly explain why this cultivar did not significantly lose its yield under *S. asiatica* infestation. Since *Striga* emergence was delayed, yield potential of the host crop might have already been determined by the time that the parasite started to inflict its damage. There was no emergence of witchweeds in some pots for cultivars SAR 19 and SAR 29. Failure of emergence can be attributable to a number of factors. It may be different resistance mechanisms that acted individually or in combination to either prevent *S. asiatica* seed germination, attachment to host roots or growth, development and subsequent emergence of the parasite above the ground. While resistance mechanisms appear to be the major contributors to failure of witchweed emergence, environmental and edaphic factors might also have contributed in a way. It is important to note that this is a common observation when witchweed resistant genotypes are being evaluated under infested conditions, irregardless of the size of population of the host genotypes. The resultant wide variation in the number of emerged *Striga* is the cause of the high coefficients of variation (C.Vs) that are encountered in screening experiments as observed in Table 2.

It is not clear why SAR 19, despite having delayed witchweed emergence and having a low *S. asiatica* count, had a significant yield loss of 52 %. This might be partly explained by the fact that SAR 19 continued to accumulate parasites and reached its maximum *S. asiatica* count 25 days after SAR 29, which was the first one to reach maximum count at 101 days after emergence (Table 2). This magnitude of yield loss is also suggestive of the operation of a single or minimum number of resistance mechanisms. Once the major resistance mechanism is overcome, then the host becomes exposed to unrestrained damage by the few attached witchweeds. Other researchers who found similar losses of yield by

resistant genotypes reported that even low levels of *Striga* species infestation could have severe effects on grain yield production of some cultivars (Gurney, Press, and Scholes 1999).

It is apparent from the results of this study that when screening host genotypes for witchweed resistance, it is very important to include a control in which the same genotypes are also screened under non-infested conditions. This does not seem to be the conventional practice when screening germplasm for witchweed resistance. Evaluation of the cultivars under non-infested conditions in this study was specifically important for two reasons. The first reason is that cultivars with a high yield potential, such as DC 75, can be incorrectly classified as tolerant because of their significantly higher yield when grown under witchweed infested conditions. Assessment of the extent of yield losses due to infestation can give a clearer picture of the response of such cultivars to witchweed infestation. Secondly, resistant cultivars that suffer massive yield reductions caused by few attached witchweeds could be identified, such as SAR 19. This cultivar is by definition resistant, due to its lower numbers of emerged *Striga*. Such cultivars might transmit such an attribute to their offspring and therefore they should be utilized with care. It was apparent that SAR 19 should not be used as the only source of witchweed resistance in a breeding programme. Rather, several sources can be utilized simultaneously so that different mechanisms of witchweed resistance can be assembled in a single genotype. It therefore turned out that in addition to breeding for low numbers of emerged *Striga*, a concomitant objective should be breeding for minimal losses resulting from few attached witchweeds.

Generally, most F2 progeny from the combined population of Crosses 2, 3, and 5 delayed witchweed emergence and consequently had low numbers of parasites per plant. Delayed emergence of *S. asiatica* in this case could be explained by mechanisms that operate after *S. asiatica* stimulation by root exudates, and probably after attachment. Mechanisms such as mechanical barriers, antibiosis and avoidance through root growth habit may have prevented early witchweed emergence in Crosses 2, 3 and 5. Lane *et al* (1995) has reported the existence of post-infectional resistance mechanisms in SAR cultivars, though specific mechanisms were not pinpointed in that investigation. It is therefore essential to screen SAR cultivars for post infectional resistance mechanisms such as *HR*, *IR* as outlined by Mohammed *et al* (2001).

3.4.2 Combining ability analysis for *S. asiatica* counts

The highly significant GCA component of genotypic variance indicated that the performance of single-cross progeny could be adequately predicted on the basis of additive gene action. Genes that act in an additive manner are preferred for selection because the superior phenotype will breed true in the next generation, provided the environment allows for the phenotype to be expressed. Additive gene action also results in the more rapid fixation of favourable alleles through exposure to selection (Stoskopf *et al.*, 1993). There was a good relationship between performance of a parent *per se* and its GCA effects for *S. asiatica* counts. Crossing cultivars SAR 19 and SAR 29, which had negative GCA effects for witchweed counts (resistant x resistant), produced progeny that significantly supported the least number of parasites. On the other hand progeny from SV-1 and SAR 16 (susceptible x susceptible), which had positive GCA effects, were the most susceptible since they had the highest witchweed counts. Intermediate parasite counts

were found among progeny from crosses involving resistant and susceptible cultivars because positive and negative gene effects were combined and produced a moderate response to witchweed infestation. A comparison of GCA effects therefore suggested that witchweed resistance is transmissible to progeny. Breeders can therefore expect to make genetic gains by selecting from segregating generations derived from crosses that include witchweed resistant genotypes.

The fixed effects model (Model 1, Griffing, 1956) assumes that the experimental material itself is the population about which inferences can be drawn. It has been reported that estimates of variance components could not be significant estimates of population parameters unless the number of parents exceeds 10 (Baker, 1978). With fewer parents, a fixed model is recommended. However, the results that were obtained in this experiment are a confirmation of reports from other diallel studies and 'line x tester' analyses which indicated the presence of quantitative genetic variation with a preponderance of additive effects for number of above ground *S. hermonthica* plants supported by sorghum in pots and the number of emerged *Striga* plants under field conditions (Hausmann *et al.*, 2000a). A recurrent selection programme, which seeks to concentrate favourable alleles in the base population (Stoskopf, Tomes, and Christie, 1993), could be an effective breeding procedure to improve *S. asiatica* resistance in sorghum. Alternatively, multiple crosses can be generated by intercrossing several resistant and high yielding lines in various combinations in order to also increase variation in the base populations. For instance, the F1 obtained from crossing SAR 19 x SV-1, and that from SAR 29 x SV-1, can be crossed with each other. Other resistant or high yielding cultivars can also be crossed to these products in various combinations. A pedigree breeding method can then be used to handle

these populations. Pedigree breeding is useful in handling the segregating generations following hybridization (Stoskopf *et al.*, 1993). Selection for plants with the desired combination of characters from the parent cultivars can be started in the F₂ generation if there is high heritability and a few genes control the traits of interest. However, selection can be delayed to later generations that have higher levels of homozygosity when heritability is low and many genes are involved.

3.4.3 Path coefficient analysis

It can be postulated that *S. asiatica* counts did not have adverse direct and indirect effects on grain yield for resistant cultivars because *S. asiatica* emerged rather late in the season for these cultivars. Mechanisms such as mechanical barriers, antibiosis and avoidance through root growth habit might have prevented or minimised early witchweed attachment and/or emergence, and hence the damage that is often caused by subterranean *S. asiatica*. Witchweed counts per plant were highly and significantly ($P < 0.01$) negatively correlated ($r = -0.992$) with DSE. This explains why the parasites were very few when *S. asiatica* emergence was delayed by the resistant cultivars. These few emerged witchweeds also had a very limited time period within which to exert adverse effects on sorghum grain yield. Similar observations were made on sorghums that were grown in *Striga*-infested pots (Hess and Ejeta, 1992). Delayed *Striga* emergence was positively and significantly correlated with normal host plant height, panicle weight and grain yield. Increase in *Striga* number was also highly correlated with reduction in sorghum height, dry weight, grain yield and delay in host plant maturity (Hess and Ejeta, 1992).

The yield of sorghum can be broken down into plants per hectare x heads per plant x seeds per head x single seed weight (Doggett, 1988). The genotypes that were used in this study did not seem to have the capacity to produce tillers. Number of heads per plant was therefore not an important component of yield in this investigation. At a given plant population, number of seeds per head is the most important component of yield (Doggett, 1988; Peacock and Wilson, 1984; Ekebil *et al.*, 1977). Having a large number of grains is depended on adequate crop development upto flowering. This development is important and leads to large leaf area indices, in well structured canopies, which in turn are the requirements for large crop growth rates during grain filling (Peacock and Wilson, 1984).

Plant characteristics that may be positively and significantly correlated with yield include plant height, length of head and circumference of head. Leaf area and stover weight may also be sometimes correlated with yield (Doggett, 1988). Head weight, days to 50 % sorghum flowering and 100 seed weight were the major yield performance traits for susceptible cultivars. Selection for yield among these cultivars under witchweed-infestation will result in earliness to flower and large seed sizes. Head weight, plant height and 100 seed weight were the major yield performance traits for resistant cultivars. Selection for higher yields under *S. asiatica* infestation among these cultivars will result in taller plants that have large seeds.

Weight of 100 seeds helped to explain head weight in this experiment. This can be deduced from the high and positive correlations between head weight and 100 seed weight for all the variable models (Figs 3-6). Unexpectedly, direct effects of 100 seed weight were negative for uninfested resistant cultivars and infested susceptible cultivars. Small

seed sizes are postulated to be the cause of this observation in all cases. The low yield potential of SARs 19 and 29 is probably manifested through small seed sizes. Witchweed infestation could have caused poor grain filling, probably by diverting nutrients from the host crop, and this resulted in small seed sizes for the susceptible cultivars SAR 16 and SV-1. There is hope for increasing grain size for both resistant and susceptible cultivars through selection. Selection for large grain size will be effective if it is combined with an independent selection for a large number of grains per head. However, it has been found out that in some circumstances, the ability of seeds to grow larger than normal when number is reduced allowed for yield compensation (Peacock and Wilson, 1984).

3.5 Conclusions

Cultivars SAR 19 and SAR 29 were resistant, while SV-1, SAR 16 and DC 75 were susceptible to *S. asiatica*. This classification was determined using a combination of data for DSE, *S. asiatica* counts, sorghum grain yield loss under witchweed infestation, and the transmission of GCA effects to progeny. It was however surprising that despite being resistant, SAR 19 suffered a significant yield reduction due to witchweed infestation. It was apparent that low levels of *S. asiatica* infestation could cause massive yield reduction on cultivar SAR 19. Cultivar SAR 19 should therefore not be used as the only source of witchweed resistance in a breeding programme. Rather, several sources should be utilized simultaneously through multiple crossings so that different mechanisms of witchweed resistance can be assembled to complement each other in a single genotype. In addition to screening for low witchweed counts, it might also be necessary to screen for minimal yield losses from a few attached witchweeds. Data on yield losses can be obtained by screening the cultivars concerned under infested and non-infested conditions. Cultivars SAR 19 and

SAR 29 were good general combiners for low witchweed numbers and they had negative GCA effects. A comparison of GCA effects suggested that witchweed resistance is transmissible to progeny. Resistant genotypes imparted witchweed resistance by reducing *S. asiatica* counts in their progeny. Likewise, susceptible cultivars transmitted susceptibility by increasing the number of parasites in their progeny. Breeders can therefore expect to make genetic gains by selecting from segregating generations derived from crosses that include witchweed resistant genotypes. Cultivars SAR 19 and SAR 29 had low yields in general and this will make it difficult for them to be accepted by smallholder farmers without an improvement in yield potential.

Grain yield parameters that were important for the cultivars tested were head weight, 100 seed weight, plant height and days to 50 % flowering. However, the direct and indirect contribution of each of these parameters to yield was influenced by the type of cultivar (resistant or susceptible) and whether there was witchweed infestation or not. The development of elite witchweed resistant cultivars therefore necessitates concurrent selection for witchweed resistance and high yield under *S. asiatica* infested conditions. In general, head weight was the most important sorghum grain yield determinant, having moderate to high direct contributions. The indirect contribution of other variables on sorghum grain yield was predominantly through an improvement of head weight. Improvement in yield should therefore be based upon selection for improved head weight, though the rest of the above parameters should form part of the selection criteria. Head weight can be assessed faster and easier than measuring grain yield. Direct effects of *S. asiatica* counts on sorghum grain yield were low. *Striga asiatica* caused yield reduction by indirectly affecting sorghum grain yield components, mostly head weight.

CHAPTER 4

RESISTANCE TO *S. ASIATICA* BASED ON LOW GERMINATION STIMULANT PRODUCTION: PARENT CHARACTERISATION AND INHERITANCE STUDIES

4.1 Introduction

Conventional breeding for *Striga* resistance is slow and greatly constrained by lack of reliable screening methods, as well as a lack of knowledge of the genetics of witchweed resistance (Haussmann *et al.*, 2000a). Hess and Ejeta (1992) attributed the lack of progress on inheritance studies and breeding for resistance to rarity of genotypes that exhibit stable resistance across geographical regions, problems of having uniform field infestations, and the difficulty encountered in evaluating individual segregating progenies for resistance. Apart from the considerable difficulty encountered when evaluating resistance in the field, there is also a lack of alternative and reliable screening assays that can adequately predict the field performance of a given genotype (Omanya *et al.*, 2001). In addition to this, most of the specific mechanisms of resistance have not been properly characterised, mainly because of lack of adequate laboratory techniques that reveal critical host-parasite interactions (Mohamed *et al.*, 2001). Of more value are methods that can expose early host-parasite interaction events and host defense reactions as they occur beneath the soil surface.

