COMPARATIVE ANALYSIS OF AGRONOMIC, METABOLIC AND NUTRITIONAL FUNCTIONAL PROPERTIES OF DIFFERENT SORGHUM (*SORGHUM BICOLOR*) GENOTYPES EXPOSED TO *STRIGA HERMONTHICA* IN ZIMBABWE

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ABSTRACT

Striga hermonthica is a widespread parasitic weed that poses a major threat to sorghum production in many parts of sub-Saharan Africa, often leading to substantial yield losses in affected regions. This study investigated the agronomic performance, biochemical response, nutritional stability, and genetic variation of 74 sorghum genotypes from Zimbabwe and South Africa under varying levels of *Striga* infestation (0 mg, 2.5 mg, and 5 mg per pot), simulating densities of up to 40,000 plants per hectare. The research aimed to identify resistant genotypes and genetic markers associated with dual resilience—*Striga* tolerance and nutritional quality—to inform breeding strategies. Agronomic assessments showed significant ($p < 0.05$) reductions in plant height (up to 52%), chlorophyll content, panicle height, and grain yield in most genotypes under *Striga* stress. At the 5 mg infestation level, 75% of genotypes experienced severe biomass loss, while 41% recorded significant dry grain weight reductions at 2.5 mg. However, a subset of genotypes—including SCSHYB012150, SCSHYB017166, CHR20, SA2133, SA3028, SA1794, SA1617, and SA4186—demonstrated stable yield and biomass, forming part of the 24.3% of genotypes clustered as highly tolerant based on Principal Component Analysis (PCA), which accounted for 74.45% of phenotypic variation. Biochemical analysis revealed a clear differential accumulation of secondary metabolites in response to *Striga*. Resistant genotypes such as SCSHYB012150, SA2133, and SA3028 exhibited significantly elevated levels of ferulic acid, gallic acid, P-coumaric acid, syringic acid, and caffeic acid—with ferulic acid showing the highest association across 74 SNP loci. These phenolic compounds play critical roles in plant defence through antioxidant, allelopathic, and antimicrobial activities. Clustering analysis of metabolic profiles further confirmed these genotypes formed distinct groups with elevated antioxidant capacity under infestation. Nutritional profiling showed that susceptible genotypes suffered sharp declines in crude protein (by up to 3.2%) and fibre content (by 1.7%). In contrast, resistant lines—including SCSHYB012150, SA1794, and SA1617—retained nutritional stability. Notably, SA2133 exhibited consistent levels of protein (~9.3%), fibre (~2.8%), and total ash (~2.1%) even under infestation. These findings suggest the presence of metabolic resistance mechanisms and highlight the potential for breeding nutrient-dense, *Striga*-tolerant cultivars. Molecular analysis using Diversity Arrays Technology Sequencing (DArTSeq) identified 27,870 high-quality SNPs across the 74 genotypes. Population structure analysis revealed three main subpopulations, while AMOVA indicated that 82% of genetic variation occurred within populations. Unique alleles associated with resistance were identified in elite lines. GWAS, using both the FASTmrMLM and FarmCPU models, mapped significant SNPs linked to key traits across chromosomes 1, 2, 3, 4, 5, and 6. For instance, SNP SB00214.1 on chromosome 1 was linked to kernel hardness and explained up to 10% of phenotypic variance. Traits such as plant height (Chr 1, 2), chlorophyll content (Chr 2), panicle height (Chr 3), grain weight (Chr 4), and phenolic content (Chr 6) were all significantly associated with specific SNPs. Additionally, SNPs for nutritional traits—including protein (Chr 3), fibre (Chr 1, 3), fat (Chr 3), and carbohydrates (Chr 8, 9)—were identified, indicating the possibility of stacking resistance and quality traits in breeding pipelines. The co-localization of resistance and nutritional trait loci confirms the feasibility of developing dual-purpose sorghum varieties—combining yield, tolerance, and health-enhancing properties. The

genotypes identified, particularly SCSHYB012150, SA2133, and SA1794, represent strong candidates for deployment in smallholder systems affected by *Striga*, as well as for use in commercial grain and brewing markets. These findings contribute to advancing marker-assisted selection (MAS), trait introgression, and the development of resilient, nutrient-rich sorghum suitable for both food and industrial uses. In conclusion, this study provides critical evidence that certain sorghum genotypes possess dual tolerance to *Striga* infestation and nutritional degradation, driven by both phenotypic resilience and favourable genetic architecture. The identification of key SNPs linked to both agronomic and nutritional traits lay the groundwork for marker-assisted selection (MAS) and genomic selection in future breeding programs. The dual-purpose genotypes—particularly SCSHYB012150, SA2133, and SA1794—are ideal candidates for developing climate-resilient, nutrient-dense sorghum cultivars. These findings have significant implications for enhancing food and nutritional security, especially for smallholder farmers in *Striga*-endemic regions. To ensure real-world impact, it is recommended that future work focuses on validating these markers through functional genomics, pyramiding resistance alleles, and integrating selected genotypes into national breeding pipelines. Additionally, participatory breeding approaches, inclusive policy support, and farmer-led adaptation strategies are essential to scale the adoption of these improved varieties. Ultimately, this study contributes a valuable genetic and phenotypic foundation to address one of the most pressing constraints in African dryland agriculture—securing high-yielding and nutritionally valuable sorghum under parasitic stress.

DECLARATION OF INDEPENDENT WORK

I, WILBERT TIGERE MUTEZO, identity number ######### and student number ########, hereby declare that this research project submitted to the Central University of Technology, Free State, for the Degree DOCTOR OF AGRICULTURE, is my independent work; complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules, and regulations of the Central University of Technology, Free State, and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

SIGNATURE OF STUDENT **DATE**

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DEDICATION

I dedicate this thesis to my beloved wife, Chengetai Magaisa, and my daughters, Ariela and Aviela Mutezo.

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CHAPTER 1

1.1 INTRODUCTION

Sorghum is a chief food staple in sub-Saharan Africa (SSA), but its production is inhibited by the parasitic plant *Striga* that binds to the roots of many cereal crops and cause unembellished stunting and loss of yield. Distant from cultivated farmland, wild sorghum varieties grow as weedy plants and have shown extraordinary immunity to *Striga*. *Striga* is also notoriously known as witchweed and can terminate a crop with up to 100% yield loss (Mbuvi *et al*., 2017). It is projected that over 60% of farmland under cultivation in SSA is infested with one or more species of *Striga*, which impacts over 300 million farmers in over 25 countries with yield losses of over seven billion dollars (Degebasa *et al*., 2022). Three species of *Striga* are particularly destructive – *S. hermonthica* (Del.) Benth. and *S. asiatica* (L) Kuntze, which attack cereals, and *S. gesnerioides* (Wild.) Vatke, which is parasitic to cowpea plants (Belay, 2022).

Sorghum is the second most significant cereal crop in Africa, after maize (Degebasa *et al*., 2022). According to statistics from the Food and Agricultural Organisation (FAO) as reported in a study conducted by Khalifa and Eltahir (2023), sorghum is the fifth most vital cereal crop worldwide and, together with maize and pearl millet, it forms the most significant dry land cereal crop for the semi-arid tropics predominantly in Africa (Khalifa & Eltahir, 2023). It is grown in at least 86 countries, on an area of 47 million hectares, with annual grain production of 69 million tonnes and average productivity of 1.45 tonnes per hectare (Ndossi *et al*., 2021). Sorghum yields in Africa nevertheless, range between 500–800 kg per hectare compared with yield levels of up to 7,000 kg (7 tonnes) per hectare in the developed world (Ndossi *et al*., 2021). The crop is the main occupation in most households. *Striga* has been identified as one of the major constraints to maize, sorghum, and finger millet production in Sub-Saharan Africa (Khalifa & Eltahir, 2023). According to these farmers, the most popular control measures are hand pulling, crop rotation, and intercropping, even though rotational systems might need a longer time-frame to reduce the soil seed bank of *Striga*. Even though the levels of *Striga* infestation and damage are always increasing in the farmers' fields, the implementation of control options is limited. The reason for the low adoption level of control methods by farmers is because they were “too risky” as there is no assurance of a direct pay-off in increased crop yield. Farmer-led evaluation and adaptation of the various *Striga* control technologies in real-life situations therefore help to facilitate the choice of appropriate options and their uptake (Samejima Sugimoto, 2018).

In the semi-arid areas of Tanzania, yield losses of sorghum [*Sorghum biocolor* (L.) Moench] due to *Striga hermonthica* (*Sh*) and *S. asiatica* (*Sa*) infestations are estimated to be 30–90% (Mrema, Shimelis, & Laing, 2017; Mrema, Shimelis, Laing, *et al*., 2017). *Striga*, the witchweed, is the biggest biological hindrance in grain production in Africa causing yield losses of more than 40%, and in some cases, they cause broad crop failure (Teressa, 2024). Sorghum is one of the most important cereals adapted to drying conditions typical of Africa. It provides up to 25% of the calorie needs of the people in in Ethiopia (Teressa, 2024). It is also a very significant source of livestock feed and is used in the brewing industry, amongst other uses.

Current varieties have yield potentials of 4.5 tons per hectare, but they are highly susceptible to *Striga*, an obligate parasitic weed (Teressa, 2024). Lately, *Striga* infestation in Africa has become more disturbing, causing farmers to abandon their lands. *Striga* has repeatedly weakened the productivity of sorghum and other cereals and grain legumes in this zone (Teressa, 2024). The deployment of *Striga* resistant varieties alongside integrated management therefore holds the key to sustaining yields in this zone.

1.2 STATEMENT OF THE PROBLEM

Witchweed, *Striga hermonthica* (hereafter, referred to as "*Striga*"), is a key biotic constraint to cereal production in sub-Saharan Africa. The parasitic plant is a socio-economic risk that has forced some resource-poor farmers to abandon their farms due to high infestation. The root hemi-parasitic weed *Striga hermonthica* is a serious restriction to production of economically important cereals in sub-Saharan Africa (Mandumbu *et al*., 2019). Due to economic constraints and illiteracy, most of the available approaches for *Striga* control are either too expensive or too erudite for African subsistence farmers. Resistant crop genotypes have long been anticipated as means of reducing losses due to *Striga* under the low-cost input subsistence farming in Africa. Due to its high genetic diversity, efforts to control Striga through breeding or agronomic practices have largely failed, with yield losses of up to 100% in sorghum reported in parts of Africa (Teressa, 2024).

The increased incidences of climate variations and unreliable rainfall patterns further highlight the urgent need for drought-tolerant crops. Sorghum is very good at adapting to climatic changes; however, the low yields of tolerant varieties have resulted in many farmers leaving sorghum production to the extremely low rainfall areas alone. Screening sorghum lines for their tolerance and good nutritional composition after *Striga* infestation will provide a solution for

farmers growing sorghum. With the increased demand of sorghum commercially for brewing purposes, among others, *Striga* resistance will help farmers to reach high yields at a low cost.