In sorghum, resistance to *S. asiatica* is a manifestation of one or more potential mechanisms that inhibit the development of parasitism (Ejeta *et al.*, 1993; Mohamed *et al.*, 2001b). In this experiment, focus was placed on the mechanism of low germination stimulant (*lgs*) production. The advantage of this mechanism is that it is able to impart field resistance to a genotype, even in the absence of other mechanisms (Hess *et al.*, 1992). Low *S. asiatica* seed germination stimulant production may form the basis of some recessive field resistances such as that reported by Hess and Ejeta (1992). However, the positive correlation between stimulant production and *Striga* emergence under field conditions depends upon the genetic materials under investigation, and also on the environmental conditions. For instance, line N 13 is a high stimulant genotype that has superior resistance to *Striga* under field conditions (Rattunde, Obilana, Haussmann, Reddy and Hess, 2000). This example illustrates that the expected relationship can be reversed in some instances.

The production of germination stimulants by different genotypes is relatively simple to assay using the agar gel technique (AGT) (Hess *et al.*, 1992). It is a simple assay used to screen for the capacity of a host genotype's root exudates to stimulate the germination of conditioned *S. asiatica* seed embedded in water agar. The capacity to stimulate germination is measured as the maximum distance from the sorghum root at which *S. asiatica* seeds germinate. In some genomic backgrounds, *lgs* production has been inherited as a major recessive gene with modifiers (Huassmann *et al.*, 2000a), and it has also been governed by different sets of alleles or genes (Huassmann *et al.*, 2000b). The fact that different sorghum genotypes differ by as much as a billion fold in the amount of

germination stimulants that they produce (Ejeta *et al.*, 1993) makes it important to investigate the inheritance of this trait in cultivars SAR 19 and SAR 29.

4.1.1 Study Objectives

- (i) To screen four sorghum cultivars for resistance to *S. asiatica* using the agar gel technique.
- (ii) To determine the inheritance mechanism of low *S. asiatica* seed germination stimulant production among F₂ progenies derived from crosses between cultivars SAR 19 and SAR 29 and SV-1.

4.1.2 Hypotheses tested

- (i) There are no differences in maximum germination distances (MGD) produced by sorghum cultivars SAR 16, SAR 19, SAR 29, and SV-1.
- (ii) Low *S. asiatica* seed germination stimulant production by sorghum cultivars SAR 19 and SAR 29 is simply inherited.

4.2 Materials and Methods

4.2.1 Surface sterilization of sorghum seeds

Sorghum seeds were soaked in 1 % sodium hypochlorite (NaOCl) solution for 45-60 minutes and rinsed with double deionised water (ddH₂O). They were then soaked in an aqueous solution of 10 % captan (48.88 % wettable powder) overnight. Seeds were rinsed with ddH₂O three times, and then incubated on moist filter paper at 27°C. After 48 hours, germinating seeds were placed in agar plates as outlined by Hess *et al* (1992).

4.2.2 Surface sterilization and conditioning of *S. asiatica* seeds

Surface sterilisation and conditioning of *S. asiatica* seeds was done following procedures described by Hess *et al* (1992). *Striga asiatica* seeds were placed in 30 ml sample bottles and rinsed three times by adding 3-5 drops of the detergent Tween 20 (polyoxy-ethylene sorbitan monolaureate) into 10 ml distilled water. Sonication was done using a ultrasonic cleaner for three minutes during the first rinse. The seeds were then rinsed once in 10 ml of 0.1 % phenol prior to sonication for three minutes with occasional swirling. Each batch of seeds was then rinsed three times with sterile ddH₂O after which they were suspended in 14 ml of sterile ddH₂O to which was added 1 ml of a 0.015 % aqueous benomyl solution. The *S. asiatica* seeds were incubated at 27⁰C for three days prior to transferring them into fresh sterile flasks containing 15 ml of a 0.001 % aqueous benomyl solution. Sample bottles were reincubated at 27⁰C for 35 days before *S. asiatica* seeds were used in the agar gel assay. The benomyl solution was changed four times during the five weeks of conditioning.

4.2.3 Assay set up

Agar assays were conducted to characterise parent and F2 genotypes for stimulant production following the methods described by Hess *et al* (1992) and Reda *et al* (1994). Basically, 100 µl of preconditioned *S. asiatica* seeds were pipetted into petri dishes. Water agar (0.7 %) was then poured over the *S. asiatica* seeds. Roots of germinating sorghum seeds were placed in the solidifying agar with the root tip pointing across the plate. The plates were incubated in the dark at 28⁰C for five days. Germinating *S. asiatica* seeds were easily visible through the bottom of the petri dish using a dissecting microscope (Wild M3B, Heerbrugg) fitted with a graduated eyepiece. Maximum germination distance

(MGD), that is, the distance between host root and the most distant germinated *S. asiatica* seed, and germination percentage were used as indices of resistance. Percentages of germinated witchweed seeds were determined by counting germinated seeds from three-microscope fields that were viewed at a magnification of 16. The fields that were examined were chosen randomly along the length of the root.

The four cultivars that were assessed for *lgs* production were SARs 16, 19 and 29, and SV-1. The experiment was laid out in a completely randomized design with five replications per parent genotype. Seventy-three and 143 F₂ progenies were analysed for the cross SAR 19 x SV-1 (Cross 5) and SAR 29 x SV-1 (Cross 6), respectively. The F₂ progeny were screened in batches that differed from 6-23 genotypes per run. A test for homogeneity of variance with unequal degrees of freedom was conducted for all the batches using the method described by Gomez and Gomez (1984). Because the variances on all the batches for Cross 6 were homogeneous ($P \geq 0.05$), data for the F₂ genotypes were put together and analysed as a single entity. However, one batch for Cross 5 had a significantly ($P < 0.05$) larger variance than that of the others and it was thus removed from the data set. Cross 5 was therefore analysed with 65 progeny instead of 73.

Analysis of variance was performed for MGD of parent genotypes for each cross. Chi-square values and their probabilities were calculated for a single gene hypothesis of 3:1 for each segregating F₂ population. Frequency distributions of MGD were plotted and standard errors calculated for each class with a frequency of more than two genotypes. Phenotypic variances were also calculated for each parent and F₂ population. Genetic variance was calculated for MGD following the partitioning of variance components for

measurements made in different generations following formulae by Strickberger (1968) as follows:

$$V_{P1} = E$$

$$V_{P2} = E$$

$$V_{F2} = V_G + E$$

Therefore, $V_G = V_{F2} - (V_{P1} + V_{P2})/2$

Where, V_{P1} , V_{P2} , and V_{F2} are the phenotypic variances of parent one, parent two and F2 generation respectively, and V_G is the genetic variance. Environmental variance is depicted by E . Broad sense heritability (H^2) was estimated according to Klug and Cummings (1994) as follows;

$$H^2 = V_G/V_P$$

where, V_P is the total phenotypic variation for each set of parents and their F2 derived progeny.

4.3 Results

4.3.1 Parent Characterisation

Generally, seeds of the sorghum cultivars that were tested germinated readily on moist filter paper and grew well in agar. There was a positive and highly significant correlation ($r=0.88$; $P<0.01$) between the percentage of *S. asiatica* seeds that germinated and the farthest distance at which they germinated after 120 hours of incubation at 30°C. Maximum germination distance was therefore recorded for the rest of the measurements where the magnitude of germination stimulant production was assessed. The four sorghum cultivars differed significantly ($P<0.01$) for MGD. Cultivar SV-1 was the highest

germination stimulant producer with a distance of 2.56 cm, followed by SAR 16 with a distance of 1.54 cm (Fig 7). Cultivars SAR 19 and SAR 29 had MGDs that were less than 1 cm.

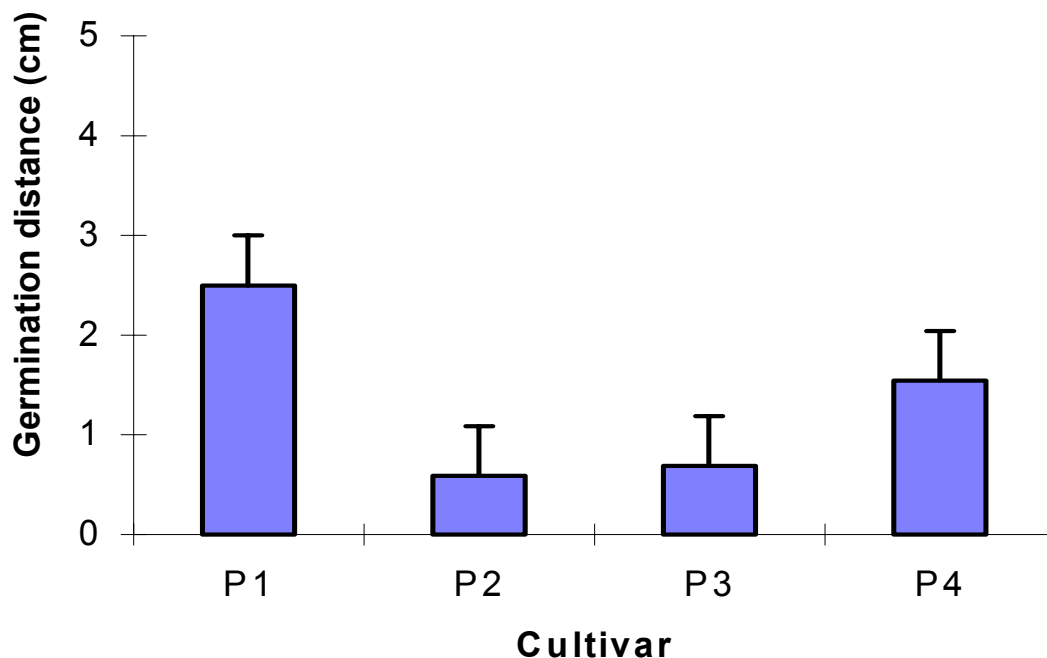


Figure 7: Germination distances from agar gel assays of four sorghum cultivars. P1=SV-1, P2=SAR 19, P3=SAR 29, P4=SAR 16. CV=44.84%

Figure 8 shows pictures of the agar gel assay. The control (Fig. 8b) was included because there has been a report on the spontaneous germination of witchweed seeds (Maass, 2001). No spontaneous germination of *S. asiatica* seeds was observed in this study as depicted by Fig. 8b.

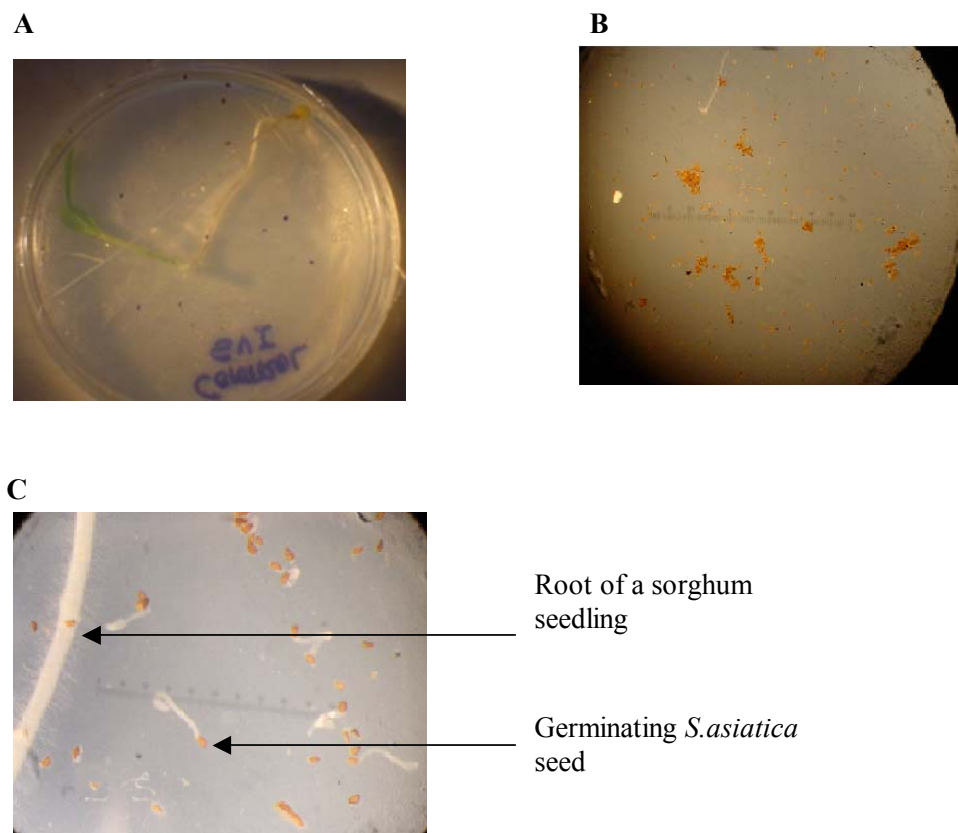


Figure 8: Pictures showing the agar gel technique (AGT). **A-** Overview of the AGT. **B-** Control showing no germination of *S. asiatica* seeds in the absence of a sorghum root. **C-** Root of a susceptible sorghum genotype that produced germination stimulants causing *S. asiatica* seeds to germinate.

4.3.2 Inheritance of low *S. asiatica* seed germination stimulant production in sorghum genotypes SAR 19 and SAR 29

Maximum germination distances ranged from 0.00 cm to 4.4 cm and from 0.00 cm to 4.55 cm for the segregating F2 populations from Crosses 6 and 5 respectively. Segregation ratios of high to low F2 stimulant producers did not differ significantly ($P \geq 0.05$) from a ratio of 3:1 (susceptible: resistant) for both populations (Table 13). It was therefore apparent that a single recessive gene controlled low witchweed seed germination stimulant

production in cultivars SAR 19 and SAR 29. Significant differences ($P \leq 0.05$) between MGD of two parental cultivars used for each of the two crosses were also observed (Table 13). Cultivars SAR 19 and SAR 29 were resistant while SV-1 was susceptible in both cases. Broad sense heritability (H^2) estimates for MGD were 0.70 and 0.52 for Crosses 6 and 5 respectively (Table 13).