1.3 JUSTIFICATION

Sorghum farmers will profit from improved yields because of increased use of *Striga* tolerant varieties. National agricultural researchers and scientists will benefit from advanced techniques in selecting for *Striga* resistance. Grain marketers will benefit from augmented supply of quality grains. Extension and advisory service agents will use new improved cultural techniques for transfer to farmers. Extensive cultivation of *Striga* tolerant varieties is expected to reduce labour demand, as weeding to control *Striga* seeds will not be essential. In addition, the control of *Striga* by the application of herbicides will become unnecessary, thus saving the environment and reduce production costs due to the elimination of herbicides.

1.4 OBJECTIVES

The overall goal of this study was to investigate the impact of *Striga* infestation on sorghum performance, nutritional and biochemical composition, and to identify high-yielding, *Striga*-resistant genotypes through phenotypic and genetic analysis.

This study pursued the following specific objectives:

- Identify the cultivated varieties that are high yielding and test for susceptibility to *Striga.*
- Evaluate the effects of *Striga* infestation on the composition of secondary metabolites in sorghum.
- Evaluate the effects of *Striga* infestation on the nutritional composition of sorghum.
- Evaluate the diversity of the selected sorghum genotypes and develop association maps for *Striga* resistance.

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Besides maize, barley, rice, and wheat, sorghum ranks fifth most consumed cereal crop in the world (Hossain *et al*., 2022). Sorghum is well suited for adaptation to technologies related to climate smart agriculture because of its extensive adaptation in arid and semi-arid land (ASAL) and climatic conditions in Africa (Hossain *et al*., 2022). As a result, it is a valuable crop for sustaining livelihoods in the difficult climate conditions due to climate change and continues to be essential to the future of Africa in both the spatial and temporal senses (Degebasa *et al*., 2022). However, the industry continues to experience output issues because of frequent and protracted droughts, necessitating the usage of drought tolerant crops like sorghum. Sorghum has been grown for a variety of reasons and is widely used due to its drought tolerance capabilities – from a common staple food through to industrial use, it is of economic importance and has social benefits such as providing food security and better livelihood for most rural households (Hossain *et al*., 2022).

Subsistence farmers, mainly in Africa, dominate the sorghum farming industry. As a result, subsistence production limitations for sorghum differ from those for commercial-scale production. The potential yield of sorghum has been decreased by numerous biotic and abiotic stresses. Two of the most frequent abiotic stresses are unreliable rainfall patterns and decreased soil health and fertility. There are also various biotic challenges which include witchweed infestation (species of *Striga*), leaf diseases, stem borers, panicle diseases, and shoot flies (Yali and Mitiku, 2022). The most widely reported issues in several countries in Africa are drought and *Striga*.

Parasitic weeds therefore pose serious damage to agricultural production in many parts of the world, resulting in huge reductions in achievable crop yields in cereal crops; for example, Broomrapes (*Orobanchaceae*) are holoparasites which depend entirely on host plants for all their water and nutrition (Teressa, 2024). Witchweeds and other members of the Orobanchaceae family, *Striga spp*., are hemi-parasites that behave like holoparasites, despite having chlorophyll and baseline photosynthetic activity. Sorghum genotypes native to ASALs are expected to employ a defence response to extreme weather conditions and entail production of several proteins responsible for altering the processing quality of grain. It is therefore

important to develop a drought-tolerant sorghum cultivar with improved grain and malting qualities and provide appropriate innovations to farmers for increased sorghum output.

2.2 *STRIGA* BIOLOGY

Striga species, including *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze, are obligate root hemi-parasites that are part of the Orobanchaceae family (Belay, 2022). In SSA, they cause catastrophic losses in the production of grain crops. Due to the parasite's capacity to produce many seeds that can survive in the ground for a period of more than fifteen years and the intricate nature of the host-parasite connection, the control of *Striga* is challenging (Belay, 2022). The complicated life cycle of *Striga* typically includes a sequential phase of germination, attachment to the host, haustoria development, vascular infiltration and formation, nutrition accumulation, flowering, and seed generation (Belay, 2022).

Over the past ten years, several *Striga* management techniques have been put forth, including host resistance, chemical, biological, cultural and agronomic measures. Managing *Striga* (witchweed) remains a significant challenge in sub-Saharan Africa despite the development of various control techniques over the past decade. Host resistance, while promising, faces obstacles such as limited availability of resistant crop varieties, the potential breakdown of resistance due to *Striga*'s adaptability, and mismatches with farmer preferences for high-yielding or marketable crops. Screening existing sorghum genotypes and developing molecular markers associated with *Striga* resistance, can offer an alternative which will save farmers from the devastating effects of this parasite.

Chemical control methods are often prohibitively expensive for smallholder farmers, pose environmental risks, and may lead to herbicide resistance if overused. Although the biological control is environmentally friendly, it is hindered by complex implementation procedures, scalability issues, and variable effectiveness influenced by local environmental factors. Cultural practices like hand-pulling and crop rotation, though effective, are labour-intensive, time-consuming, and constrained by land limitations common among smallholders. Agronomic measures, including intercropping and soil fertility management, face adoption barriers due to limited farmer knowledge, resource constraints, and the need for site-specific solutions. Additionally, cross-cutting issues such as inadequate extension services, persistent *Striga* seed banks that can remain viable for up to 20 years, socio-economic challenges, and the unpredictable impacts of climate change further complicate management efforts.

Addressing these challenges requires integrated, context-specific approaches supported by robust agricultural extension services, farmer education, and affordable access to inputs.

2.2.1 Mode of action

Specific features that enable parasitic effects or indicate the ability to lead a parasitic lifestyle have emerged in parasitic plants. The following are crucial phases in the lifecycle of an obligatory root parasite: (i) finding an appropriate host, which links seed germination and development to the existence and location of a possible host; (ii) gaining access to the host's water supply and nutrition, which requires creation of an efficient haustorium; and (iii) finishing the life cycle on the host, which comprises the creation of parasitism and maintaining it until seeds are developed (Belay, 2022). These phases are further unpacked in the sections to follow.

2.2.1.1 Germination – Identification of host root system

Striga and similar plants that parasitise roots partake in the development of effective mechanisms to guarantee reproduction (David *et al*., 2022). These mechanisms comprise the dispersal of many tiny seeds that have a long lifespan to establish a seed bank that is very persistent (David *et al*., 2022). These seeds, which are like dust, can be easily spread by water, wind, people, and contaminated crop seed. Additionally, *Striga* seeds remain viable beyond a period of ten years before they start to grow (David *et al*., 2022). The process of germination is dependent on the perception of the host as the endosperm of *Striga* can only support growth in the absence of a host for the initial three to four days. During this time, *Striga* must infect the host or else it will die.

The germination of *Striga* predominantly relies on perceiving the germination stimulants which are produced in the host's root system activity. To respond to these stimulants, *Striga* requires a period of conditioning which consists of adequate moisture and high temperatures for 7–14 days (David *et al*., 2022). During that period, if no stimulation is perceived, *Striga* enters a secondary dormancy stage. A lot of different germination stimulants were identified, namely jasmonate, coumarin, strigolactones, sesquiterpene, dihydrosorogoleone, kinetin, ethylene, and fungal by-products. Strigolactones are certainly the most extensively researched and highly effective persuaders of *Striga* germination (Mashiguchi *et al*., 2021). Strigolactones are characterised with the inhibition of shoot and root branching. Additionally, they also promote

hyphal branching of arbuscular mycorrhizal (AM) fungi, likely to attract them in nutrient-depleted situations.

2.2.1.2 Haustorium development

Once germination stimuli are received, the seed develops a radicle which grows towards the host plant. Upon contact, the *Striga* radicle tips cease growth, adhere to the roots, and develop a haustorium which enters the root cortex of likely hosts (Aoki *et al*., 2022). A lot of plant species that are not hosts do not stop adherence or entry. An exclusion to this is *Phtheirospermum japonicum* (Thunberg) Kanitz, a partially parasitic plant of East-Asian origin and comparatively interrelated to *Striga* (Aoki *et al*., 2022). The root secretion from *P. japonicum* can stimulate *Striga* germination, but radicle tips seldom enter the root system.

After a period of 12 hours post-attachment, restructuring of the *S. asiatica* meristematic tissue starts developing (Mashiguchi *et al*., 2021). Identification of haustoria-inducing molecules is essential for this step. There are numerous naturally available factors which induce haustoria development and such have been linked to 2,6-dimethoxypbenzoquinone (DMBQ), a product of lignin oxidation and decarboxylation of phenolic acids found in plant cell walls (Dossa *et al*., 2023). The recent theory of DMBQ observation is founded primarily on studies carried out on *S. asiatica* and *Triphysaria versicolor* fish (Dossa *et al*., 2023). To summarise, this theory suggests that DMBQ is produced in the host cell walls and ultimately goes into the parasite's cells. The NAD (P)H-dependent quinone reductase QR1 reduces DMBQ to generate an unstable semiquinone intermediate essential for haustorium growth. *Triphysaria* QR1 is transcriptionally upregulated in response to host root extracts and QR1 knockdown roots are impaired in haustoria formation (Dossa *et al*., 2023). A second quinone reductase (QR2) does not respond to host root extracts but to DMBQ and may act as a parallel detoxification pathway. The DMBQ observation theory is closely related to *Striga* resistance in sorghum, as it sheds light on the molecular interactions that facilitate *Striga* attachment and parasitism. Sorghum varieties that exhibit resistance to *Striga* may do so by altering the production or composition of quinones like DMBQ in their root cell walls, thereby disrupting the chemical signals required for haustorium formation. Additionally, resistant sorghum genotypes may impede the activation of QR1 in *Striga*, preventing the reduction of DMBQ to the semiquinone intermediate essential for parasite development. Understanding these biochemical pathways offers valuable insights for breeding sorghum varieties with enhanced *Striga* resistance by targeting key processes involved in host-parasite recognition and attachment.

An equilibrium between detoxification and production of the haustorium-inducing semiquinone could generate a balance-dependent threshold mechanism where continuous exposure to DMBQ is required for haustoria formation. Additional to chemotrophic signals, a thigmotropic response is vital for *Striga* to develop typical haustoria (Aoki *et al*., 2022).

Within 24 hours of contact, the radicle stops to divide, and a hypertrophic growing stage commences (Makaza *et al*., 2023). Penetration into the host cuticle is facilitated by the elongation of distal cells in the protoderm or cuticle and basic ground tissue, trailed by cycles of periclinal and anticlinal multiplication of these cells, resulting in advancement into the bark of host plants. Upon reaching the host's endodermis, the most distal cells of the haustorium elongate and divide, forming a palisade of cells. The breakthrough of the endodermis is often delayed, but once successful, vascular connections are established. In general, penetration is complete within 48–72 hours after contact with a host root (Makaza *et al*., 2023). No information is currently available to point to which *Striga* genes are needed for successful infestation of host plants. The development of the haustorium utilises cellular mechanisms such as organogenesis which has been studied in other autophytic plants (St John-Smith and Abed, 2022).