Table 13: Characterisation of parent cultivars (SV-1, SAR 19 and SAR 29) and their F2-derived progeny for *S. asiatica* seed germination stimulant production

Genotype	No. of seedlings	Mean [‡]	H^2 [†]	Expected [§] Ratio	Observed ratio [¶]		χ^2	P
					High	Low		
SV-1xSAR 29	134	2.08 <i>ab</i>	0.70	3:1	106	28	1.20ns	0.54
SV-1	5	2.66 <i>a</i>						
SAR 29	5	0.70 <i>c</i>						
SV-1xSAR 19	65	1.80 <i>a</i>	0.52	3:1	55	10	3.20ns	0.08
SV-1	5	1.84 <i>a</i>						
SAR 19	5	0.62 <i>b</i>						

[‡]Means followed by the same letter are not significantly different ($P \leq 0.05$)

[†]Broad sense heritability = $[V_{F2} - (V_{P1} + V_{P2})/2] / V_{F2}$

[§]Expected segregation ratio for a single dominant gene for high stimulant production

[¶]High: maximum distance for germination of *S. asiatica* seeds was ≥ 1.0 cm. Low: maximum distance for germination of *S. asiatica* seeds was < 1.0 cm.

ns- non-significant, $P \leq 0.05$

Frequency distributions of F2 progenies from Crosses 5 and 6 for MGD are shown in Fig 9 and Fig 10. The class interval for the frequency distributions of MGD among the F2 progenies was 0.2 cm. Block arrows in the graphs show mean parental values obtained during the time that their respective F2 progeny were screened. The F2 progeny of both crosses were generally put into two groups, low and high *S. asiatica* seed germination stimulant groups. There was, however, considerable variation for MGD within each group (Figs 9 and 10). Standard error of means ranged from 0.01 to 0.04 for all different classes of the two populations.

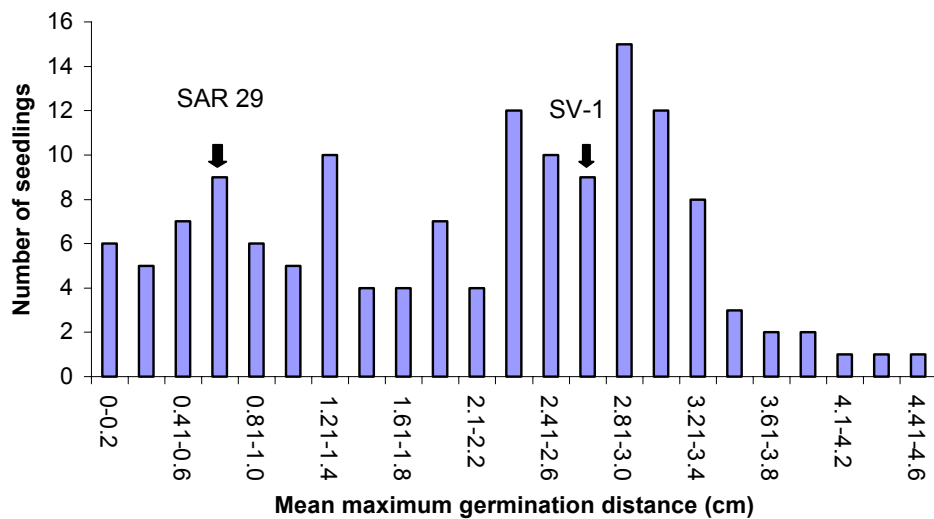


Figure 9: Frequency distributions of segregating F2 progeny from crosses between SV-1 (high germination stimulant producer) and SAR 29 (low germination stimulant producer).

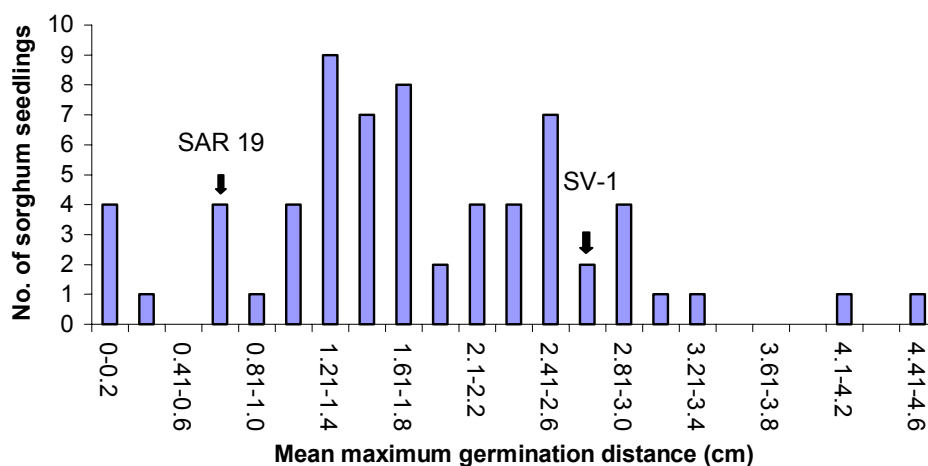


Figure 10: Frequency distributions of segregating F2 progeny from crosses between SV-1 (high germination stimulant producer) and SAR 19 (low germination stimulant producer).

4.4 Discussion

The results of screening cultivars SV-1 and SAR 16 for stimulant production revealed that both cultivars had germination distances of more than 1 cm, and were therefore susceptible to witchweeds. This classification is based on the stipulation that genotypes with a germination distance of less than 1 cm are resistant while those with more than 1 cm are susceptible to *S. asiatica* (Hess *et al.*, 1992). The same genotypes were also shown to be susceptible to *S. asiatica* when they were screened using the pot culture technique (Chapter 3). These findings are contrary to earlier reports in which SAR 16 has been reported to be resistant to *S. asiatica* from pot screening experiments (Obilana and Ramaiah, 1992; Mabasa, 1996). Lack of correspondence between current results and earlier reports may be due to the confounding effects of intraspecific physiological strains of *S. asiatica* that might have been used in the different experiments. It has been reported that physiological strains of *S. asiatica* occur in Zimbabwe (Musimwa *et al.*, 2000).

Different physiological strains and/or biotypes of witchweeds are expected to have different virulence towards their host genotypes. However, there was no deliberate testing of *S. asiatica* seeds from diverse geographical regions in both agar gel and pot screening experiments in this study. The reason for the differences in classification of the response of SAR 16 to witchweeds therefore remains inconclusive. As suggested in Chapter 3 of this write-up, differential sorghum genotypes should be identified for use in Eastern and Southern Africa (Hess, 2001). These sorghum lines will then be used to identify different witchweed pathotypes among collections from all agro-ecological zones of Zimbabwe. Potentially resistant cultivars would have to be screened using these pathotypes before their release.

Agar gel-screened genotypes should also be screened in multilocal trials, in *S. asiatica* “hot spots”, over at least three seasons. This will ensure that the genotypes will be exposed to both intraspecific and interspecific physiological strains of *S. asiatica*. Genotypes that will be selected under these conditions will have broad-based and stable resistance to witchweeds.

Since cultivars SAR 19 and SAR 29 had MGDs that were less than 1 cm, they were classified as resistant. Results obtained using the agar gel assay for SARs 19 and 29 agree with results obtained from pot-screening experiments (Chapter 3), and in general, field screening experiments that were conducted in Zimbabwe and the Southern African Development Community (SADC) region (Mabasa, 1996). Cultivars SAR 19 and SAR 29 can therefore be good sources of genes for the *lgs* trait in breeding programmes. These SAR cultivars have also been reported to have other postinfectious resistance mechanisms (Lane *et al.*, 1995), which still have to be identified through the use of appropriate screening techniques. The total field resistance to witchweeds that has been shown by these cultivars is therefore most likely to be a result of a combination of resistance mechanisms.

A single recessive gene largely controlled low *S. asiatica* seed germination stimulant production in cultivars SAR 19 and SAR 29. This was in agreement with results from similar investigations with other sorghum genotypes (Vogler *et al.*, 1996; Haussmann *et al.*, 2000a; Greiner *et al.*, 2001). However, considerable variation for MGD was observed among F₂ progeny within the low and high germination stimulant groups. This suggested that there were additional minor genes or modifiers that modified MGD in the F₂ populations. It has been reported that witchweed host plants produce several compounds that stimulate *Striga*

seed germination. In sorghum, four active compounds that are found in root exudates have been shown to act as germination stimulants. These sorghum derived germination stimulants are sorgoleone, sorgolactone, strigol and a water-soluble compound with a quantitative biosynthetic pathway that has not yet been identified (Siame, Weerasuriya, Wood, Ejeta, and Butler, 1993; Vogler *et al.*, 1996). The major stimulant from sorghum is sorgolactone and the minor stimulants are structurally related to strigol (Siame *et al.*, 1993).

In related genetic studies, general combining ability (GCA) effects for MGD of *S. hermonthica* indicated that different sets of alleles or genes were responsible for *lgs* production in cultivars 555 and Framida (Haussmann *et al.*, 2000b). Stuber, Polacco and Senior (1999) reported that traits that are considered to be simply inherited might be “semi-quantitative”. Many genes, such as a major gene plus several modifiers, govern trait expression in this case. For example, researchers working on Yam mosaic virus (YMV) in white yam observed that variation for YMV resistance was continuous, though a single major gene was found to control YMV resistance (Mignouna, Abang, Onasanya, Agindotan, and Asiedu, 2002). Genotypes that were resistant to YMV showed mild mosaic symptoms (symptom severity score ≤ 2), just like *S. asiatica* resistant genotypes support fewer emerged *Striga* under field conditions, or have short germination distances in agar gel assays (< 1 cm). Having some level of *S. asiatica* seed germination would suggest a continuity in variation for *lgs* production as depicted by Figs 9 and 10, and hence the existence of modifier genes.

High and low germination stimulant groups of F2 progenies derived from Cross 6 were able to approximate parental values at their peaks (Fig 2). However, F2 progeny of Cross 5 failed to approximate parental values at their peaks (Fig 3). This lack of correspondence may be due

to the confounding effects of intraspecific physiological strains of *S. asiatica* that might have been used in the different experiments. In addition, this can also be explained by the existence of four different stimulant compounds which all act variably to induce *S. asiatica* seed germination in sorghum (Siame *et al.*, 1993). The implication is that the segregating F2 progeny may have variably expressed the stimulant compounds, for Cross 5 and not for Cross 6.

The observed variation for MGD was predominantly genetically determined and quite comparable with results from other investigations. Heritability estimates for MGD ranged between 0.65 and 0.89 when sorghum recombinant inbred lines (RILs) were screened for *S. hermonthica* resistance (Haussmann *et al.*, 2001). Vogler *et al* (1996) and Haussmann *et al* (2000a) also reported high H^2 estimates for MGD from agar assays. Vogler *et al* (1996) reported H^2 estimates ranging from 0.62 to 0.75 for three crosses that were screened for germination stimulant production against *S. asiatica*. Haussmann *et al* (2000a) screened two sets of the same population at different times and found heritability (H^2) estimates of 0.65 and 0.89. In addition, three to five quantitative trait loci (QTL) were also detected for MGD when the same recombinant inbred population was screened using different *S. hermonthica* populations. The QTLs only explained up to a maximum of 39 % of the genetic variance, indicating the presence of a major gene that controls the rest of the genetic variance. These results show that genetic gains can be made if selection for the *lgs* trait is imposed upon appropriate segregating populations.

Since *lgs* production appears to be inherited as a single recessive gene, it should be relatively simple to manipulate in breeding programmes relative to field resistance, which is normally

quantitatively inherited. The major recessive gene for *lgs* production can be transferred to desirable genomic backgrounds by the backcrossing procedure (Poehlman and Sleper, 1995). However, the process may be long due to a requirement to self the backcross progeny in each backcross generation in order to facilitate expression and selection for the recessive trait using the AGT.

The value of selecting for different mechanisms of *S. asiatica* resistance with the idea of pyramiding them depends on a number of factors, in addition to having an appropriate technique for use in selecting. These factors include the correlation of each mechanism with witchweed resistance under field conditions, the degree of its genetic variability in crop germplasm, and the heritability of that mechanism. While the inheritance of *lgs* production mechanism was examined for SARs 19 and 29 in these studies, further investigations are recommended to generate information that will elaborate the above factors for each mechanism of witchweed resistance. Avoidance and tolerance mechanisms (itemised in section 2.5) could also be investigated further. Apart from giving an indication of the mechanisms to place major focus on, the gathered information will also facilitate assemblage of various mechanisms in single genotypes.