2.2.1.3 Establishment of parasitism and life cycle completion

After the connections have been completed on xylem vessels, *Striga* develops an adventitious root system. These roots can develop lateral haustoria on the same or other host plants. Secondary haustoria are thought to have evolved before primary or terminal haustoria. Normally, host plants are frequently parasitised by number of *Striga* plants, and the parasites rapidly feed from photo-assimilates and plant nutrients (Mbuvi *et al*., 2017; Zarban *et al*., 2022). When compared, *Striga* contains nitrogen levels twice as high as host plants. The exhaustion of nitrogen disrupts the host physiology and causes them to produce low photosynthetic assimilates which are commonly linked with *Striga* damage. Numerous photosynthetic parameters in sorghum start declining once attacked by *Striga*. This includes the electron transport frequency via the photosystem II and photochemical quenching (Mutinda *et al*., 2023). However, it is unknown whether *Striga* interferes with host hormonal system and the consequential results of parasitism.

Once germination is completed, *Striga* begins photosynthesis. Nevertheless, *Striga*'s carbon dioxide fixation rates are very low, and it possesses increased night respiration rates which result in negative carbon gain. *Striga* remains extremely dependent on the host for its growth

(David *et al*., 2022). Additionally, *Striga* leaves are known to contain few chloroplasts per cell compared to other plants. This causes low photosynthesis which is supported by transcriptome data from studies on RNA isolated from the *S. hermonthica* tissue (Aoki *et al*., 2022).

Compared to gene expression in *T. versicolor,* it was observed that *Striga* has low gene expression for chlorophyll synthesis and photosynthetic activity (Yali & Mitiku, 2022). *Striga* exhibits high transpiration rates which might point to the fact that host photoassimilates are depleted through transpiration. This supports evidence which has been reported that *Striga* development is harshly affected by high humidity (Yali & Mitiku, 2022). In fact, stomatal activity shows high rates of respiration and conduction with little response to being closed by darkness. Compared to its hosts, *Striga* has a less favourable leaf area index and compensates for this with increased stomatal conductivity (Mutinda *et al*., 2022). It was observed that *Striga*-infested maize plants did not show significant increase in water consumption up to the 63-day post infection period (Mutinda *et al*., 2022). This suggests that during the early stages of infestation, *Striga* may focus on establishing its parasitic connection by developing haustoria and extracting nutrients, rather than causing immediate water stress to the host. The delayed effect on water uptake could represent a parasitic strategy to avoid triggering the host plant's defence responses. Recognizing this early phase of minimal water impact presents an opportunity for timely interventions before *Striga* significantly disrupts the host's water balance and growth.

Disease symptoms are seen before *Striga* emerges and this makes management control very difficult through using manual weeding or herbicides. Nonetheless, these methods are still useful to prevent *Striga* from reproducing and increasing the seed bank. *Striga asiatica* flowers in four weeks after emergence while other species have been reported to flower earlier (Makaza *et al*., 2023). The flowers are organised in spikes or racemes, and each bears numerous flowers. The *Striga* can secrete its protein material directly into its host. It was found that 3,375 putative secreted proteins were present in *Striga*, various identified to be like *A. thaliana* secreted proteins, giving an indication for secretion into the extracellular space delivered (Makaza *et al*., 2023; Yali & Mitiku , 2022). On average, the proteins secreted by *S. hermonthica* were comparatively smaller and had increased levels of cysteine compared to the rest of the proteome. Genes associated with the proteins released were seen to be clustered when compared to other genes in the genome. This reveals that they are possibly arranged in a row and emanate from a large gene family (Amadou *et al*., 2022). This clustering pattern suggests

that the genes are possibly organized in a linear sequence and originate from a large gene family, indicating potential coordinated regulation and related biological functions.

2.3.2 Habits of *Striga* in relation to its effectiveness to parasitise sorghum

2.3.2.1 Diversity

The genus *Striga* includes a lot of different widely spread species, with different host requirements. It has been observed that other *Striga spp.* can only attack a limited range of hosts. In contrast, some can damage several host species. This variability of *Striga* in host preferences facilitates the search for suitable hosts, thus contributing to the expansion of their range. As a result, *Striga* has been spread to over 40 countries in Africa from its origin in Ethiopia and Sudan (Makaza *et al*., 2023). On infested fields, *Striga* causes crop failures of 20–100% (Hossain *et al*., 2022).

In terms of precise species spread and host range, the range of *S. hermonthicas* spreads across East Africa and Southern Africa. It is a parasite of millet, rice, and sorghum. However, *S. gesnerioides* poisons dicots and severely limits cowpea production in most West African countries (David *et al*., 2022; Degebasa *et al*., 2022). The species diversity is therefore a prominent issue in combatting it.

2.3.2.2 Prolificacy

Fertility and fecundity are used in biology to describe an organism capable of producing many viable seeds for further reproduction. A solitary *Striga* plant can produce 50 to 500,000 seeds in one season (Chenyin *et al*., 2023). This increased level of fecundity gives *Striga* a high evolutionary capacity as the pathogen population increases exponentially from one generation to the next.

2.3.2.3 Agility

The *Striga* seeds are small (approximately 0.2 mm long) and dusty (Belay, 2022). This gives it the ability to be quickly spread by water, wind, and polluted plant seeds. It can also stick to people and animals, contributing to the spread of the seeds and contamination of uninfected fields (Zarban *et al*., 2022). On the other hand, germination must occur only when the host is available to avoid suicidal germination of the *Striga* seeds.

2.3.2.4 Synchrony

The existence of the *Striga* is closely associated with the presence of host plants and includes the following phases: germination, haustorium formation, and penetration. The initiation of emergence only begins on the availability of an appropriate host, which is identified with the help of secreted plant hormones termed strigolactones (Begum *et al*., 2023). In the natural context, strigolactones play a role in inhibiting shoot branching. It also serves as a cue for colonisation by phosphate (P)-mobilising symbionts like arbuscular mycorrhizal fungi (AMF). In response to insufficient soil phosphorus concentrations, plant root system secretes strigolactone to enhance activity of AMF (Begum *et al*., 2023). However, *Striga* appropriates this physiological process and attaches to the roots. The strigolactone then binds to the Karrakin-Insensitive 2 (Kai2)/Hypersensitive to Light (HTL) receptors in *Striga* seeds, resulting in the receptor allowing entrance of an F-box protein that destroys germinative transcriptional regulators (Sourabié *et al*., 2022). This series of events triggers the germination of *Striga* seeds.

2.3.2.5 Dormancy

Without receiving chemical germination cues, *Striga* shows relatively high dormancy and longevity in the soil. The seeds can lie dormant for up to ten years, in anticipation of proper host to infest (Kawa and Brady, 2022). The ability of witchweed to remain dormant over a long period is critical to its successful life cycle as this makes the parasite evade both chemical control and putrefaction-causing organisms.

2.3.2.6 Deviousness

Striga reprograms the host in a way conducive for parasitism. Reports confirm *Striga* can change the concentrations of critical hormones, for example, abscisic acid, cytokinins, and gibberellins in the infection phase (Thilakarathna *et al*., 2022). It can possess genes from host plants and reprocess them for parasitism (Thilakarathna *et al*., 2022). Additionally, *Striga* has been shown to be a cunning host immunity manipulator. It is plausible that *Striga* can suppress host defence by releasing a range of chemical molecules (effectors), similar to what bacteria and fungi produce. Although the isolation and characterisation of these effector genes from *Striga* has not yet been performed, the breed-specific triggering of the host's defence response gives credibility to this claim. For example, in research conducted in Nigeria and Niger, S.

gesnerioides-resistant cowpea cultivar (b301) showed immunity to hypersensitive reactions (HR) when exposed to the hypervirulent *S. gesnerioides* race 3 (SG3) (Makaza *et al*., 2023; Yali & Mitiku, 2022).

2.3.2.7 Mysteriousness

Striga's common name, "witchweed" was derived from the wilting, drought-like symptoms and necrosis which affected hosts show prior to emergence of the parasitic weed from the ground. These symptoms appear within a period of one week after infection and cannot be explained by only referring to *Striga'*s ability to deprive the host of nutrients. It has long been suspected that *Striga* injects its hosts with unknown phytotoxins during parasitism, resulting in the beguiling phenotype. Even though research is still pending to characterise the toxin and its mode of action in detail, the theory that *Striga* possesses secondary metabolites which assist it to damage its hosts can be observed from phytochemical trials of *Striga* extracts, such as iridoid glucosides (Soumaïla *et al*., 2022). Although iridoglycosides are not toxic themselves, they rapidly get converted into a toxic aglycone moiety that destroys cells by denaturing proteins.

2.3 AGRONOMIC FUNCTIONAL PROPERTIES OF SORGHUM POST *STRIGA* INFESTATION

2.3.1 Plant growth

Research conducted to evaluate effects of *Striga* on grain yield and other yield parameters of sorghum at the Institute of Agricultural Research in Samaru, Nigeria, showed a decrease in height of plants, length of the panicle, wet panicle weight, weight of 1,000 grains and dry grain yield by 13.7%, 35.9%, 52.9%, 64.5% and 52.6%, respectively (Ousseini *et al*., 2022). The reduction of photosynthetic activity in leaves of sorghum after infection is believed to be the most critical mechanism for growth reduction of sensitive genotypes (Makaza *et al*., 2023). It was observed that the maximum decrease in leaf photosynthetic capacity was 40–50% and this decline was achieved when just a few *Striga* plants infest the host (Ousseini *et al*., 2022). This happens long before the initial *Striga* plant has appeared above ground (Makaza *et al*., 2023). Consequently, the extent of *Striga* infestation will notably magnify the extent of photosynthetic damage by promoting rapid disruption of photosynthetic activity in the early phases of infection.

2.3.2 Yield metrics

Evidently, *Striga* infestation has a greater damage on the susceptible genotypes, although the parasite disturbs growth and dry matter distribution for all genotypes of sorghum. A decline in above-ground biomass is followed by a relatively increased partitioning of dry matter to the root system (Ousseini *et al*., 2022). The leaf area index is reduced because of reduced individual leaf size and rapid senescence. However, the number of leaves per plant is not affected. The decrease in yield is because of a reduced number of grains per panicle which translates to a reduced 1,000 kernel weight (Ousseini *et al*., 2022). Grain filling is largely maintained by flag leaf photosynthesis, and this is impaired in grains infested with *S. hermonthica* (Makebe and Shimelis, 2023). The negative impacts of these changes in grain filling can have far-reaching implications for the nutritional components of the grain, as seed protein quality is easily compromised in *Striga* infected sorghum.

Generally, the negative impacts of *Striga* on plant development are ascribed to two damage mechanisms: (i) The parasites act as an additional sink for water, carbon, and inorganic solutes; (ii) The parasite develops phytotoxins or pathological effects on the host. A 25–45% reduction of the crop biomass in sorghum was observed only a month after emergence (Sirany *et al*., 2022). At this time, the parasite attachments are too small to be visible to the naked eye.

Striga damage on pre-flowering characteristics results in a 14–50% negative impact on seedling vigour and prolonged flowering from 2–9%. It was also observed that panicle weight decreased from 8–37% and grain weight from 5–45% (Widowati and Luna, 2022).