4.5 Conclusion

Cultivars SV-1 and SAR 16 had MGDs of more than 1 cm and they were therefore susceptible to *S. asiatica*. Cultivars SAR 19 and SAR 29 had MGDs of less than 1 cm and they were classified as resistant. The low *S. asiatica* seed germination stimulant trait in SARs 19 and 29 was inherited as a single recessive gene. However, minor genes modified *lgs* production, judging by the variation for MGD that was observed among the low and

high germination stimulant groups of F2 progeny. Broad sense heritability (H^2) values obtained for Crosses 5 and 6 revealed that genetic factors largely explained the observed phenotypic variation for MGD in this experiment. Selection for low *S. asiatica* seed germination stimulant production should be successful if sorghum breeding programmes for resistance against witchweeds are able to identify and utilize good sources of resistance, preferably those with multiple mechanisms of resistance. The agar gel assay can be used as an efficient tool to select for *lgs* production, especially in the segregating generations where single plant selection is important. It is suggested that *S. asiatica* collections from Zimbabwe should be classified into pathotypes using differential sorghum lines. Screening of sorghum genotypes in water agar should then be done using these witchweed pathotypes. Agar gel-screened genotypes should also be screened in multilocal trials, in *S. asiatica* “hot spots”. Sorghum cultivars selected under these conditions should have broad-based and stable resistance to witchweeds.

CHAPTER 5

A SEARCH FOR RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND SIMPLE SEQUENCE REPEAT (SSR) MARKERS THAT ARE LINKED TO *STRIGA ASIATICA* RESISTANCE IN SORGHUM

5.1 Introduction

Molecular (DNA) markers can provide a powerful arsenal for use by the Plant Breeder. They permit identification and mapping of genes for individual, monogenic resistance mechanisms (like the *lgs* locus) and of QTL involved in polygenic, quantitative resistance to witchweeds under field conditions. Detecting resistance genes by their linkage to DNA markers makes it possible to screen for many different resistance mechanisms simultaneously, without a need for inoculation with the *Striga* parasite. Pyramiding of resistance mechanisms to provide durable witchweed resistance is therefore facilitated. Integration of MAS into *S. asiatica* resistance breeding could permit more rapid movement of desirable genes among varieties, and the transfer of novel genes from wild crop species (Haussmann *et al.*, 2000b). However, molecular markers are not replacements for classical resistance breeding techniques. They only assist in improving selection efficiency by allowing selection to be based upon the genotype and not the phenotype of an individual.

Bulked segregant analysis (BSA) has been used in many studies to "tag" both monogenic and polygenic traits of economic importance. This is a rapid and efficient method to detect

markers in target genomic regions by using segregating populations. It involves screening for differences between two pooled DNA samples derived from a segregating population that originated from a single cross (Michelmore, Paran and Kesseli, 1991). Each pool, or bulk, contains individuals selected to have identical genotypes for a particular genomic region (target locus) but random genotypes at loci unlinked to the selected region. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the bulks (Michelmore *et al.*, 1991). Linkage between the polymorphic markers and genes of interest is confirmed and quantified by analysing individual plants belonging to the susceptible class which is also obtained from the same segregating population (Zhang, Shen, Dai, Saghai-Marooof and Li, 1994). Bulk-extreme and recessive class analysis hence provide a fast and cost effective means for identifying chromosomal regions that are likely to contain genes of interest (Zhang *et al.*, 1994). By contrast, the use of a random F₂ population will be time-consuming, laborious and expensive.

The enormous attraction of RAPDs is that the technique is quick, simple, and efficient, uses small amounts of DNA, sample throughput can be high and the procedure is automatable (Karp *et al.*, 1997). There is also no requirement for DNA probes or sequence information for primer design, and only the purchase of a thermocycling machine and agarose gel apparatus will be required. When a combination of markers is used, RAPD markers can bypass the limitation of being dominant. In this case, a RAPD marker linked in coupling phase is used in association with another marker linked in repulsion phase to the same allele, to constitute a codominant pair (Kelly, 1995). In the case of the recessive *lgs* locus, a repulsion-phase RAPD marker would identify the recessive allele (or resistant

genotypes, - “aa”) while a coupling phase marker would identify the dominant allele (or susceptible genotypes, - “AA and Aa”). If both markers are scored it should then be possible to identify heterozygous genotypes. Selection based on linked RAPD markers can also be as efficient as codominant markers when the RAPD markers are used on advanced generation RILs, where the level of heterozygosity is minimal (Kelly, 1995). Reports by Morrell *et al* (1995), Weeden *et al* (1992) and Hoffman and Bregitzer (1996) suggest that well optimised RAPDs can be very reproducible and useful markers.

Microsatellites or SSRs are important gene markers in a number of crop species, including sorghum. They are abundant, dispersed throughout the genome, and show higher levels of polymorphism than other molecular markers (William, Dorocicz, and Kasha, 1997). Variations in the length of tandem repeats can be identified by amplification of the region containing the repeat motif by PCR using primers that correspond to conserved regions flanking the SSR. The SSR technique has high levels of reliability due to stringent assay conditions involving the use of long primers and high annealing temperatures.

It was important to identify markers that were linked to the genes for witchweed resistance in resistant sorghum genotypes that were available to us. Ideally, such markers have to be identified for all resistance mechanisms, including QTLs for field resistance to witchweeds. These markers should facilitate the expeditious deployment of host resistance genes into elite sorghum cultivars through MAS. Other researchers have identified markers that are linked to the *lgs* trait in sorghum and rice (Bennetzen *et al.*, 2000; Ejeta, 2000; Haussmann *et al.*, 2000b, 2001). However, these markers were unknown to us at the inception of this research project. Furthermore, the identity of these markers is still

unknown to us today and they also have to be tested for their usefulness in screening our germplasm for their capacity to stimulate *S. asiatica* seed germination. There are possibilities that the markers may not even distinguish our resistant and susceptible genotypes, and might therefore be unlinked to the trait.

5.1.1 Study objective

To identify molecular markers that are linked to low *S. asiatica* seed germination stimulant (*lgs*) production using bulked segregant analysis (BSA).

5.1.2 Hypothesis tested

There are no molecular markers that are linked to the locus for low *S. asiatica* seed germination stimulant production in sorghum cultivar SAR 29.

5.2 Materials and methods

Second filial (F₂) generation progeny from crosses between SAR 29 and SV-1 (Cross 6) were utilized in this experiment.

5.2.1 Screening of parent and F₂ genotypes for stimulant production and growing of sorghum seedlings

Parental genotypes and F₂ generation progeny were screened for stimulant production using the AGT (Hess *et al.*, 1992). The index of resistance was maximum germination distance (MGD), as explained in section 4.2.3. Genotypes with a germination distance of less than 1 cm were classified as resistant and those with a distance greater than or equal to 1 cm were classified as susceptible. See section 4.2 for a full description of this assay.

After classifying parental and F2 plants as high or low stimulant producers in agar, the same seedlings were transplanted into kaolite crates filled with river sand.

The seedlings were initially rinsed in a 1 g/l solution of thiram (a fungicide) prior to transplanting in order to avoid fungal infections. The seedlings were kept under clear plastic sheeting, under which jets of water were supplied to form a mist and keep humidity high during the hot summer afternoons. After three days, the river sand was drenched with a 0.004 % solution of nutrofil (a liquid nitrogenous fertilizer) so that the plants would grow quickly. After seven days, the seedlings were transplanted again into 16 cm diameter by 16 cm depth clay pots that were filled with red clay soil. The seedlings were allowed to grow in the greenhouse for 14 days prior to DNA extraction.

5.2.2 DNA extraction

Genomic DNA was extracted from the above AGT-classified parental and F2 genotypes. Extraction of DNA was done using 0.5 g of fresh leaf tissue and a buffer containing sodium dodecyl sulfate (SDS) following a method that was modified from Picknett, Sanders, Ford and Holt (1997). Sodium dodecyl sulfate eliminates polyphenolic compounds, which are usually high in sorghums. Basically, following cell wall disruption by crushing freeze-dried leaf material using a pestle and mortar, cells were lysed using SDS buffer. Nucleic acids were precipitated using potassium acetate, and further precipitated using isopropanol. Messenger RNA (mRNA) was removed by digestion using RNase 'A', and protein thereof digested with proteinase K, followed by extraction of the protein (enzyme) with phenol:chloroform (1:1). The DNA was finally precipitated using sodium acetate and absolute ethanol. Any remaining salts were washed away using 70 %

ethanol, prior to re-suspending the pelleted DNA in sterile water. Genomic DNA was extracted from about ninety individual F2 progeny. At least five DNA samples were extracted from each parent genotype so as to have large quantities of parent DNA for use in the investigations.

5.2.3 DNA quantification and construction of resistant and susceptible DNA bulks

Deoxyribonucleic acid (DNA) concentration for the extracted samples was measured using a Biospec-1601 uv-visible spectrophotometer (Shimadzu Corporation). For quality assessment 3 ul of the concentrated DNA solutions were mixed with 5 ul of loading buffer III (0.25 % bromophenol blue, 0.25 % xylene cyanole FF and 30 % glycerol) in 0.5 ml reaction tubes. This mixture was loaded on a 1 % agarose gel and then electrophoresed at 5 v/cm. Lambda DNA digested with *Hind* III was used as the molecular weight marker. The gel was then stained with ethidium bromide and photographed under UV transillumination. The DNA samples were visually assessed for RNA contamination and shearing.

The PCR protocol used for this study required DNA at a concentration of 10 ng/ul. On the basis of the spectrophotometer readings, the correct amount of DNA per genotype was mixed with sterile water. These 10 ng/ul solutions were stored at 4⁰C and then used for the amplification reactions. Forty microlitres of a 10 ng/ul solution of each genotype were used in the construction of DNA pools. Resistant and susceptible bulks were prepared by pooling DNA from extremely resistant (MGD<1 cm) and susceptible (MGD>2.5 cm) F2 genotypes, respectively. The resistant bulk had 16 genotypes while the susceptible bulk had 25 F2 genotypes.

5.2.4 Primer Screening and segregation analysis of polymorphic markers

Parent genotypes were screened for polymorphisms using RAPD and SSR primers. Primers that were polymorphic between the parents were then used to screen DNA bulks for differences in DNA sequence at the *lgs* locus. When using BSA, a primer that can detect differences between bulked DNA samples is linked to the trait on the basis of which the contrasting DNA pools would have been constructed (Michelmore *et al.*, 1991). After failing to detect polymorphisms between bulked DNA samples, the strategy was then to conduct an analysis of the segregation of individual molecular markers among a total of 77 F2 progeny. Second filial generation progeny that were genotyped consisted mostly of the extremely resistant and susceptible F2 genotypes, with a few samples having intermediate values for MGD. Ninety-nine of the primers that had been polymorphic between SAR 29 and SV-1 were used to amplify DNA of the 77 F2 progeny. Those primers that failed to segregate in the F2 generation were excluded from the analysis.

5.2.5 RAPD procedures

A total of 440 RAPD primers were screened in this study. They consisted 12 primer kits from Operon technologies (Kits A, B, C, G, H, I, J, K, L, M, Q and R) and two from the University of British Columbia, Canada (UBC100 and UBC400 series). The final concentrations of PCR reagents that were used for amplification with RAPD primers were; 30 ng template DNA, 1.75 units Taq DNA polymerase (Roche Molecular Biochemicals, South Africa), 400 μ M dNTPs, 0.6 μ M primer and 1X buffer (50 mM Tris pH 8.5, 2 mM $MgCl_2$, 20 mM KCL, 5 μ g/ μ l Bovine serum albumin, 25 g/l of Ficoll 400 and 0.2 g/l xylene

cyanole). Amplification was performed using a 15 µl reaction mixture in 0.2 ml PCR reaction tubes.

A GeneAmp PCR System 9700 (Perkin-Elmer Applied Biosystems) thermocycler was used for all RAPD and SSR thermal cycling reactions in this study. The RAPD samples were subjected to two preamplification cycles of denaturation at 91⁰C for 2 minutes, annealing at 40⁰C for 30 seconds and extension at 72⁰C for 1 minute 10 seconds. The subsequent 38 cycles had denaturation time set at 91⁰C for 30 seconds, annealing at 40⁰C for 30 seconds and extension at 72⁰C for 1 minute 10 seconds. However, the final elongation step was extended to 5 minutes.

5.2.6 Agarose gel electrophoresis and scoring of polymorphisms

After amplification, the products were separated by gel electrophoresis in 1.5 % agarose gels in TBE (tris/borate/EDTA, pH 8.0) buffer at 5 v/cm. The reaction products were first mixed with 5 µl of loading buffer III. Eight microlitres of molecular weight marker XIV (0.25 µg/µl) were loaded either in the first well or the middle well of each row to enable estimation of the sizes of the amplified products. Two twenty four-teeth combs of a high throughput gel electrophoresis tank (Sigma, South Africa) were used to create wells for loading 15 µl of the amplified RAPD products per well. After electrophoresis, the gel was stained with ethidium bromide to a concentration of 5 µg/µl and viewed under 302 nm UV light using a Gene Genius Bioimaging system [Syngene, Synoptics Ltd). Gel pictures were taken and stored in the computer prior to scoring of polymorphisms.

The bands that were scored had to be clear and of high intensity, and the phenotypes that were scored were presence or absence of the RAPD fragment. Each marker that could be unambiguously scored was considered to be amplified from a different locus and to be a dominant marker for that locus. In instances where more than two phenotypic classes were discernible as band intensity differences, the intensity variants were summed up in a single presence class. This was done to minimise errors when scoring, particularly because progenies that were amplified by the same primer would be run on more than one gel. Heterozygotes could therefore not be distinguished from homozygotes because of the scoring procedure which was used in this study.