2.4 ROLE OF METABOLIC SYSTEMS IN DEFENCE AGAINST *STRIGA* INFESTATION

2.4.1 Secondary metabolites in plant biochemical interactions

When consumed, sorghum provides secondary metabolites which act as antioxidants or can be utilised for medicinal purposes. Plants have different secondary metabolites, and they possess a unique structure (Widowati and Luna, 2022). Sorghum has a variety of secondary metabolites, such as flavonoids, alkaloids, tannins, steroids, polyphenolic compounds, saponins, and terpenoids (Amadou *et al*., 2022; Aoki *et al*., 2022). Alkaloids are a class of molecules containing aromatic nitrogen. Secondary metabolites can be divided into three main classes:

1. Terpenoids: Terpenoid compounds mainly have carbon and hydrogen and are processed through the mevalonic acid pathway (Aoki *et al*., 2022). Examples of these are monoterpenes, sesquiterpenes, diterpenes, triterpenes and terpene polymers.
2. Phenolic: These are made up of simple sugars, and phenolic compounds comprise benzene, hydrogen, and oxygen rings in their chemical structure (Amadou *et al*., 2022). Examples are flavonoids, coumarins, lignin, phenolic acids, and tannins.
3. Nitrogenous compounds. Examples are glucosinolates, tanpa-tanda, and alkaloids. Plants produce secondary metabolites to defend themselves and compete with other living organisms in their environment (Thilakarathna *et al*., 2022).

Plants which produce secondary metabolites prevent other plants from growing around them through a process called allelopathy. The role of these metabolites is discussed in the section to follow. Allelopathy is closely linked to the production of secondary metabolites, which are non-essential compounds produced by plants that serve a variety of ecological functions, including defence and competition. These secondary metabolites, such as phenolics, terpenoids, and alkaloids, are often released into the surrounding environment, either through root exudates, leaf litter, or volatilization, where they inhibit the growth, germination, or establishment of nearby plants. By producing these chemicals, plants can reduce competition for resources like water, light, and nutrients, giving them a competitive edge. This use of secondary metabolites in allelopathy is especially important for invasive species or dominant plants in an ecosystem, as it allows them to suppress other species and maintain control over their habitat. Understanding the role of these metabolites in allelopathy provides valuable insights into plant interactions and offers potential applications in agriculture, such as developing crops that can naturally control weeds.

2.4.2 Role of secondary metabolites in shaping plant defence mechanisms

Plant secondary metabolites are critical in the defence against phytopathogens, for example, alkaloids (Zhang *et al*., 2023). It is believed that endophytes cause the secretion of secondary metabolites by host plants. Therefore, co-cultivation must be applied to drive the many fold production of secondary metabolites by the plant cells. Secondary metabolites play an evolutionary role as a repellent or signalling molecule in biological associations and with environmental problems, and are not necessarily for plant growth. These secondary metabolites have been grouped into different functional properties, namely benzopyranones, alkaloids,

quinones, phenolic acids, xanthones, tetralones, steroids, quinones, flavonoids, tannins, saponins, terpenoids, and many others.

Secondary metabolites include different chemical molecules developed by plants performing an important function in how plants adapt to their environmental conditions (Widowati and Luna, 2022).

2.5 CHANGES IN NUTRITIONAL FUNCTIONAL PROPERTIES AND ENERGY VALUES OF SORGHUM VARIETIES AFTER *STRIGA* INFESTATION

Sorghum acts as a very good source of food for livestock and pets. The nutritional composition of sorghum for different livestock species is 95% or above that of yellow tooth corn (Makebe & Shimelis, 2023; Sirany *et al*., 2022). Brown sorghum is believed to have 80–85% the nutritional value of corn as it contains condensed tannins that reduce digestibility (Makaza *et al*., 2023). Not considering the type of sorghum genotype, adequate processing to improve its utilisation is required. Steam flaking, popping and reconstitution are used to produce sorghum grain on beef cattle farms in the United States. Pelleting, crushing, milling, and rolling are utilised for poultry and pig feed (Yali & Mitiku, 2022). Sorghum is like maize in terms of its amino acid content and nutritional composition. However, sorghum has a lower fat content, has marginally lower calories and thus metabolizable energy. The tryptophan content is higher than in corn. It was observed that varieties that contain about 50% more lysine would achieve better weight gain in weaned rats (Irondi *et al*., 2022). Simple fermenting and malting procedures can change the nutritional value of sorghum positively.

In a research study, it was found that crude fibre constituted 2.49%, ash content 1.91%, protein content 9.34%, and fatty acids 5.14% in the JN 3 variety (Irondi *et al*., 2022). Contrastingly, it was observed by Irondi *et al.* (2022) that the LZ 19 crude fibre values (1.58%) and fatty acid content (2.98%) were low, while JX had the lowest protein content of 6.24%. JX was shown to have 78.46% carbohydrate content, starch content of 77.42%, a 69.43% starch yield and a starch recovery rate of 89.68% compared to other genotypes (Irondi *et al*., 2022). Sorghum contains two important anti-nutritional metabolites, namely total phenols and tannins found in the grain. The main negative impacts of tannins are a decline in feed uptake, thereby reducing digestibility and absorption of nutrients, and reducing metabolic activity while increasing toxicity levels in livestock production. The quantity of tannins obtained in sorghum is a key factor reducing the nutritional composition for the food and non-food processing industries.

2.6 IMPLICATIONS OF *STRIGA* DAMAGE TO SORGHUM ON FOOD SECURITY

Striga is an affiliate of the Orobanchaceae family, and a root parasite of most cereal crops that poses an enormous negative impact to food security, poverty and malnutrition in a lot of African countries (Khalid *et al*., 2022). Emptying the reserves of undesirable seed soil creates a sustainable route to minimising sub-surface damage to cereal crops. Sorghum production provides significant socio-economic value in Africa in terms of food security and meeting increased commercial use due to increased population and climate related challenges (Njinju *et al*., 2022). Nonetheless, the application and production of the crop is yet to be explained in terms of profitability and financial benefits. The traits of a sorghum variety favoured by large-scale farmers are related to improved yield, resilience to drought, and tolerance to witchweed**.** Sorghum stands as the fifth most produced in the world, after wheat, corn, rice, and barley (Sirany *et al*., 2022; Thilakarathna *et al*., 2022). Aside from food, breweries continually offer a ready market for a vast amount of sorghum which is expected to continue to increase over time. The rich diversity of sorghum in the ASAL areas makes it amenable to adaptation to climate friendly agriculture and technology innovation farming practices. This presents it as an important crop for subsistence farmers in view of the harsh climatic conditions resulting from changes in climatic conditions.

2.7 FUTURE OPPORTUNITIES IN SORGHUM PRODUCTION AND UTILISATION

Sorghum presents huge opportunities for broad adoption due to its agronomic properties and nutritional composition (Pontieri *et al*., 2022). However, before sorghum can be widely used as a manufactured food, some serious technical problems need to be solved. In bread making, more research focus should be placed on creating a viscoelastic protein domain for improved texture and shelf-life of gluten-wheat-free baked goods (Thilakarathna *et al*., 2022).

In brewing industries, sorghum malting in the absence of exogenous enzymes appears to demand genetic manipulation of the grain to lower their requirements of starch gelatinisation temperatures and increase their malt amylase activity (Khalid *et al*., 2022). Alternatively, pre-cooking some of the grain before malting might temporarily solve this problem. As a result of its low protein digestibility, and specifically its decline thereof during cooking, sorghum can be utilised to reduce energy intake in Western populations who wish to control their consumption rates (Yali & Mitiku, 2022).

In addition, the use of sorghum to produce nutraceutical ingredients, functional foods with antioxidant polyphenols, incorporation of porridge/muesli as a substrate for probiotic drinks, and as a gluten-free substitute for celiac patients may offer opportunities for new products development for a typically viable niche market for sorghum utilisation (Hossain *et al*., 2022). Finally, the production of sorghum-based ready meals speaks to the potential uses of this promising crop.

Sorghum provides tremendous opportunities for broader use, but the hurdle of improving sorghum use is yet to come. Improved use of sorghum can be achieved using modern varieties, innovation, and changes in governmental polices to encourage native grain varieties (Njinju *et al*., 2022). Although the technology required to convert sorghum into useful products is generally feasible, profitability and the access to good quality sorghum flour are crucial factors reducing the use of sorghum. Sustainability of sorghum production requires weight to be placed on the development/identification of lines suitable for selected end products. The prevalence of identity-preserving genotypes on the market might very well result in an increased utilisation of sorghum in different products. For example, the release of white, food-grade sorghum in Mali has resulted in production of biscuits containing 5–20% sorghum flour and sorghum-flour baking mixes made from identity-preserving white, food-grade sorghum is also available (Thilakarathna *et al*., 2022). Sorghum foods are also playing a bigger role in the healthcare market, targeting specific nutritional groups such as B. Celiac disease patients (gluten intolerance).

The production of specific food products for diabetic patients, solving indigestion issues, low carbohydrate foods, breakfast cereals and weaning foods warrants the selection of appropriate varieties. The health-improving capabilities of sorghum need to be demonstrated, and relevant nutritional certifications should be obtained from nationalised laboratories, while at the same time promoting sorghum as an industrial crop. Sorghum is arguably a tropical grain which presents opportunities for future use in malt-based products. It is vital to conduct research to improve awareness of the malting processes which decrease the production outlays. Research advances in sorghum brewing and malting technology, together with genetic improvement, can improve the potential for sorghum's commercial uses.

The difficulties of sourcing good and dependable value sorghum can therefore be solved by intercession of players in seed propagation and contracted cultivation in appropriate agro-ecological zones for the targeted products (Khalid *et al*., 2022). The development of processing

standards and the establishment of value-added products to promote health and nutritional benefits, and functional health food certification can further expand the field of sorghum (Sirany *et al*., 2022). To get sorghum back into cultivation, cooperation between all stakeholders, especially seed companies, food processors, farmers, breeders, and the governmental institutions is therefore very important.

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CHAPTER 3

EVALUATION OF SORGHUM GENOTYPES FOR *STRIGA (S. HERMONTHICA)* TOLERANCE

ABSTRACT

Striga infestation and transmission, and the adverse impact of *Striga* on crop yield can essentially be diminished through selection of resistant genotypes. The study screened X sorghum genotypes for *Striga hermonthica (Striga)* tolerance based on their physiological responses to the parasitic effects of this weed. Seventy-five grain sorghum [*Sorghum bicolor* (L.) Moench] genotypes were subjected to three levels (0 mg, 2.5 mg and 5 mg/pot) of *Striga* at Rattray Arnold Research Institute in Zimbabwe which are equivalent to 0 mg/pot → 0 g/ha, 2.5 mg/pot → 50 g/ha and 5 mg/pot → 100 g/ha. One-way analysis of variance results showed that there were significant differences among genotypes on plant height, chlorophyll content, leaf number, field weight, grain weight, panicle height for *Striga**genotype interaction for all 74 sorghum genotypes (p<0.001). The correlation matrix indicated that fresh panicle weight and grain dry weight showed a very high correlation (0.948) at p<0.05. Panicle height and fresh panicle weight were highly correlated (0.736) at p<0.05. Similarly, panicle height and grain dry weight were also highly correlated (0.718) at p<0.05. Results from the heatmap analysis showed that for yield components of sorghum, the fresh weight of the sorghum seed was measured, and results indicated that 75% of the genotypes suffered severe reduction in weight at 5 mg of *Striga*, 41% still showed a huge decrease in weight at 2.5 mg of *Striga,* while 13% of the genotypes had low fresh weights even in the absence of *Striga* damage. The freshly harvested seeds were then oven dried at 25^0C, and it was observed that 67% of the genotypes showed significant reductions in weight at 5 mg of *Striga*, 35% showed reduced weights at 2.5 mg of *Striga,* and 11% of the genotypes showed that their yields were low even without *Striga* damage. A yield component indicator (panicle height) was measured to see whether *Striga* affected it, and the results showed that 45% of the genotypes were affected by 5 mg of *Striga*, 27% were affected by 2.5 mg of *Striga,* while 4% of the genotypes indicated that they had smaller panicles even in the absence of *Striga* damage. Principle component analysis using the scree plot Eigen values showed that the first factor contributed 58% of the cumulative variation. Two principal axes (F1 and F2) were selected, which explained approximately 74.45% of the total variation. Neighbour-joining hierarchical clustering analysis led to the formation of five groups for *Striga**genotype interaction. It can thus be concluded that *Striga hermonthica* negatively affects crop morpho-physiological aspects such as plant height, chlorophyll content and leaf number, as well as yield-determining components such as field weight and panicle height which ultimately reduce the yield of sorghum. The existence of high variability in the response to *Striga hermonthica* infestation in the 74 sorghum genotypes provides room for breeding interventions to improve tolerance to this parasitic weed.

Key words: agronomic components, yield, resistance, infestation, sorghum

3.1 INTRODUCTION

The benefits of using *Striga*-resistant/tolerant sorghum genotypes are limited if soil nutrients are insufficient. However, these genotypes are strong contenders to be included in ongoing breeding programs. In this way, future research efforts may be directed at understanding host resistance mechanisms, improvement of field screening and infestation techniques, and the improvement of strong, high-yielding, *Striga*-resistant varieties which may give farmers an advantage. In Ethiopia, it was discovered that some sorghum genotypes support considerably fewer *Striga* plants to germinate than others and these gave higher grain yield (Bejiga, 2019). On the other hand, some genotypes showed considerably higher yield reduction than others under the same level of infestation while others are highly susceptible and would not give yield at all.

The presence of this wide range of variability in *Striga* resistance and tolerance traits among sorghum genotypes suggests an opportunity to develop high yielding and resistant/tolerant genotypes through hybridisation or genetic engineering to improve on the resistance traits (Bejiga, 2019). Similarly, in Nigeria, negative correlations were observed between *Striga* and height of infected Sorghum (-0.371), as well as between number of *Striga* and number of Sorghum plants (-0.818) (Akomolafe *et al*., 2018). All these findings point to the need to develop breeding strategies to increase cultivar tolerance to *Striga* attack. Additionally, *Striga,* also known as witchweed, is notoriously recognised as causing a yield loss of up to 100% (Kountche *et al*., 2019). It has been reported that more than 60% of arable land under development in sub-Saharan Africa (SSA) is pervaded with at least one type of *Striga*, which influences more than three hundred million farmers in more than 25 nations, resulting in a yield loss of more than seven billion dollars (Kountche *et al*., 2019). Three types of *Striga* are especially ruinous – *S. hermonthica* (Del.) Benth, also *S. asiatica* (L) Kuntze, which attack oats, and *S. gesnerioides* (Willd.) Vatke, which is parasitic to cowpea plants.

Striga infestation and transmission, and the adverse impact of *Striga* on crop yield can essentially be diminished through selection of resistant genotypes. Above ground, germinated *Striga* number is a solid measure for tolerance. *Striga* inflorescence dry weight can be utilised to recognise varieties that decrease *Striga* multiplication. The most extreme relative yield loss is an appropriate choice measure for resilience in susceptible genotypes, while for progressively tolerant genotypes, the relative yield loss per *Striga* contamination appears to be

increasingly suitable. For these resistance measures, yield appraisal of nearby uninfected controls is fundamental.

However, chlorophyll fluorescence, especially photochemical extinguishing and electron transport rate, may empower screening for resistance without this necessity. A two-fold pot system to screen an enormous assortment of sorghum genotypes for low germination stimulant-based protection from *Striga* was developed (Ennami *et al*., 2020). In this approach, seven-day-old sorghum seedlings were grown in sterile quartz sand in a pot with a punctured base, which was set in another pot without apertures to gather root exudates (Ennami *et al*., 2020). An aliquot of the root exudate was applied to precondition *Striga* seeds to evaluate its germination-instigating action. Utilisation of the two-fold pot and other comparative methods brought about the distinguishing proof of a few low-stimulant genotypes. Similarly, pot trials have been used in this research to evaluate tolerance to *Striga* (*Striga aciata*) for 75 sorghum genotypes. *Striga* germination stimulants, *Striga*lactones, in the root exudates of 36 sorghum genotypes were analysed and assessed for *Striga* germination and infection in Sudan (Ennami *et al*., 2020).

In another study by Mohemed *et al*. (2018), showed that the *Striga*lactones profile in the root exudate of sorghum has a large impact on the level of *Striga* infection. High concentrations of 5-deoxystrigol result in high infection, while high concentrations of orobanchol result in low infection (Mohemed *et al*., 2018). This knowledge should help to optimise the use of low germination stimulant-based resistance to *Striga* by the selection of sorghum genotypes with *Striga*lactones profiles that favour normal growth and development but reduce the risk of *Striga* infection or genetic manipulation of related genes responsible for the production 5-deoxystrigol (Mohemed *et al*., 2018).

The aim of this research was to identify high *Striga acaita* stimulated sorghum lines and genotypes to be used to for genetic engineering process using Crispr Cas9 technology to confer resistance to these lines. The specific objective of this study was to evaluate the effects of *Striga hermonthica* on selected sorghum genotypes based on growth parameters.

3.2 MATERIALS AND METHODS

3.2.1 Site description

The pot trials were carried out at Rattray Arnold Research Station (RARS) in Harare, Zimbabwe, from December 2020 to April 2021. The site is located 30 km northeast of Harare

central business district, along Shamva road, with coordinates Latitude: 17° 40′ 20.07″ S and Longitude: 31° 12′ 41.35″ E. The station falls under ecological region II b with an altitude of 1,354 metres above sea level (masl), mean annual temperature range of 16–19°C, and mean annual rainfall of 800 mm. The soil type is red clay soil.

3.3 PLANTING MATERIAL

Striga hermonthica seeds were collected in 2019 from parasitised sorghum plants in Rushinga, Mt Darwin, Mashonaland West province, Zimbabwe. Sorghum seeds were stored in a black plastic bag in the dark at room temperature until use. Seventy-four sorghum genotypes were used for this experiment as shown in Table 1. These included 53 genotypes from Seed Co Limited, Zimbabwe, and 21 grain sorghum genotypes from South Africa's Agricultural Research Council. Sorghum seeds were surface-sterilised by immersion for 2 min. in 70% ethanol, followed by 2 min. in 5% sodium hypochlorite solution containing 0.02% (v/v) Tween20, and rinsed three times in sterilised, distilled water.

Table 1: List of sorghum genotypes from South Africa and Zimbabwe used in this study

No.	Genotype code	Source	No.	Genotype code	Source	No.	Genotype code	Source
1	SCSHYB012150	Zimbabwe	26	SCSHYB017081	Zimbabwe	51	SCSHYB015027	Zimbabwe
2	SCSHYB013103	Zimbabwe	27	SCSHYB017139	Zimbabwe	52	SCSHYB015028	Zimbabwe
3	SC SILA	Zimbabwe	28	SCSHYB017166	Zimbabwe	53	SCSHYB017082	Zimbabwe
4	SCSHYB01150	Zimbabwe	29	SCSHYB017165	Zimbabwe	54	SA2133	South Africa
5	SA1595	Zimbabwe	30	SCSHYB017086	Zimbabwe	55	SA1617	South Africa
6	CHR19	Zimbabwe	31	CHR 25	Zimbabwe	56	SA1939	South Africa
7	MACIA	Zimbabwe	32	SCSHYB012101	Zimbabwe	57	SA1800	South Africa
8	SC SMILE	Zimbabwe	33	SCSHYB017075	Zimbabwe	58	SA2527	South Africa
9	SCSHYB011120	Zimbabwe	34	SCSHYB012116	Zimbabwe	59	SA1796	South Africa
10	CHR 28	Zimbabwe	35	SCSHYB012112	Zimbabwe	60	SA2490	South Africa
11	SA1600	South Africa	36	SCSHYB015002	Zimbabwe	61	SA3280	South Africa
12	CHR 20	Zimbabwe	37	SCSHYB017090	Zimbabwe	62	SA1873	South Africa
13	SA1596	South Africa	38	SCSHYB017084	Zimbabwe	63	SA3100	South Africa
14	CHR22	Zimbabwe	39	SCSHYB017085	Zimbabwe	64	SA1618	South Africa
15	SCSHYB017089	Zimbabwe	40	SCSHYB017083	Zimbabwe	65	SA2538	South Africa

16	SCSHYB017087	Zimbabwe	41	SCSHYB015017	Zimbabwe	66	SA3028	South Africa
17	SCSHYB012108	Zimbabwe	42	SCSHYB015018	Zimbabwe	67	SA2362	South Africa
18	SCSHYB017088	Zimbabwe	43	SCSHYB015019	Zimbabwe	68	SA2548	South Africa
19	SCSHYB012121	Zimbabwe	44	SCSHYB015020	Zimbabwe	69	SA2311	South Africa
20	SCSHYB012156	Zimbabwe	45	SCSHYB015021	Zimbabwe	70	SA2475	South Africa
21	SCSHYB012120	Zimbabwe	46	SCSHYB015022	Zimbabwe	71	SA2070	South Africa
22	SC SILA(KRC) 0P45	Zimbabwe	47	SCSHYB015023	Zimbabwe	72	SA4186	South Africa
23	SCSHYB012157	Zimbabwe	48	SCSHYB015024	Zimbabwe	73	SA1794	South Africa
24	SCSHYB012155	Zimbabwe	49	SCSHYB015025	Zimbabwe	74	SA2093	South Africa
25	SCSHYB012160	Zimbabwe	50	SCSHYB015026	Zimbabwe			

3.4 PLANTING PROCEDURE

The experiment was conducted in pots in the greenhouse to investigate the effects of three levels (0 mg, 2.5 mg and 5 mg) of *Striga hermonthica* on different sorghum genotypes' growth parameters which is equivalent to 0 mg/pot → 0 g/ha, 2.5 mg/pot → 50 g/ha and 5 mg/pot → 100 g/ha. Disinfected sorghum seeds (3/pot) were planted in plastic pots (40 cm in diameter), filled with ferruginous soil collected from a *Striga hermonthica*-free area, and immediately irrigated. In each pot, 15 g of compound D (7N:14P:7K) fertiliser was applied and thoroughly mixed with the soil in the pot. *Striga hermonthica* inoculation was achieved by mixing 2.5 and 5 mg of *Striga hermonthica* seeds in the top 6 cm soil in each pot, respectively. The pots were watered every day to prevent moisture deficit. After emergence, the plants were thinned to two plants per pot. Weeds other than *Striga hermonthica* were regularly handpicked.