5.2.7 Simple sequence repeat (SSR) procedures

5.2.7.1 Amplification with sorghum SSR primers

Twenty-nine *Xtxp* sorghum microsatellite, or SSR, primers that were originally developed at Texas A & M University were used in this study. The final concentrations of PCR reagents that were used for amplification with sorghum SSRs primers were; 30 ng template DNA, 1.75 units Taq DNA polymerase (Roche Molecular Biochemicals, South Africa), 250 μ M dNTPs, 30 ng of forward and reverse primers and 1X buffer (50 mM Tris pH 8.5, 2 mM $MgCl_2$, 20 mM KCL, 5 μ g/ μ l Bovine serum albumin, 25 g/l of Ficoll 400 and 0.2 g/l xylene cyanole). The total volume of reaction mixture that was amplified was 25 μ l. Initial denaturation of the template DNA was accomplished at 94⁰C for 4 minutes. The samples were then subjected to 35 repeats of the following thermal cycle: denaturation at 94⁰C for 1 minute and elongation was for 1 minute at 72⁰C . Annealing temperature would vary with the primer that was being used. The primers had annealing

temperatures of 50⁰C, 55⁰C and 60⁰C .The final elongation step was extended to 10 minutes at 72⁰C.

5.2.7.2 Amplification with Maize SSR primers

A total of seven maize primers from the *phi* and *nc* series were sourced from CIMMYT (International Maize and Wheat Improvement Centre) Mexico and used in this study. Polymerase chain reaction (PCR) conditions and cycling profile were based on a protocol published by Senior (<http://www.agron.missouri.edu>). The final concentrations of PCR reagents that were used for amplification with maize SSR primers were; 30 ng template DNA, 1.75 units Taq DNA polymerase (Roche molecular biochemicals, South Africa), 400 µM dNTPs, 50 ng of forward and reverse primers, 2,5 mM MgCl₂ and 1X buffer (50 mM Tris pH 8.5, 20 mM KCL, 5 µg/µl Bovine serum albumin, 25 g/l of Ficoll 400 and 0.2 g/l xylene cyanole).

A touchdown PCR programme was used for maize primers as described by Senior (<http://www.agron.missouri.edu>, 2001). The initial two cycles had denaturation at 95° C for one minute, annealing at 65° C for 1 minute and extension at 72° C for 1 minute 30 seconds. Two cycles were further performed for each one-degree decrement in annealing temperature, until the temperature got to 55⁰C. Twenty cycles were therefore performed at 10 different temperature settings. The temperature regime for the succeeding 25 cycles was then; denaturation: 95° C for 1 minute; annealing: 55° C for 1 minute; extension: 72° C for 1 minute 30 seconds. In the final cycle the extension period was 10 minutes.

5.2.8 Electrophoresis of SSR products

Microsatellite PCR products were electrophoresed using 6 % non-denaturing polyacrylamide gels. Acrylamide gels were prepared by polymerising acrylamide with a cross-linking agent (bis acrylamide) in the presence of a catalyst/chain initiator mixture thus producing a cross-linked matrix of variable pore size. TEMED (N, N, N, N' - tetramethylenediamine) was used as the catalyst, and the ammonium persulphate ion ($S_2O_8^{2-}$) was used as the initiator following protocols described by Sambrook, Fritsch and Maniatis (1989). The non-denaturing polyacrylamide gel was composed of a 40 % acrylamide stock (38 g acrylamide and 2 g *N,N'*-methylenebisacrylamide, topped up to 100 ml with distilled H_2O).

Small or Mini Protean 11 cell vertical (14 cm height; Biorad) and medium sized adjustable height (16 cm (width) X 14 cm (height) glass plates; (Sigma, MO, USA) electrophoresis rigs were used when running polyacrylamide gels. It was made certain that genotypes amplified by a specified SSR primer would be run on either of the two gels, and not on both, to make comparisons possible. After electrophoresis, ethidium bromide (0.5 $\mu\text{g/ml}$) was used to stain the gel at room temperature for 35 minutes. The gel was then photographed using Polaroid film (type 667) under UV light. Other gel pictures were documented using a Gene Genius Bioimaging system (Syngene, Synoptics Ltd). Molecular sizes of amplified fragments were estimated in base pairs by comparing the fragments with Molecular Weight Marker XIV (Roche Molecular Biochemicals, South Africa).

5.2.9 Segregation and Linkage analyses

Segregation of individual markers was analysed by a chi-square test for goodness-of-fit to a 1:2:1 (ABH) and 3:1 (present:absent) ratios for the codominant SSRs and dominant RAPDs, respectively. Linkage analysis of the entire set of markers was performed using the computer software MAPMAKER/EXP 3.0b (Lander, Green, Abrahamson, Barlow, Daly, Lincoln, and Newburg, 1987). Mapmaker uses the Lander and Green algorithm to calculate the maximum likelihood genetic linkage map for any given order of loci. This means that the software searches over 20 different maps and then chooses the best map on account of its having the highest probability of fitting the data. Mapping analysis was conducted using a likelihood of odds ratio (LOD) threshold score equal to or above 4.0 and a 0.50 maximum recombination frequency. A molecular linkage map of the resulting linkage groups was subsequently drawn using the software Mapplot.

5.3 Results

Overall, 440 RAPD primers were screened on the genomic DNAs of SAR 29 and SV-1. A total of 2532 bands were amplified and thus 7.3 loci were amplified and examined per primer. The number of RAPD fragments that were amplified ranged from one to 14, and the sizes ranged from 200 to about 2 500 bp. One hundred and eighty-seven (7.3 %) RAPD primers gave polymorphic bands between the parent genotypes. This low rate of polymorphisms for RAPDs may be attributed to the fact that the variation is intraspecific. Out of 29 sorghum *Xtxp* primers, five failed to amplify while 10 (34.48 %) of them amplified bands that were polymorphic between the parents. The remaining 14 *Xtxp* sorghum SSR primers amplified bands that were monomorphic between SAR 29 and SV-

1. Out of seven maize SSR primers, one failed to amplify, two of them generated polymorphisms while four primers were monomorphic between the parent genotypes.

Figure 11 shows some gel pictures of RAPD and SSR amplification products that were generated in this experiment. Sorghum and maize SSR primers were used to amplify the amplification products in Fig 11a and Fig 11b, respectively. Lane 9 of Fig. 11a is empty probably because it may represent a null allele. It has been reported that mutations in the binding region of one or both of the microsatellite primers may inhibit annealing that may result in the reduction or loss of a PCR product (Callen, Thompson, Shen, Phillips, Richards, Mulley and Sutherland, 1993). The null allele thus appears as an empty lane because of failure of amplification of target DNA.

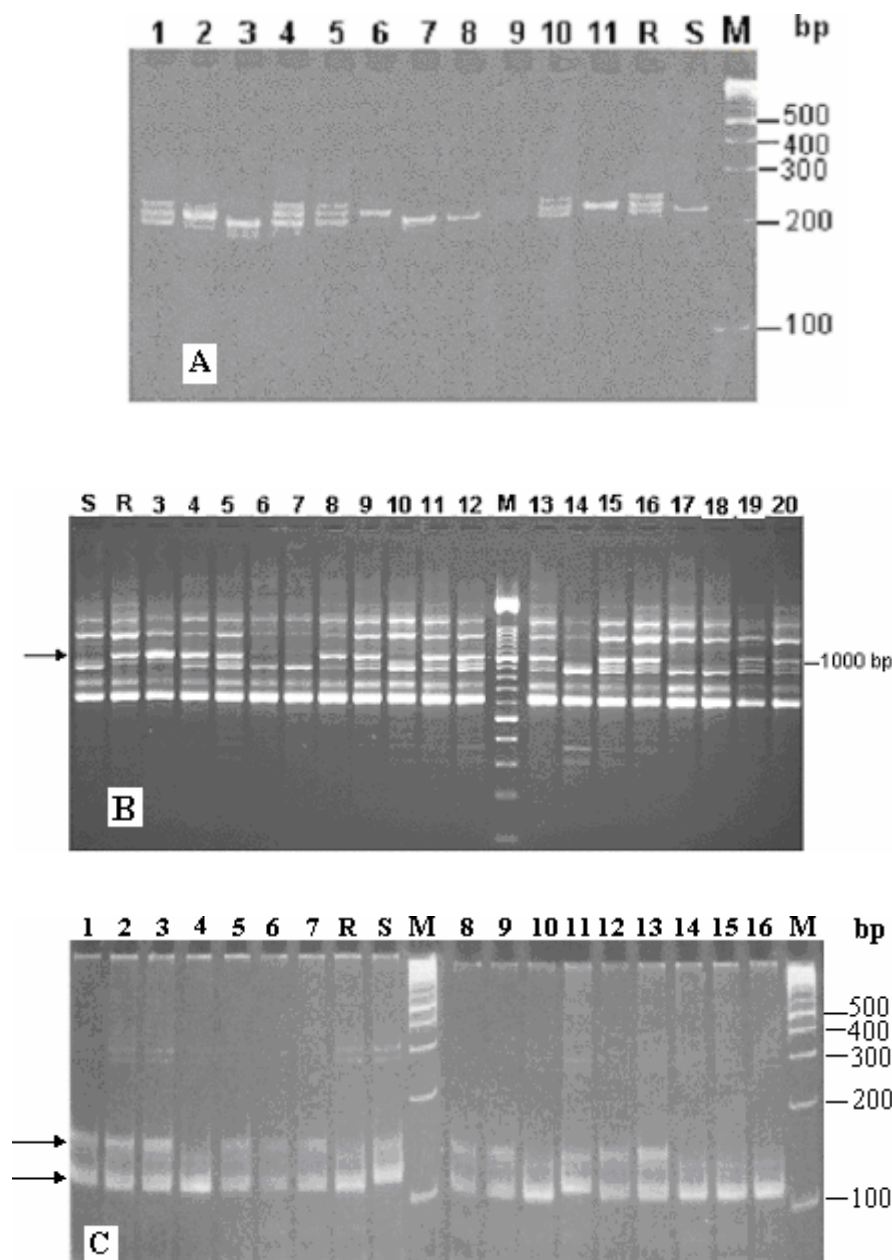


Figure 11: Gel pictures of RAPD and SSR amplification products. **A)** Sorghum SSR primer *Xtxp7*, **B)** RAPD primer UBC415 and **C)** Maize primer *phi074*. Lanes marked "M" contain the molecular size standard, Marker XIV (Roche Molecular Biochemicals). Lanes marked "R" and "S" contain the resistant (SAR 29) and susceptible (SV-1) parents respectively. The rest of the numbered lanes contain segregating F2 progenies derived from crossing SV-1 and SAR 29. The arrow on the left of Figures **B** and **C** show the amplification products that were polymorphic between SV-1 and SAR 29.

None of the primers that produced polymorphisms between the parent lines were able to distinguish the bulked DNA samples. The primers were thus used to amplify individual F2 progeny DNA in order to investigate their possible cosegregation with the *lgs* locus. A total of 99 marker loci (10 SSR and 89 RAPD) were scored in the segregating population consisting of 77 F2 progeny. The average number of F2 genotypes per primer was 60.44. This was because some of the F2 DNA got finished before amplification with other primers. Such samples would have been having a low DNA concentration after extraction. Sorghum SSRs, however, also had sufficient primers for at most 50 progenies per primer and this weighed down the average number of F2s screened.

Segregation analysis of individual marker loci using the chi-square goodness-of-fit test revealed that 63 (63.6%) of the loci segregated normally while 36 (36.4%; 1 SSR and 35 RAPD) exhibited distorted segregation ($P \leq 0.05$). At a LOD grouping threshold of 4.0, 13 linkage groups (LGs) with 2-8 markers were identified. Five LGs (groups 9, 10, 11, 12 and 13) consisted of two markers. The shortest LG was LG 10, with 14.1 cM. The longest LG (group 1) had 6 loci and covered a distance of 95.5 cM (Table 14 and Fig 12). The final map consisted of 45 (2 SSR and 43 RAPD) markers spanning a total of 494.5 cM (Haldane) (Table 14, Fig 12). Fifty-four loci (54.5 %) were completely unlinked and could not be included in the map. Unlinked loci included the locus for low germination stimulant production. The average, minimal and maximal distances between markers were 10.99 cM, 2.3 cM and 34.9 cM, respectively.

The chromosomal locations of sorghum SSR markers were used as a basis for identifying the possible chromosomes to which some LGs might belong. Marker *Xtxp* 15 and *Xtxp* 30 are both located on chromosome 'J' of sorghum (Bhatramakki, Dong, Chhabra and Hart, 2000). In this study, however, they were located on LGs 1 and 10, respectively. Therefore, LGs 1 and 10 might be part of the same linkage group. The chromosomal location of sorghum primer *Xtxp*29 (the remaining sorghum SSR primer that was mapped on chromosome 11 in this study) in the sorghum genome is not known (Bhatramakki, Dong, Chhabra and Hart, 2000).