3.5 EXPERIMENTAL DESIGN

Pots were laid out in a split plot design with three *Striga hermonthica* levels (0, 2.5 and 5 mg/pot) as the main factor and 74 genotypes (sub factor), and with two replications.

3.6 MEASUREMENTS

Data were collected from each pot from both plants. *Striga hermonthica* un-infested control was included for comparison purposes. Chlorophyll content was measured starting from week three after emergence to week 10 after emergence using a photometer. The MC-100 chlorometer was used to directly measure and display chlorophyll concentration from intact

leaf samples without damaging the plant material. The meter was calibrated to measure chlorophyll concentration with units of µmol of chlorophyll per m^2. Sorghum height and leaf number were measured at 4, 5, 6, 7, 8, 9 and 10 weeks after sowing (WAS). Sorghum panicle fresh weight, panicle length and grain dry weight were also measured at physiological maturing using weighing scale for fresh weight and dry grain weight while a measuring stick was used for panicle height.

3.7 DATA ANALYSIS

All data were subjected to descriptive statistics and analysis of variance (ANOVA) using computer program SAS (XLSTAT 2020.5.1.1046). For the statistical analysis, one-way analysis of variance (ANOVA) was used, followed by least significant differences of means (5% level). Principal component analysis (PCA) of agronomic traits was conducted, Pearson's correlation coefficient for all variables was also determined, heatmap analysis was carried out and agglomerative hierarchical clustering (AHC) was also conducted using the Ward method.

3.8 RESULTS AND DISCUSSION

3.8.1 Analysis of variance

A one-way analysis of variance was carried out to investigate the effects of three levels of *Striga hermonthica* (0, 2.5 and 5 mg) on 74 sorghum genotypes. The results showed that there were significant differences (p<0.001) for *Striga**genotype interaction for plant height, chlorophyll content, number of leaves, fresh weight, grain dry weight and panicle height for the sorghum genotypes. Considering plant height, some sorghum genotypes are inherently short while others are tall. However, in this research, *Striga hermonthica* significantly reduced the plant heights of susceptible sorghum genotypes which later affected the other agronomic attributes of those susceptible ones. Chlorophyll changes were noted even before the *Striga* had started emerging above ground. This indicated that *Striga* damage occured prior to *Striga* emergence above ground. The number of leaves varied significantly with each response of the different lines and genotypes to *Striga hermonthica* infestation. Some of the genotypes (SCSHYB013103, SC SILA, SA1595, CHR19, MACIA, SC SMILE, SCSHYB011120, CHR 28, SA1596, SCSHYB017089, SCSHYB017087, SCSHYB017088, SCSHYB012156, SCSHYB012120, SC SILA(KRC) 0P45, SCSHYB012157, SCSHYB012155, SCSHYB012160, SCSHYB017081, SCSHYB017139, SCSHYB017165, SCSHYB017086,

CHR 250) developed shorter than normal leaves, at the same time showing delayed growth as evidenced by reduced plant height and fewer leaves compared to the control experiment. The fresh weight reduced drastically for those genotypes which were highly susceptible to *Striga*. Contrastingly, the genotypes which exhibit some level of tolerance did not show significant decreases in fresh panicle weight at harvesting for example SCSHYB012150, CHR 20, SCSHYB017166, SCSHYB015022, SCSHYB015023, SCSHYB015025, SCSHYB015027, SCSHYB017082, SA2133, SA1617, SA1800, SA1796, SA2538, SA3028, SA2311, SA2070, SA4186, and SA1794. The panicle heights varied in most of the highly susceptible genotypes such as SCSHYB012101, SCSHYB017075, SCSHYB012116, SCSHYB012112, SCSHYB015002, SCSHYB017090, SCSHYB017084, SCSHYB017083, SCSHYB015017, SCSHYB015018, SCSHYB015021, SCSHYB015024, SCSHYB015028, SA2527, SA2490, SA3100, SA2475, and SA2093. They exhibited short, prematurely terminated panicle development and, in some cases, failed to even produce the panicles. The dry weight of the grains varied significantly amongst the genotypes. The susceptible genotypes produced fewer, smaller, and lighter weight grains while the tolerant ones produced well matured grains resulting in the difference in grain weight among the genotypes.

Table 2: Summary of ANOVA of sorghum genotypes agronomic attributes to *Striga*

Source	DF	Plant height	Chlorophyll	Leaf number	Fresh weight	Grain dry weight	Panicle height
Striga	**2**	0.008	<0.001	0.065	<0.001	<0.001	0.011
Cultivar	**74**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
***Striga* x Cultivar**	**148**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Error	**219**	**4.947**	**1.961**	**0.5355**	**2.406**	**2.531**	**1.0452**

3.8.2 Pearson's correlation matrix

The correlation matrix showed that all the variables had positive correlation for all the parameters that were measured. A reduction in the plant height, chlorophyll content and leaf number had a negative impact on the yield components of sorghum genotypes. However, the yield-determining components showed that they were highly correlated. Fresh panicle weight

and grain dry weight showed a very high correlation (0.948) at $p<0.05$. The highly susceptible varieties failed to produce good panicles and, in some instances, did not even form panicles. A reduction in the panicle size translated to a decrease in the grain weight and vice versa. Panicle height and fresh panicle weight were highly correlated (0.736) at $p<0.05$. The panicles which were short failed to produce grain and, in some instances, produced fewer grains which caused them to be lighter in weight. The well-developed panicles produced good panicle height and grain filled properly, allowing an increase in the fresh grain weight for the tolerant genotypes. Panicle height and grain dry weight were also highly correlated (0.718) at $p<0.05$.

Table 3: Summary of correlation matrix of sorghum genotypes agronomic attributes to *Striga hermonthica*

Variables	Height	Chlorophyll	Number of leaves	Fresh panicle weight	Grain dry weight	Panicle height
Height	**1**					
Chlorophyll	**0.404**	**1**				
Number of leaves	**0.446**	**0.407**	**1**			
Fresh panicle weight	**0.469**	**0.475**	**0.353**	**1**		
Grain dry weight	**0.429**	**0.475**	**0.355**	**0.948****	**1**	
Panicle height	**0.404**	**0.403**	**0.371**	**0.736****	**0.718****	**1**

*Values in bold are different from 0 with a significance level alpha=0.05, ** shows very high correlation*

3.8.3 Heatmap analysis for agronomic components

Plant growth characteristics were measured, and results are as shown in **Figure 1**. Results from the heatmap showed that for plant height, 36% of the plants were severely affected by *Striga* at 0.5 mg application rate, 12% were severely affected at 2.5 mg of *Striga,* while only 4% of the sorghum genotypes have inherently short plant heights. It was also observed that 85% of the genotypes were severely affected in terms of chlorophyll content when 5 mg of *Striga* was applied, and 73% were severely affected with 2.5 mg of *Striga* applied. However, it was noted that 37% of the genotypes had inherently low chlorophyll content as well. When the number of leaves were counted, it was observed that 44% of the genotypes showed a severe reduction

in number of leaves when 5 mg of *Striga* was applied, while 36% of the genotypes exhibited a reduction in number of leaves at 2.5 mg of *Striga*. It was observed that 4% of the genotypes had fewer leaves even in the absence of *Striga* damage.

Yield components for sorghum were measured and results are as indicated in **Figure 2**. When fresh mass of the sorghum seed was measured, the results indicated that 75% of the genotypes suffered severe reduction in weight at 5 mg of *Striga*, 41% still showed a huge decrease in weight at 2.5 mg of *Striga,* while 13% of the genotypes showed that they had low fresh weight even in the absence of *Striga* damage. After the freshly harvested seeds were thedried, it was observed that 67% of the genotypes showed significant reductions in weight at 5 mg *Striga*, 35% showed reduced weights at 2.5 mg *Striga,* and 11% of the genotypes had low yields even without *Striga* damage. A yield component indicator (panicle height) was measured to see whether *Striga* affected it, and the results showed that 45% of the genotypes were affected by 5 mg of *Striga*, 27% were affected by 2.5 mg of *Striga,* while 4% of the genotypes indicated that they had smaller panicles even in the absence of *Striga* damage.

Figure 1: Heatmap analysis of plant height, chlorophyll and number of leaves per plant for 74 sorghum genotypes exposed to *Striga*

Figure 2: Heatmap analysis of fresh grain weight, dry grain weight and panicle height of 74 sorghum genotypes exposed to *Striga*

3.8.4 Principal component analysis

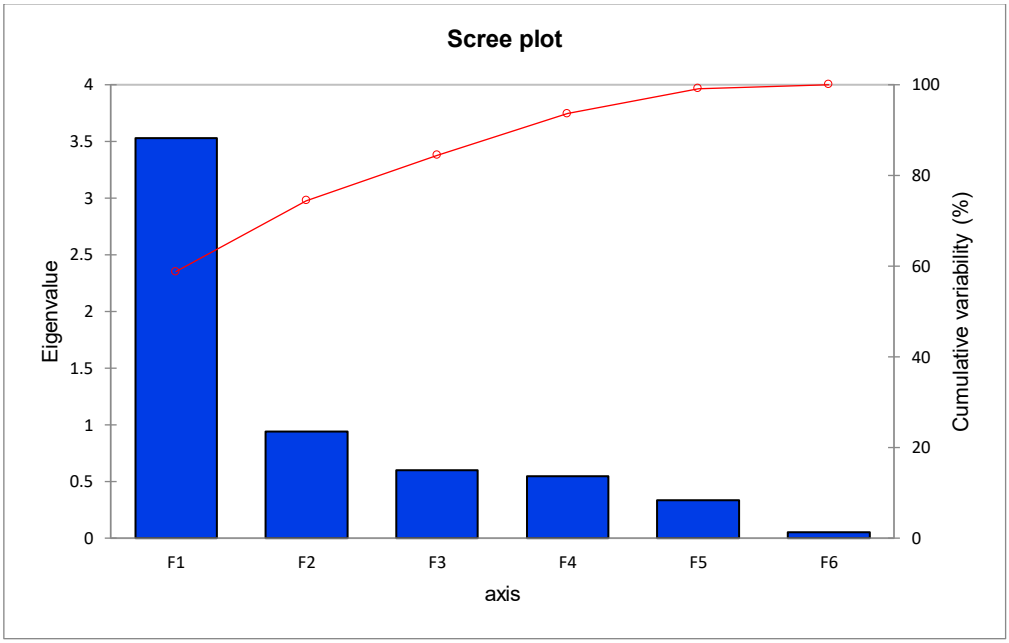

Figure 3: Scree plot for eigenvalues for sorghum physiological and yield components

The first factor contributed 58% of the cumulative variation. This shows how the *Striga hermonthica* infestation contributed to the total variability accounted for in this research. F2, F3 and F4 contributed 16%, 10% and 9%, respectively. Cultivar difference also played a critical role in explaining the differences in their ability to tolerate *Striga* damage. It is therefore very important to come up with breeding strategies which will leverage on these natural defence mechanisms to reduce the effects of *Striga hermonthica*.

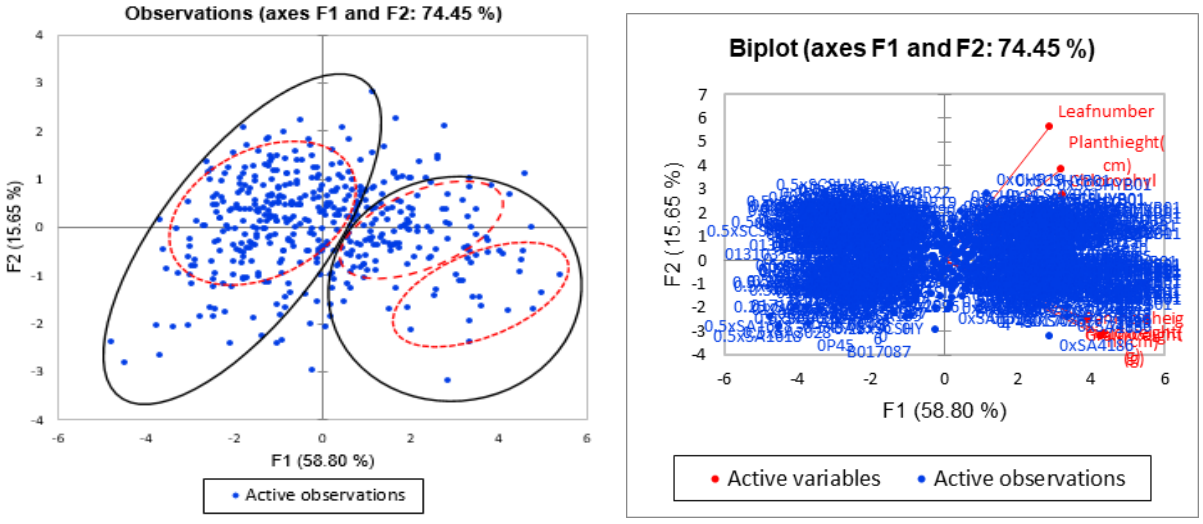

Figure 4: Active observations and box plot for sorghum agronomic attributes of 75 lines and genotypes

Two principal axes (F1 and F2) were selected, which explained 74.45% of the total variation (measured by the inertia). The contribution of variables to the first principal axis (F1) (also shown on the principal plane spanned by F1 and F2 in **Figure 4**) was due mostly to the fresh panicle weight, dry grain weight and panicle height. The second principal axis (F2) mainly contributed plant height, chlorophyll content and leaf number. The findings from the PCA analysis computed with the complete dataset showed a clear clustering along the first component (describing 58.80% of the total variance) based on cultivar tolerance to *Striga hermonthica*. Most genotypes were found on the negative axis and, interestingly, with a negative loading for fresh panicle weight, dry grain weight and panicle height.

3.8.5 Neighbour-joining hierarchical clustering analysis

Neighbour-joining hierarchical clustering analysis led to the formation of five groups (**Figure 5**). Group 1 contained 132 interactions between *Striga* and cultivar (86~0.5 mg *Striga*, 40~0.25 mg *Striga* and 8~0 mg *Striga*). This group included lines and genotypes which were highly affected by *Striga* infestation. The other eight interactions with 0 mg showed that there were some lines or genotypes which had inherently low yields even before being attacked by *Striga*. Differences have also been noted for the level of *Striga* (**Figure 5**). Group 2 contained 135 interactions between *Striga* and cultivar (40~0.5 mg *Striga*, 53~0.25 mg *Striga* and 42~0 mg *Striga*. This included lines and genotypes which were moderately affected by *Striga* infestation. Group 3 contained 92 interactions (19~0.5 mg *Striga*, 33~0.25 mg *Striga* and 40~0 mg *Striga*). This group included interactions with low susceptibility to *Striga* infestation. Group 4 contained (5~0.5 mg *Striga*, 24~0.25 mg *Striga* and 60~0 mg *Striga*). This group indicated interactions where lines and genotypes were highly tolerant to *Striga* infestation and the control. Evidently, five interactions showed lines and genotypes that were highly tolerant to *Striga* damage (SCSHYB015022, SCSHYB015017 and SCSHYB015020). The other 24 interactions showed that there were lines and genotypes which showed tolerance to *Striga* when the *Striga* levels were low. The other 60 interactions showed the control with no *Striga* application.

The genotypes found in Group 1 were the ones selected for further studies to perform genetic engineering. Those lines or genotypes which appeared in Group 1 were also selected using heatmap analysis for yield components, were then designated for further research. The selected varieties for further screening using secondary metabolite analysis were SCSHYB013103, SCSHYB012112, SC Sila, Macia, SA 1617, SA 1618, SA 3028 and SA 2311. These varieties

were highly susceptible to *Striga* attack from the heatmap analysis and neighbour join clustering.

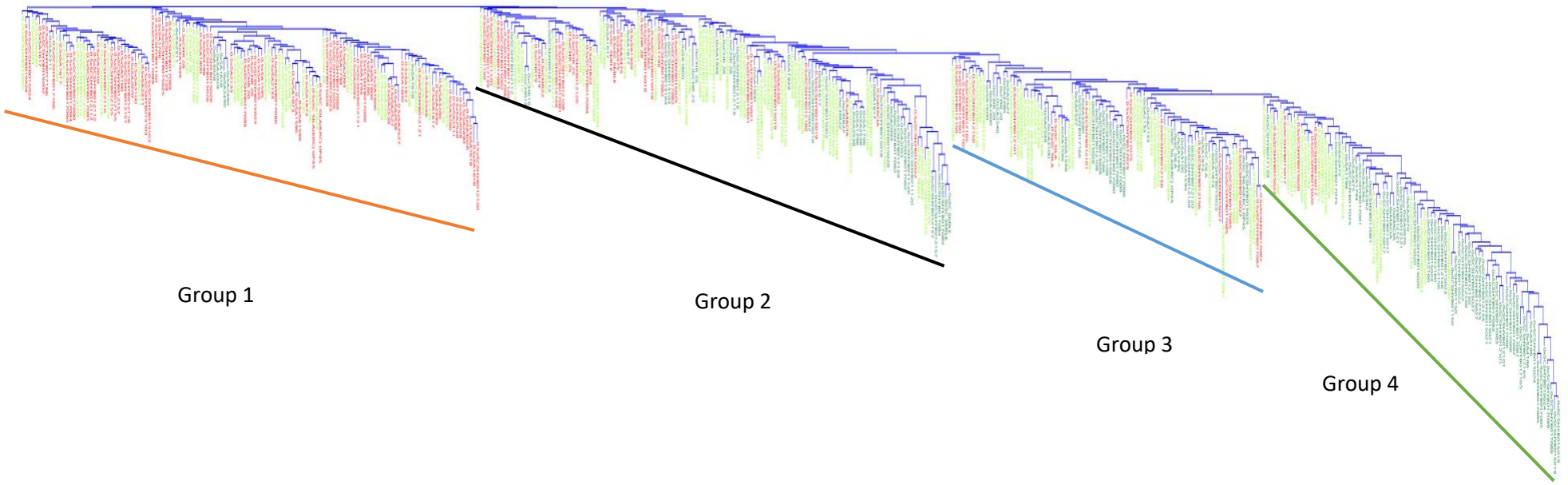

Figure 5: Neighbour join clustering analysis for *Striga**cultivar interaction for 74 sorghum genotypes

3.8.6 Discussion

One-way analysis of variance was carried out for *Striga**genotype interaction of 74 sorghum genotypes. The results showed that there were significant differences (p<0.001) for *Striga**genotype interaction for plant height, chlorophyll content, number of leaves, fresh weight, grain dry weight and panicle height for the sorghum genotypes. The differences in plant height were as a result of varietal differences, as well as the contribution made by *Striga hermonthica* infestation. In similar research by Gwatidzo *et al*. (2020) carried out in Zimbabwe, it was also observed that *Striga hermonthica* influenced sorghum metabolism, regardless of the amount of parasitism to which the host was exposed, causing a reduction of the processes affecting carbon acquisition, and eventually growth. Plant height is regarded as one of the utmost delicate parameters which show the effects of witchweed on plants (Gwatidzo *et al*., 2020). It was also demonstrated that some genotypes showed some levels of tolerance to *Striga hermonthica* infestation. For example, it was observed that the height and internode lengths of Mahube and ICSV 111 IN varieties were not negatively affected by *S. hermonthica* (Gwatidzo *et al*., 2020). This clearly shows that some varieties do not succumb to the dwarfing effects of the parasitic weed, *S. hermonthica*.

The reduction in the chlorophyll content of crops, especially in the early stages, remains key in the ability of *Striga hermonthica* to successfully parasitise its host. It therefore means that, to minimise damage, there is need to develop genotypes that disrupt the parasite-host interaction in the early stages of crop growth. The existence of this wide range of inconsistency in *Striga hermonthica* resistance and tolerance traits amongst sorghum genotypes proposes a chance to develop high-yielding and resistant/tolerant genotypes through hybridisation (Seyoum *et al*., 2019). The number of leaves varied significantly in this research. Some genotypes were highly affected by *Striga* and remained shorter and produced very few leaves. Contrastingly, in another similar research conducted by Gebremedhin *et al*. (2000), it was reported that leaf number was not changed due to *Striga hermonthica* invasion in the development of crops in both susceptible and resistant sorghum varieties. It was observed that there were no substantial reduction in stem height in SRN 39 (Gebremedhin *et al*., 2000). In this research, there were tolerant genotypes which produced normal and good panicles which then later translated into the high fresh weight. The fresh weight of the sorghum panicles differed significantly in this study with tolerant genotypes showing higher fresh weights and susceptible ones showing a huge reduction in the fresh weight Some of the panicles completely failed to produce any

grains, while others had premature abortion of grain, and others did produce grain in differing quantities.

The correlation matrix showed that fresh panicle weight and grain dry weight showed a very high correlation (0.948) at $p<0.05$. Panicle height and fresh panicle weight were highly correlated (0.736) at $p<0.05$. Similarly, panicle height and grain dry weight were also highly correlated (0.718) at $p<0.05$. These variables are the ones which are very important in the determination of the final yield of different genotypes. In a similar trial in Ethiopia, some sorghum genotypes supported the germination of considerably fewer *Striga hermonthica* plants and produced greater grain yield than others (Seyoum *et al*., 2019). It was also reported that various genotypes resulted in slighter yield reductions than others under the same level of infestation (Seyoum *et al*., 2019). This supports results obtained in this research where some genotypes have shown that they are extremely susceptible and would not give yield at all, as evidenced by their panicle fresh weight, panicle height and grain dry weights. Heatmap analysis results indicated that *Striga* has the ability to severely affect sorghum plant height, chlorophyll content, and number of leaves per plant. This happens in the early phases of crop growth and, in most instances, will result in severe crop losses. It can also be reported that yield-determining factors such as fresh grain weight, dry grain weight and panicle height are also affected by the parasitic weed *Striga*. It is therefore clear that the parasitic effects of the weed can result in severe loss for most of the genotypes tested.