Table 14: **Number of markers and length (in cM) of the 13 linkage groups produced using F2 progeny from the cross SAR 29 x SV-1.**

Linkage Group	Number of Markers	Length [Haldane cM]	Distorted segregation
1	8	52.3	0
2	4	32.1	1
3	4	49.8	1
4	4	34.9	1
5	6	95.5	6
6	3	32.7	1
7	3	46.8	2
8	3	51.6	2
9	2	17.2	1
10	2	14.1	0
11	2	22.4	2
12	2	17.6	0
13	2	27.5	2
13	45	494.5	19

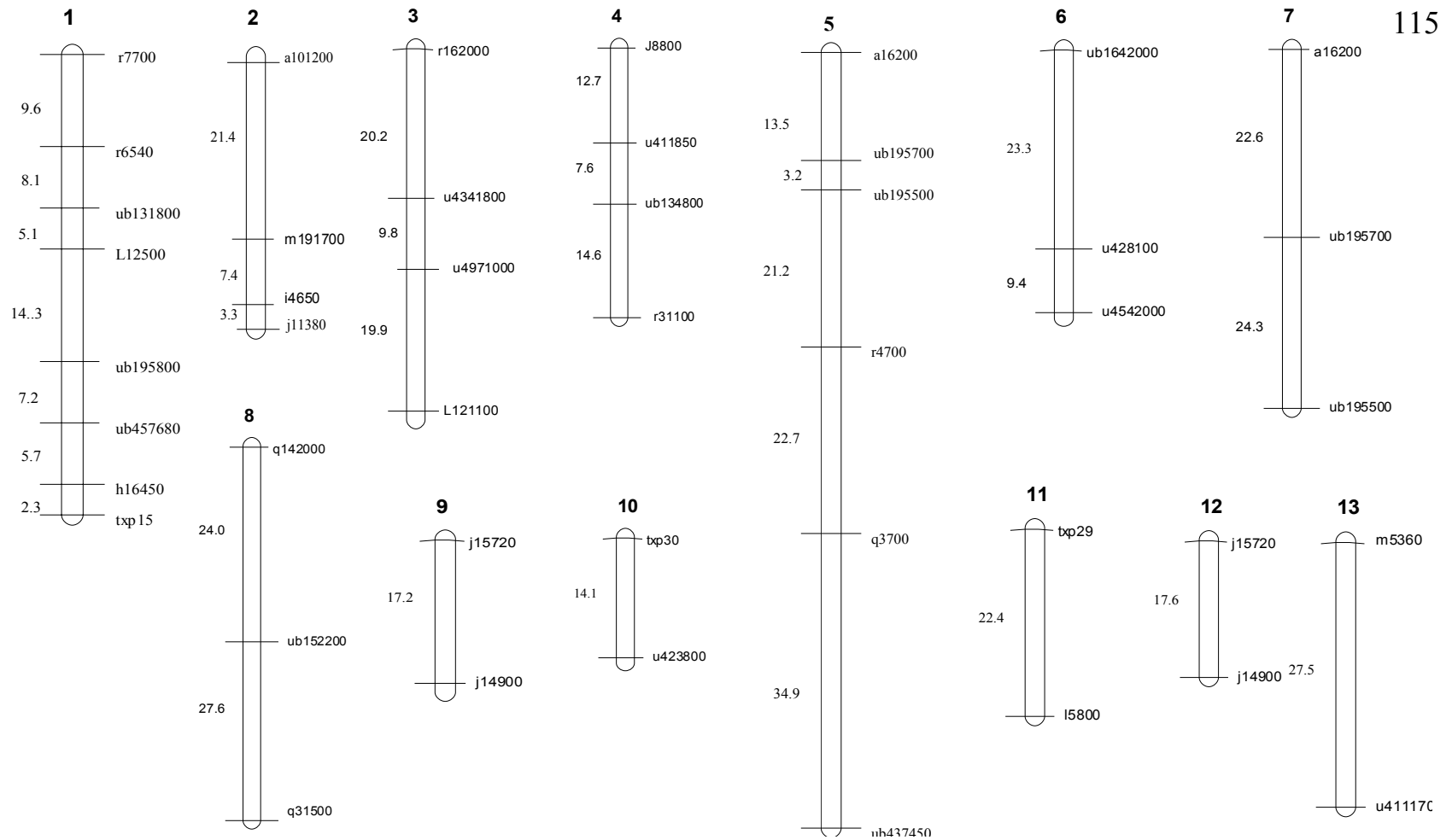


Figure 12: Linkage map constructed from F2 progeny from crosses between SV-1 and SAR 29. Names on the right of each linkage group are the marker names. Numbers on the left of each linkage group indicate distance between markers in cM (Haldane).

Of all the mapped markers, 19 showed distorted segregation. With the exception of LGs 1, 10 and 12, all the other LGs contained at least one marker with distorted segregation. All the six markers on LG 5 exhibited distorted segregation. This might be confirmation of clustering of markers with distorted segregation in sorghum (Haussmann *et al.*, 2002) and several other crops (Menendez, Hall and Gepts, 1997; Lu, Sosinski, Reighard, Baird and Abbott, 1998). It has been reported that segregation distortion for any marker is dependent on the overall segregation distortion of the genomic region where they are located (Winter *et al.*, 2000). Some genomic regions are more prone to distorted segregation than others.

5.4 Discussion

Bulked segregant analysis and segregation analysis of individual molecular markers were both unable to detect a marker that was linked to witchweed resistance. It has been reported that a high level of genetic uniformity exists in *S. bicolor* (Tao, Manners, Ludlow, and Henzell, 1993). This implies that a large number of primers may have to be screened in order to identify the ones that will generate polymorphisms that are associated with *S. asiatica* resistance. Bulked segregant analysis has been used in many studies to tag both monogenic and polygenic traits of economic importance (Michelmore *et al.*, 1991; Barua, Chalmers, Hackett, Thomas, Powell, and Waugh 1993; Zhang *et al.*, 1996; Heller, Schondelmaier, Steinrucken, and Jung 1996). It was however observed that one individual in 10 carrying a certain RAPD locus was enough to contaminate an otherwise null genotype bulk, resulting in the amplification of a RAPD product

where there should be no amplification (Grattapaglia, Bertolucci, Penchel, and Sederoff, 1996). Failure of BSA in detecting linkage in this investigation could only have possibly resulted from errors in sampling during bulk composition. Such errors would result from the classification of a susceptible F2 genotype as being resistant, or a resistant one as being susceptible. However, the chances of having such a misclassification were minimised through the selection of genotypes that were on the extremes of resistance (>2.5 cm) and susceptibility (<1.0 cm) when pooling the DNA samples.

RAPD markers are inherited as dominant genetic markers. This limits the application of this marker type, particularly in cases where one would like to distinguish homozygous from heterozygous genotypes. More than two phenotypic classes were observed as band intensity differences for some RAPD fragments. However, only the presence or absence of a RAPD band was considered. Segregation of phenotypes that differ in RAPD fragment staining intensities may represent true genetic variation, although heritability of these allelic phenotypes is not clear. There is evidence that RAPD fragments can arise from mismatched sequence hybridisations between primer and the DNA template (Williams, Kubelik, Livak, Rafalski and Tingey, 1990). Therefore, sequence variation at specified primer sites may not account for all the intensity variants that were observed in this study.

The PCR amplification that generates RAPD fragments of interest is very sensitive to specific reaction conditions (Karp and Edwards, 1997). RAPD markers are therefore not reproducible outside mapping populations in which they have been detected. A different population will

produce different amplification profiles using the same primers. This is what necessitates the conversion of a RAPD marker that is linked to a gene of interest into a more breeder friendly marker such as a sequence characterised amplified region (SCAR). The cloned RAPD fragment can also be labeled non-radioactively and used as a witchweed specific RFLP probe. A codominant SCAR/RFLP probe will be quite suitable for most applications in plant breeding such as inheritance studies, genome mapping and MAS (Garcia, Stalker, Shroeder and Korchert, 1996).

The observation that LGs 1 and 10 might be part of the same group suggests that if additional markers are mapped, then the gaps between these two linkage groups can be filled. Coincidentally, it is also in this linkage group that other researchers have located the *lgs* locus (Bennetzen, Gong, Xu, Newton and de Oliveira, 2000). Mapping more markers will also obviously result in presently unassigned markers being assigned to certain LGs. It will be advisable to use sorghum microsatellite or simple sequence repeat (SSR) markers to 'tag' the genes for witchweed resistance. This may be a very focused approach since locus specific SSRs will be utilised. Microsatellite markers are codominant and highly reproducible in different populations, even in those in which the markers have not been mapped. AFLP markers have also been used extensively in the construction of sorghum genetic linkage maps (Haussmann *et al.*, 2000a; 2001; 2002). High reproducibility, rapid generation and a high frequency of identifiable polymorphisms make AFLP DNA analysis an attractive technique for use to tag *S. asiatica* resistance. Both SSRs and AFLP can also be useful as anchors for the ten chromosomes of sorghum.

It is also suggested here that a recombinant inbred population be used to identify DNA markers that are linked to *S. asiatica* resistance and to saturate the available molecular linkage map. The advantage of recombinant inbred lines (RILs) over F₂s is the opportunity that will be provided to test the genotypes for *S. asiatica* resistance in multilocal trials over several years. This is because RILs represent a permanent population in which segregation is fixed. Therefore very reliable segregation data can be obtained from them for use in QTL analysis of *Striga* resistance. Relatively stable QTLs can potentially be identified with such replicated data. Additional markers and traits of economic importance such as yield can be placed on the developed map at any time. Recombinant inbred lines can be developed in a minimum of six to seven seasons. There was therefore a time limitation to the development of RILs in this study.

Segregation distortion was most pronounced for RAPDs mainly because the majority of markers that were analysed were RAPDs. In other studies, the extent of segregation distortion was not dependent on the marker type, but more on the overall segregation distortion of the region where they resided (Winter *et al.*, 2000). Distorted segregation can be detected with almost any kind of genetic marker, including morphological mutant markers, isozymes and DNA markers (Xu, Zhu, Xiao, Huang and McCouch, 1997). The genetic basis of segregation distortion may be the abortion of male or female gametes or the selective fertilization of particular gametic genotypes. Distortion at a marker locus is also caused by linkage between the marker and the gametophyte gene (*ga*) that confers lower pollinating ability, and it is also

referred to as a gamete eliminator or pollen killer causing abortion of gametes (Xu *et al.*, 1997), or 'outlaw genes' (Zamir and Tadmor, 1986). The tighter the linkage between a marker locus and distortor locus means that more intense skewing will be observed for that marker (Xu *et al.*, 1997). This explains why some genomic regions are prone to higher levels of segregation distortion than others, and why some markers show more extreme skewing than others.

In cases where crossovers are clustered or suppressed in certain regions rather than being randomly distributed, the genetic map will be a distortion of the physical distances separating loci on the chromosomes. This is because the distance between points on a genetic map is just a representation of the recombination frequency between those two points. The genetic distances that are shown on LG 5 may not be a true reflection of the actual physical distance between the loci since there appears to be a clustering of distorted loci on this LG. It has been suggested that the unequal segregations should be considered in breeding programmes (Xu *et al.*, 1997; Zamir and Tadmor, 1986). If a target locus is known to be linked to a segregation distortion locus and is underrepresented in the population, the frequency of the favourable allele can be increased by using molecular markers to select for recombinants in the region of interest (Xu *et al.*, 1997). Segregation distortion has been reported to be around 20 % on average for most segregating populations (Winter *et al.*, 2000). The higher level of distortion observed in this study, 36 %, may be attributed to a relatively small sample size of an average of 60.44 F₂ progeny per primer. Competition also occurs in the amplifications of RAPD fragments and may have been a source of genotyping errors and thus contributed to the

observed distorted segregation. Hallden *et al* (1996) defined competition in RAPD analyses as the repeatable disappearance of an expected band in certain genotypes but not in others. They reported that certain primers and bands are more liable to errors due to competition than others.

The currently available molecular map was derived from an intraspecific cross, the parent materials having been chosen from the cultivated gene pool of sorghum. Such a map is very useful for practical breeding applications. This is because the markers that are identified are polymorphic within the cultivated gene pool, and they are likely to be present in other crosses between cultivated genotypes that are of interest to breeders (Menendez, Hall, and Gepts 1997). It is recognized that most sorghum genetic maps have been constructed using wide crosses (Hausmann *et al.*, 2002). The disadvantage with such maps is that loci that are identified may be polymorphic only between divergent genotypes. Molecular maps based on crosses involving wild progenitors also have little direct application in breeding programmes that usually exploit intraspecific variation within cultivated forms (Menendez *et al.*, 1997).

5.5 Conclusion

A total of 440 RAPD, 24 sorghum SSRs and six maize SSRs were used to screen SV-1 and SAR 29 for polymorphisms. Out of these, 199 markers (187 RAPD; 10 *Xxtp* Sorghum and 2 maize SSR) were polymorphic between parental genotypes. None of these molecular markers was linked to the loci for low *S. asiatica* seed germination stimulant production. Ninety-nine marker loci that were polymorphic between the parent genotypes (10 SSR and 89 RAPD) were

scored in the segregating F2 population consisting of 77 progeny. Linkage analysis resulted in the subsequent construction of a molecular marker linkage map consisting of 45 markers that were distributed over 13 linkage groups and spanning a total distance of 494.5 cM (Haldane). Fifty-four loci were completely unlinked and could not be included in the map. Unlinked loci included the locus for low *S. asiatica* seed germination stimulant production. The molecular marker linkage map that was generated consisted of markers that are polymorphic within the cultivated gene pool of sorghum. This map is therefore potentially very useful for practical plant breeding applications because the polymorphisms that were identified are likely to be detected again in other crosses between cultivated sorghum genotypes.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Parent characterization, combining ability and path coefficient analyses

Cultivars SAR 19 and SAR 29 were resistant while SV-1, SAR 16 and DC75 were susceptible to *S. asiatica*. This classification was determined from results of screening using the pot culture and agar gel techniques. It was also apparent from pot screening experiments that despite being resistant, low levels of *S. asiatica* infestation could cause massive yield reduction on cultivar SAR 19. It was therefore apparent that SAR 19 should not be used as the only source of witchweed resistance in a breeding programme. Rather, several sources should be utilized simultaneously so that different mechanisms of witchweed resistance can be assembled in a single genotype. The multiple mechanisms in such genotypes will complement each other to prevent high yield losses that might be associated with a single and/or a few witchweed resistance mechanisms. In addition to breeding for low numbers of emerged *Striga*, a concomitant objective could be breeding for minimal losses resulting from few attached witchweeds. The magnitude of yield loss for a particular sorghum genotype can be quantified by screening it under both infested and non-infested conditions.

Cultivars SAR 19 and SAR 29 were good general combiners for low witchweed numbers. A comparison of GCA effects suggested that witchweed resistance is transmissible to progeny. Resistant genotypes imparted witchweed resistance by reducing *S. asiatica* counts among

their F2 progeny. Likewise, susceptible cultivars transmitted susceptibility by increasing the number of parasites in their progeny. Breeders can therefore expect to make genetic gains by selecting from segregating generations derived from crosses that include witchweed resistant genotypes. However, cultivars SAR 19 and SAR 29 had low yields in general and this would make it difficult for them to be accepted by SH farmers without an improvement of their yield potential. Cultivar SV-1 is drought tolerant, high yielding and well adapted to marginal rainfall areas of Zimbabwe. This cultivar has to be improved for witchweed resistance or alternatively, its good yield potential can be transferred to the SAR cultivars. Progeny derived from crosses between SARs 19 and 29, and cultivar SV-1 therefore provide a good population from which to select for a combination both *S.asiatica* resistance and high yielding ability. However, multiple crosses involving these basic parents and many more should be generated in order to increase variation in the base populations. For instance, the F1 obtained from crossing SAR 19 x SV-1, and that from SAR 29 x SV-1, can be crossed with each other. Other resistant or high yielding cultivars can also be crossed to these products in various combinations.

Grain yield and plant parameters that were important for the cultivars tested were head weight, 100 seed weight, plant height, leaf width and days to 50 % flowering. However, the direct and indirect contribution of each of these parameters to yield was influenced by the type of cultivar (resistant or susceptible) and whether there was witchweed infestation or not. The development of elite witchweed resistant cultivars therefore necessitates concurrent and independent selection for witchweed resistance and high yield under *S. asiatica* infested

conditions. This is important because there were no consistent yield components under infested and non-infested conditions. In general, head weight was the most important sorghum grain yield determinant, having moderate to high direct contributions. Improvement in yield should therefore be based upon selection for improved head weight, though the rest of the above parameters should form part of the selection criteria. Head weight can be assessed faster and easier than measuring grain yield. Direct effects of *S. asiatica* counts on sorghum grain yield were low. *Striga asiatica* caused yield reduction by indirectly affecting sorghum grain yield components, mostly head weight. It is suggested that investigations be done to find out if there is no linkage or association between the presence of witchweed resistance factors and low yield potential in sorghum.

6.2 Germplasm characterization and inheritance of low witchweed seed germination stimulant production

A single recessive gene and some modifiers controlled the inheritance low *S. asiatica* seed germination stimulant production trait in SARs 19 and 29. Minor genes were observed to modify *lgs* production as judged by the variation for MGD that was found among the low and high germination stimulant classes of F2 progeny. Selection for low *S. asiatica* seed germination stimulant production should be successful if sorghum breeding programmes for resistance against witchweeds are able to generate sufficient genetic variability for the trait. This requires the identification of good sources of low stimulant producers and their subsequent use in various crossing programs.

The agar gel assay would be a simple and efficient tool for use in selecting for low *Striga* seed germination stimulant production, especially in the early segregating generations. However, it is recommended that host differential sorghum lines be developed. These genotypes could be used to identify and classify different *Striga* collections into pathotypes that occur in Zimbabwe. Promising witchweed resistant sorghum cultivars would be tested against the major pathotypes in water agar. Multilocal field screening of the same materials in witchweed “hot spots” over a number of seasons should follow agar or pot screening. Screening under field conditions will cater for all resistance mechanisms, since field resistance to *S. asiatica* infestation is a result of the expression of all mechanisms within a certain genotype. Cultivars that will show resistance after such rigorous screening will be having truly stable and broad-based resistance to *Striga*, and should be suitable for release to farmers.

It is also important to develop or to refine laboratory assays for use in screening for specific resistance, tolerance and avoidance mechanisms as they have been itemised in section 2.5. Since laboratory assays are capable of screening individual progeny, early generation selection will be made possible. Early generation selection can result in enormous savings of resources because the current practice is to defer selection for witchweed resistance until true breeding progenies are derived. This is costly since a large number of progeny have to be maintained for several generations prior to evaluation in field trials.

6.3 Search for molecular markers that are linked to *S. asiatica* resistance and prospects for their use in marker assisted breeding

A total of 199 markers (187 RAPD; 10 *Xtp* and 2 maize SSR) were polymorphic between cultivars SAR 29 and SV-1. Molecular markers that are linked to the gene(s) for low *S. asiatica* seed germination stimulant production could not be identified using both BSA and individual molecular marker segregation analysis. Instead, a molecular marker linkage map was constructed and it consisted of 45 markers that were distributed over 13 linkage groups and spanned a total distance of 494.5 cM (Haldane). Fifty-four loci were completely unlinked and could not be included in the linkage map. Unlinked loci included the locus for low *S. asiatica* seed germination stimulant production. The molecular marker linkage map that was generated consisted of markers that are polymorphic within the cultivated gene pool of sorghum. This map is therefore potentially very useful for practical plant breeding applications because the polymorphisms that were identified are likely to be detected again in other crosses between cultivated sorghum genotypes.

Many more molecular markers would have to be screened to identify the ones that will generate polymorphic amplification products that are linked to *S. asiatica* resistance. It would be necessary to score the markers using a bigger segregating population. However, it is recommended that a recombinant inbred population be used to identify markers that are linked to *S. asiatica* resistance and to saturate the available molecular linkage map. Recombinant

inbred lines (RILs) will facilitate identification of stable QTLs for both field resistance to *S. asiatica* and other traits of economic importance such as yield. It is advisable to use sorghum SSRs for this purpose. Simple sequence repeat (SSR) markers that are placed every 20 cM along all the chromosomes of sorghum should be selected for segregation and linkage analysis. If there are sufficient financial resources and facilities, then AFLP and RFLP markers could also be used in combination with sorghum SSRs. Apart from the higher information content revealed per primer and/or probe due to codominance of the above markers, they will also serve as anchors that will facilitate assignment of LGs to the chromosomes of sorghum.

Random amplified polymorphic DNA markers can still be utilised in combination with the above markers. The advantage of using RAPD markers in this population is that those primers that are polymorphic between parental cultivars SAR 29 and SV-1 have already been identified. So there will be no need for primer screening between parents again, apart from screening the primers with a few segregating progenies to identify those that will be segregating in the population. Random amplified polymorphic DNA markers will help to saturate the map since they are expected to detect higher levels of variation due to the sampling of non-coding regions which are prone to mutation than the more highly conserved coding regions of the above codominant markers. If a RAPD marker is linked to *S. asiatica* resistance, then it can be converted to a codominant SCAR marker that is more suitable for MAS applications in plant breeding.

Availability of a saturated molecular linkage map of sorghum will facilitate identification of molecular markers that ‘tag’ genes for other *Striga* resistance mechanisms. Once other resistance mechanisms such as *HR*, *IR*, antibiosis and avoidance are ‘tagged’, MAS techniques can be developed and used to increase the efficiency of *Striga* resistance breeding in sorghum. The concept of gene pyramiding could easily be pursued when more than one *S. asiatica* resistance mechanism can be assembled in a single genotype through the use of MAS. It is also worthwhile to screen markers that have already shown linkage to *Striga* resistance genes in rice and sorghum. Their usefulness in detecting *lgs* producing genotypes in Zimbabwe can thus be assessed.

6.4 General strategies for breeding and control of *S. asiatica*

For effective *Striga* control, resistant cultivars must be integrated with other control methods such as weeding, cereal/legume intercropping, fertilisation and crop rotation. The chosen treatments and cultural methods should basically reduce witchweed emergence, prevent *Striga* reproduction, reduce seed in the soil bank and also reduce damage to the host crop. A joint development of integrated *Striga* management strategies by breeders, agronomists, pathologists and farmers should therefore contribute to more sustainable sorghum production in *Striga* infested areas of semi-arid Africa. Farmers should participate in identification of parents for use in backcross programs and in determining the most important selection traits for each target environment. Extensive feedback between farmers and breeders will ensure

that the cultivars developed are adapted to farmer circumstances and meet end-user preferences. Cultivars that are developed with farmer participation will also be readily adopted when they are released because the farmers will be able to identify with them.

Various breeding strategies have been proposed to develop sorghum cultivars with long-term and polygenic resistance to witchweeds. A recurrent selection programme or pedigree method is recommended for adoption, and should develop and utilize breeding populations with multiple sources of resistance, respectively. Multiple mechanisms of resistance can be obtained by utilising multiple crosses that involve several resistant sorghum genotypes. However, standard measures of multitrait improvement for self-pollinated crops are essential to combine *Striga* resistance with grain yield and other specified traits required for different environments and/or end-users. Efforts should also be made to understand the patterns of variability of *S. asiatica* within and between different regions. This will enable breeding programmes to target sources of resistance at different areas and to understand the nature of resistance required.

When field screening is undertaken, emphasis should be placed on genotypes that support few witchweed plants under infested conditions. Early generation selection is recommended for isolation of genotypes with individual resistance mechanisms using laboratory procedures. The agar gel and extended agar gel assays can be used to screen for the *lgs* production, *lhf*, and *HR*. The paper roll technique (PRT) can also be used to screen for *HR* and *IR*. However, the PRT still needs some refinement for it to be employable on a larger number of progenies

in high throughput breeding programs. Techniques that can be used to screen for the rest of the resistance mechanisms that have been cited in this study still need to be developed. The efficiency of *Striga* resistance breeding in sorghum could thus be improved by combining all laboratory assays with field evaluation, and by developing marker-assisted selection techniques. Multilocal trials are recommended for all promising witchweed resistant cultivars to ascertain their performance across different environments and different physiological strains. Breeding lines with different resistance mechanisms could be combined to form heterogeneous synthetic cultivars. The different mechanisms of resistance will complement each other and hence impart some durability to the resistance of the synthetics.

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APPENDICES

Appendix 1: Formulae for combining ability analyses (Griffing, 1956)

1.1 Calculation of variances

$$\text{Variance } (g_i - g_j) = 2\sigma^2 / (p-2)$$

$$\text{Variance } (S_{ij} - S_{ik}) = 2(p-3) \sigma^2 / (p-2)$$

$$\text{Variance } (S_{ij} - S_{kl}) = 2(p-4) \sigma^2 / (p-2)$$

1.2 Estimation of GCA effects

$$g_i = \{pX_{i.} - 2X_{..}\} / p(p-2)$$

1.3 Estimation of SCA effects

$$S_{ij} = X_{ij} - (X_{i.} + X_{.j}) / (p-2) + 2X_{..} / [(p-1)(p-2)]$$

1.4 Genetic Model for Griffing's method 4, -one set of F1's but neither parents nor reciprocal F1's.

$$X_{ij} = u + g_i + g_j + s_{ij} + \sum e_{ijkl} / bc_{kl}$$

Where;

u = the population mean,

g_i and g_j = general combining ability (GCA) effects of the i^{th} and j^{th} parents, respectively

s_{ij} = specific combining ability (SCA) effects (interaction of the i^{th} and j^{th} parents)

e_{ijkl} = error effect peculiar to the $ijkl$ observation

bc_{kl} = block effect

($k=1, 2, \dots, r$; $i=j=1, 2, \dots, p$) for p inbred lines and r replications

Restrictions $\sum g_i = \sum s_{ij} = 0$ are imposed on the combining ability effects

Appendix 2: ANOVA tables

Table 2.1: ANOVA table for sorghum grain yield pot^{-1} of parent genotypes whose pots were not infested with *S. asiatica* at Henderson Research Station, 1998/99 season

Source of Variation	D.F.	S.S	M.S	F	P
Replication	4	0.06	3.101	0.08	0.970
Treatment	3	12.41	0.020	12.88	0.000
Error	12	2.89	0.241		
Total	19				
LSD (0.05)		0.676			
CV%		15.88			

Table 2.2: ANOVA table for sorghum grain yield pot^{-1} of parent genotypes that were infested with *S. asiatica* at Henderson Research Station, 1998/99 season

Source of Variation	D.F.	S.S	M.S	F	P
Replication	4	4.27	1.424	3.15	0.065
Treatment	3	4.86	1.216	2.69	0.083
Error	12	5.43	0.452		
Total	19				
LSD (0.05)		1.036 ns ^Y			
CV%		33.93			

^Y ns- non-significant

Table 2.3: ANOVA table for combined analysis of sorghum grain yield pot^{-1} of infested and non-infested parent genotypes.

Source of Variation	D.F	S.S.	M.S	F	P
Location	1	320.23	320.23	28.24	0.002
Error	6	68.04	11.34		
Factor A	4	513.55	128.39	16.16	0.000
LA	4	103.23	25.81	3.25	0.029
Error	24	190.63	0.347		
Total	39				
LSD (0.05)	2.908				
CV%	40.33				

Table 2.3: ANOVA table for sorghum grain yield plot^{-1} of infested F2 progenies derived from diallel crosses between SAR cultivars and SV-1.

Source of Variation	D.F.	S.S	M.S	F	P
Replication	4	0.28	0.070	2.62	0.0653
Treatment	5	0.05	0.010	0.36	0.868
Error	20	0.54	0.027		
Total	29				
LSD (0.05)	0.217 ns ^Y				
CV%	25.76				

^Y ns- non-significant**Table 2.4:** ANOVA table for *S. asiatica* counts plot^{-1} of F2 progenies derived from diallel crosses between SAR cultivars and SV-1.

Source of Variation	D.F.	S.S	M.S	F	P
Replication	4	113.24	28.309	1.07	0.396
Treatment	5	1849.17	369.833	14.01	0.000
Error	20	527.94	26.397		
Total	29				
LSD (0.05)	6.78				
CV%	51.98				

Table 2.5: ANOVA table for days to *S. asiatica* emergence of F2 progenies derived from diallel crosses between SAR cultivars and SV-1.

Source of Variation	D.F.	S.S	M.S	F	P
Replication	4	672.86	168.216	0.21	0.932
Treatment	5	10647.24	2129.448	2.62	0.056
Error	20	16283.74	814.187		
Total	29				
LSD (0.05)		37.64 ns ^Y			
CV%		34.64			

^Y ns- non-significant

Table 2.6: ANOVA table for *S. asiatica* seed germination distances of four parent genotypes (SV-1 and SARs 16, 19 and 29) assays.

Source of Variation	D.F.	S.S.	M.S	F	P
Replication	4	1.66	0.415	1.14	0.3830
Treatment	3	12.62	4.206	11.57	0.0007
Error	12	4.36	0.363		
Total	19				
LSD (0.05)		0.93			
CV%		44.84			

Table 2.7: ANOVA table for *S. asiatica* seed germination percentages for four parent genotypes from agar assays.

Source of Variation	D.F.	S.S	M.S	P
Replication	4	73.51	18.378	0.7749
Treatment	3	1546.34	515.445	0.0005
Error	12	496.82	41.402	
Total	19			
LSD (0.05)		9.913		
CV%		55.76		

Appendix 3: Table of Means

Table 3.1: Sorghum mean grain yields plot⁻¹ for F2 progenies at Henderson Research station, 1998/99 season

	Grain Yield (g ⁻¹)	
	Untransformed	Square -root Transformed
SAR 16 X SAR 19	4.950	0.692
SAR 16 X SAR 29	3.521	0.597
SAR 16 X SV-1	3.403	0.577
SAR 19 X SAR 29	3.883	0.631
SAR 19 X SV-1	4.060	0.645
SAR 29 X SV-1	5.232	0.674
P	0.521	0.8675
LSD (0.05)	0.143 ns	0.217 ns ^Y
CV%	42.62	25.76

-Numbers in parenthesis are transformed by squareroot (x+0.5)

^Y ns- non-significant

Table 3. 2: Table of means for agar assay maximum germination distances (MGD) and germination percentages

	Germination distance (cm)	Germination Percent
SAR 19	0.586c	3.34b
SV-1	2.560a	22.70a
SAR 16	1.544b	17.58a
SAR 29	0.688c	2.50b
P	0.0007	0.0005
LSD (0.05)	0.929	9.913
CV %	44.84	55.76
Correlation Coefficient (r) = 0.883		

Appendix 4: Correlation coefficients of sorghum grain yield and witchweed components.

Matrix 1: Infested resistant parents

	C3 [¶]	C4	C5	C6	C7	C8	C9	C10	C11
C4	0.686								
C5	0.637	0.974**							
C6	0.768*	0.568	0.536						
C7	0.906**	0.776*	0.777*	0.573					
C8	0.748*	0.663	0.652	0.473	0.831*				
C9	0.741*	0.529	0.587	0.806*	0.723*	0.446			
C10	0.467	0.047	0.061	0.452	0.327	0.155	0.671		
C11	0.748*	0.173	0.194	0.604	0.647	0.676	0.676	0.651	
C12	-0.018	-0.261	-0.317	-0.024	-0.101	-0.036	0.097	0.708*	0.263
C13	-0.350	-0.091	-0.134	-0.772*	-0.155	0.003	-0.769*	-0.670	-0.486
C14	0.481	0.061	0.092	0.705	0.310	0.017	0.837**	0.795*	0.595
	C12	C13							
C13	-0.246								
C14	0.332	-0.922**							

[¶] C3=stover weight; C4=head weight; C5=yield; C6=100 seed weight; C7=plant height; C9=head width; C10=leaf width; C11=leaf length; C12=50% flowering; C13=days to *Striga asiatica* emergence; C14= *Striga asiatica* counts

*,**Significant (P<0.05) and (P<0.01) respectively

Matrix 2: Non-infested resistant parents

	C3 [¶]	C4	C5	C6	C7	C8	C9	C10	C11
C4	0.260								
C5	0.284	0.989**							
C6	-0.427	0.010	0.009						
C7	0.414	0.581	0.617	-0.626					
C8	0.364	0.795*	0.797*	-0.185	0.410				
C9	0.543	0.500	0.480	-0.349	0.462	0.602			
C10	0.367	-0.648	-0.673	-0.591	-0.074	-0.375	0.000		
C11	-0.072	0.292	0.385	-0.215	0.677	0.154	-0.171	-0.299	
C12	-0.051	-0.836**	-0.802*	0.248	-0.707	-0.667	-0.466	0.403	-0.430

[¶] C3=stover weight; C4=head weight; C5=yield; C6=100 seed weight; C7=plant height; C9=head width; C10=leaf width; C11=leaf length; C12=50% flowering; C13=days to *Striga asiatica* emergence; C14= *Striga asiatica* counts

*, **Significant (P<0.05) and (P<0.01) respectively

Matrix 3: Infested susceptible parents

	C3 [¶]	C4	C5	C6	C7	C8	C9	C10
C4	0.045							
C5	-0.015	0.991**						
C6	0.549	0.489	0.457					
C7	0.505	0.472	0.442	0.645				
C8	0.318	0.878**	0.849**	0.646	0.728*			
C9	0.234	0.898**	0.875**	0.606	0.697	0.941**		
C10	0.715*	0.499	0.423	0.607	0.676	0.582	0.529	
C11	-0.179	0.086	0.117	-0.017	0.546	0.090	0.133	0.168
C12	0.641	0.105	0.034	0.778*	0.611	0.417	0.251	0.595
C13	-0.318	0.244	0.245	0.239	0.165	0.176	0.054	0.119
C14	-0.573	0.453	0.502	-0.426	-0.374	0.163	0.239	-0.364
	C11	C12	C13					
C12	0.033							
C13	0.391	0.262						
C14	-0.116	-0.701	-0.025					

[¶] C3=stover weight; C4=head weight; C5=yield; C6=100 seed weight; C7=plant height; C9=head width; C10=leaf width;
C11=leaf length; C12=50% flowering; C13=days to *Striga asiatica* emergence; C14= *Striga asiatica* counts

*,**Significant (P<0.05) and (P<0.01) respectively

Matrix 4: Non-infested susceptible parents

	C3 [¶]	C4	C5	C6	C7	C8	C9	C10
C4	-0.229							
C5	-0.278	0.993**						
C6	0.026	-0.683	-0.650					
C7	0.469	0.424	0.384	-0.552				
C8	-0.550	0.845**	0.876**	-0.506	-0.016			
C9	-0.446	0.761*	0.793*	-0.543	0.297	0.777*		
C10	-0.010	0.683	0.668	-0.859**	0.687	0.437	0.637	
C11	0.122	0.490	0.459	-0.758*	0.642	0.180	0.390	0.912**
C12	0.494	-0.882**	-0.882**	0.627	-0.163	-0.873**	-0.745*	-0.618
	C11							
C12	-0.506							

[¶] C3=stover weight; C4=head weight; C5=yield; C6=100 seed weight; C7=plant height; C9=head width; C10=leaf width;
C11=leaf length; C12=50% flowering; C13=days to *Striga asiatica* emergence; C14= *Striga asiatica* counts

*,**Significant (P<0.05) and (P<0.01) respectively

Appendix 5: Characteristics of SSR primer sets

5.1 Sorghum SSR primer sets

Primer Pair	Linkage group	Sequence of forward primer	Sequence of Reverse primer	Size	Ann. T ⁰ C
Txp 6	B	AATACTAGGTGTCAGGGCTGTG	ATGTAACCGCAACAACCAAG	173	55
Txp 7	B	AGCAGGCGTTTATGGAAG	ATCCTCATACTGCAGGACC	232	50
Txp 9	I	ATCGGATCCGTCAGATC	TCTAGGGAGGTTGCCAC	120	50
Txp 12	D	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	193	55
Txp 14	J	GTAATAGTCATGACCGAGG	TAATAGACGAGTGAAAGCCC	149	50
Txp 15	J	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	215	55
Txp 18	H	ACTGTCTAGAACAAGCTGCG	TTGCTCTAGCTAGGCATTTC	231	55
Txp 24	D	TTGTGTAGTCCATCCGATGC	TTCTAAGCCCACCGAAGTTG	145	60
Txp 25	B	CCATTGAGCTTCTGCTATCTC	CATTTGTCACCACTAGAACCC	139	55
Txp 26	unkn.	AAGTGTAGTAGCAGTTTAGTCTC	TAGGTATCAAAGGACCAAGG	184	50
Txp 29	unkn.	TAGGGCAGTGGTTAGTCGTG	TACAAGTGGTGGTCCGAGG	338	60
Txp 30	J	AAAAAGGACGCGCAGCTG	CTGGTCTCCACCATCCGTAG	273	60
Txp 32	A	AGAAATTCACCATGCTGCAG	ACCTCACAGGCCATGTGC	133	60
Txp 43	A	AGTCACAGCACACTGCTTGTC	AATTACCTGGCGCTCTGC	171	60

5.2. Maize SSR primer sets

Sourced from MaizeDB, <http://www.agron.missouri.edu> -, 2001

Bins	SSR	AT_Locus	Repeat	Forward_Primer	Reverse_Primer	Source	GenBank
1.09	phi055	glb1	GAA	GAGATCGTGTGCCCCGCACC	TTCCTCCTGCTCCTCAGACGA	Chin, E	X59085
4.04	phi074	zp22.1	CAA	CCCAATTGCAACAACAATCCTTGGCA	GTGGCTCAGTGATGGCAGAAACT	Chin, E	J01245
4.11	phi076	cat3	AGCGGG	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC	Pioneer	L05934
8.08-	phi015	gst1	AAAC	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG	Pioneer	M16900
9.03	phi061	wx1	TTCT-GTAT	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	Chin, E	M24258
4.03	phi021	adh2	AG	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA	Chin, E	X02915
4.05	nc005	gpc1	CT	CCTCTACTCGCCAGTCGC	TTTGGTCAGATTTGAGCACG	Senior,L	X15596

Appendix 6: Reagents and solutions for RAPDs and SSRs

6.1 Reagents used for DNA isolation

SDS extraction buffer

1 M Tris-HCL (pH 8.0)	100 ml
0.5 M EDTA (pH 8.0)	4 ml
3 M NaCl	167 ml
Polyvinyl poly-pyrrolidone	10 g
10 % SDS	170

Make up to 1000ml with distilled water

6.2 Reagents for the RAPDs

dNTP + MgCl₂ solution

25 mM dNTP mix	80 µl (20 µl of each 100 mM dNTP stock)
25 mM MgCl ₂	100 µl
Sterile H ₂ O	820 µl

5 X buffer (PCR)

1 M Tris base (pH 8.5)	25 ml
1 M KCL	10 ml
BSA	0.25 g
Ficoll 400	7.5 g
Xylene cyanole	0.05 g

5 X TBE

Tris base	54 g
Boric acid	27.5 g
0.5 M EDTA (pH 8.0)	20 ml

Make up to 1000 ml with distilled water

6.3 Reagents and solutions for the SSRs

40 % acrylamide

acrylamide	38 g
N,N'-methylenebisacrylamide	2 g

Make up to 1000 ml with H₂O. Heat solution to 37 °C to dissolve the chemicals

5 X TBE

Tris base	54 g
Boric acid	27.5 g
0.5 M EDTA (pH 8.0)	20 ml

Make up to 1000 ml with distilled water

10 % Ammonium persulfate

Ammonium persulfate	0.1 g
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Make up to 1000 µl with distilled water in an eppendorf tube

6 % polyacrylamide gel

40 % acrylamide	7.5 ml
5 X TBE	10 ml
Water	32.1 ml
10 % Ammonium persulfate	400 µl
TEMED	20 µl

6 X buffer type III (Loading dye)

0.25 % bromophenol blue
 0.25 % xylene cyanol FF
 30 % glycerol in water