Principal component analysis using the scree plot Eigen values showed that the first factor contributed 58% of the cumulative variation. Two principal axes (F1 and F2) were selected, which explained 74.45% of the total variation. The first group had leaf number, plant height and chlorophyll content while the other group was comprised of panicle height, field weight and grain weight. The first group contains the morpho-physiological features while the second group represents the yield-determining parameters. In both cases, *Striga hermonthica* significantly affected the genotypes in some diverse ways for those that were susceptible as compared to those that were tolerant. This suggests that *Striga* infection starts even before the parasite itself is above ground (Gebremedhin *et al*., 2000). *Striga hermonthica* germination is not the only important measure of the influence this parasite has on plants. It is also crucial to elucidate activities before *Striga hermonthica* germination as they have been shown to have greater impact on the observed yield components later in the production stage of the crop. Fresh panicle weight and grain weight results showed that some of the varieties even failed to produce meaningful grain due to the parasitic effects of *Striga hermonthica*. There are however

promising genotypes which have shown tolerance to *Striga hermonthica* as seen with the grain yield which was only slightly affected such as SCSHYB012150, CHR 20, SCSHYB017166, SCSHYB015022, SCSHYB015023, SCSHYB015025, SCSHYB015027, SCSHYB017082, SA2133, SA1617, SA1800, SA1796, SA2538, SA3028, SA2311, SA2070, SA4186, and SA1794.

These genotypes also showed very few *Striga hermonthica* plants which emerged, and the source and mechanism of this resistance will be key in improving other varieties. It is highly likely these genotypes have low germination stimulation activity to the *Striga hermonthica* parasite.

Neighbour-joining hierarchical clustering analysis led to the formation of five groups for *Striga**genotype interaction. Highly susceptible genotypes were found clustering in the same groups at 5 mg of *Striga*. However, the behaviour of these genotypes changed when exposed to lower doses of *Striga* (2.5 mg). Grain and stem weight were reduced in susceptible genotypes, while leaf and root biomass were preserved in tolerant ones. Damages in host production resulted from two processes: export of carbon to the parasite and parasite-induced declines in host photosynthesis. The latter transpires prior to the emergence of *Striga hermonthica* above ground and explains the 80% anticipated loss in production over the lifecycle of the association. *S. hermonthica* is reliant on the carbon exported from the host, as the plant has low rates of photosynthesis combined with high rates of respiration. Host-derived carbon thus accounts for nearly one-third of the total parasite carbon requirement (Mwangangi *et al*., 2024). Producing *Striga hermonthica* resistant genotypes is therefore the most promising, practical, and cost-effective approach to reduce the effects of *Striga hermonthica*. Furthermore, resistance in genotypes is a key component of integrated control packages. Consequently, impending research efforts should be focused towards understanding host resistance mechanisms, enhancement of field screening and infestation procedures, and development of stable high-yielding *Striga hermonthica* resistant varieties that are satisfactory to farmers.

3.9 CONCLUSION

It can be concluded that *Striga hermonthica* negatively affects crop morpho-physiological aspects such as plant height, chlorophyll content and leaf number, as well as yield-determining components such as fresh panicle weight, panicle height and dry grain weight which ultimately reduce the yield of sorghum. The existence of high variability in the response to *Striga hermonthica* infestation in the 74 sorghum genotypes provides room for breeding interventions

to improve tolerance to this parasitic weed. The use of gene editing in manipulating the *Striga hermonthica* germination stimulation activity provides enormous breakthrough potential for future research in developing resistant genotypes. It is very important to explore natural resistance mechanisms exhibited in the wild type varieties to come up with novel breeding strategies aimed at reducing negative effects of *Striga hermonthica* in sorghum. The sorghum lines which showed all three the parameters, short panicle height, low fresh panicle weight and low dry grain weight, were selected for further screening using secondary metabolites data to confirm their susceptibility to *Striga*.

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CHAPTER 4

EFFECTS OF *STRIGA* (*STRIGA HERMONTHICA*) INFESTATION ON THE CONCENTRATION OF SECONDARY METABOLITES AND QUANTITY OF NUTRITIONAL COMPONENTS IN SORGHUM

ABSTRACT

Striga hermonthica and *S. asiatica* are widely distributed throughout most African countries, wreaking havoc on sorghum production and productivity. The study investigated the effects of *Striga hermonthica* on levels of secondary metabolites and nutritional composition of 75 sorghum breeding lines and released varieties from South Africa and Zimbabwe. One-way analysis of variance was carried out to analyse the effects of three levels of *Striga* (0 mg, 2.5 mg and 5 mg) on the levels of phenolic compounds and nutritional composition in the sorghum seed of 74 genotypes. There was a significant difference (p<0.01) on *Striga**genotype interaction for protocatechuic acid, ferulic acid, p-coumaric acid, syringic acid, gallic acid, cinnamic acid and hydroxybenzoic acid. There were significant differences (p<0.001) for ash content, fat content, protein content, fibre content and fibre content for the sorghum genotypes. Correlation matrix showed that hydroxybenzoic acids and protocatechuic acid have a very strong correlation (0.756) at p<0.05. P-coumaric acid and vanillic acid (0.738), P-coumaric acid and caffeic acid (0.701) and P-coumaric acid and cinnamic acid (0.777) have very strong correlations as well. Caffeic acid and vanillic acid (0.931) and vanillic acid and cinnamic acid (0.734) have very strong correlations. Cinnamic acid and caffeic acid (0.702) exhibited strong correlations. Bothe fibre and fat percentages had very strong correlation (0.791) at p<0.05, protein percentage and fat percentage (0.823) at p<0.05, and protein percentage and fibre percentage (0.895) at p<0.05. Principle component results revealed that two principal axes (F1 and F2) were selected, which explained about 67.84% of the total variation for phenolic compounds while, for nutritional composition, it explained about 74.45% of the total variation. Neighbouring-joining clustering results showed four groups for the NJ for phenolic compounds of the analysed 74 sorghum genotypes. There were five groups for the interaction of *Striga**genotype on nutritional composition of 74 sorghum genotypes.

Key words: phenolic compounds, carbohydrates, natural defence mechanism, *Striga* resistance, infestation

4.1 INTRODUCTION

Several biotic, abiotic, and socio-economic restrictions constrain Africa in crop production. *Striga* is one of the biggest biotic limitations in the region, causing up to 90% losses in sorghum production (Rouamba *et al.,* 2024). *Striga hermonthica* and *S. asiatica* are widely dispersed in these areas, wreaking havoc on sorghum production and productivity. However, there are several *Striga* management tactics that can be combined to tackle the weed in a synergistic manner. For instance, the utilisation of resistant sorghum genotypes compatible with *Fusarium oxysporum* f.sp. *Strigae* (FOS), a *Striga* biocontrol agent, in combination with host plant resistance, could help to promote integrated *Striga* management (ISM) (Kavuluko *et al*., 2021).

In most Sub-Saharan African nations, where sorghum is a staple food crop for millions of people, this method is yet to be explored. Low germination stimulant production, low haustorial initiation factor, mechanical barriers, incompatibility, antibiosis, and *Striga* avoidance owing to root development habits, are all components of *Striga* resistance in cereals and legumes (Mandumbu *et al*., 2019). The effective utilization of resistance strategies has been limited by several challenges: the evolution of *Striga* races with host-specific compatibility, genetic variability that enables adaptation to newly introduced resistance alleles, the presence of alternative host species that maintain the parasite population even without cereal crops, and poor soil fertility due to continuous nutrient depletion in smallholder farming systems (Mandumbu *et al*., 2019). The findings suggest that various resistance mechanisms may be required in genetically heterogeneous types, or that cereals with different resistance mechanisms could be cycled in the same field. Soil management should therefore be a part of any comprehensive *Striga* management plan.

Polyphenols or plant phenolics are the secondary metabolites which have multiple roles to alleviate various abiotic (heat, drought, cold, salt or heavy metal) and biotic (bacterial, fungal, viral, insect or weed) stressors (Tak and Kumar, 2020). Phenolic chemicals generated via the common phenylpropanoid pathway operate as signalling molecules and can act as agents in plant shielding (Johnson *et al*., 2021; Kumari *et al*., 2023). Phenolic chemicals fulfil a key function in the control of seed germination and collaborate in regulating the development of plants, including taking part in defensive responses during viral or bacterial infection, excessive sun or light exposure, wounds and heavy metal stressors (Tak and Kumar, 2020). One of the key purposes of phenolic compounds is their antimicrobial activity in plants, to act as protecting substances against disease causing agents such as fungus, bacteria, nematodes, weeds and other pathogens.

Striga is known for its diminishing effects on head biomass in several sorghum genotypes, showing that the parasite has the potential to influence photo assimilate allocation (Gwatidzo *et al*., 2020). It was also discovered that when *Striga* is present, the parasite directs all photo assimilates to itself rather than the host plant (Tak and Kumar, 2020). Based on sorghum height, chlorophyll content, and root shoot/ratio data, it was determined that the Mahube, ICSV 111 IN, and SV4 genotypes resisted *Striga* infection, but Kuyuma, SV2, Macia, and Wahi genotypes were susceptible (Gwatidzo *et al*., 2020). The area of sorghum nutrition composition after *Striga* attack is less explored. This area provides an exciting opportunity for research to elucidate the effects of *Striga* on different populations of sorghum genotypes. Much of the

existing research has been focused on the effects of *Striga* attack on agronomic morpho-physiological characteristics and yield of sorghum genotypes. This research assessed the impacts of *Striga* infestation on production of secondary metabolites and the nutritional composition of 74 sorghum genotypes from South Africa and Zimbabwe.

4.2 MATERIALS AND METHODS

4.2.1 Site description

Refer to section 3.2.1

4.3 EXTRACTION OF SECONDARY METABOLITES IN SORGHUM SEED

4.3.1 Plant material

Refer to section 3.3

4.3.2 Lipids

Extraction of solid material is performed as solid liquid extraction or leaching. For lipids, hexane was used as the extraction solvent. Mass spectrometry was used for fatty acids analysis. Isolation of lipids of intermediate polarity was analysed using the Folch method (Folch, Lees and Stanley, 1957) and the Bligh and Dyer (B&D) method (Bligh and Dyer, 1959).

4.3.3 Total phenolic content

The total phenolic content in the samples/extracts was determined by the Folin-Ciocalteu colorimetric method, using gallic acid as the standard (Luo *et al*., 2018).

4.3.4 Antioxidant assays

The evaluation of antioxidant capacity may vary according to the methods used. In this research, it was determined using the Trolox equivalent antioxidant capacity (TEAC) assay using 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid radical) (Luo *et al.,* 2018).

4.3.5 Total flavonoid content

The total flavonoid concentration was measured by colorimetric method at 500 nm using a chromogenic system with aluminium and antioxidant activity by ABTS and DPPH radical scavenging method (Luo *et al*., 2018).

4.3.6 Anthocyanins

Ultrasound-assisted extraction (UAE) was used to extract the anthocyanins from pericarp and lipids from the seeds of sorghum, as described by Luo and colleagues in 2018 (Luo *et al*., 2018).

4.4 EXTRACTION OF NUTRITIONAL COMPONENTS IN SORGHUM SEED

4.4.1 Moisture determination

Seed moisture content was ascertained using the AOAC 925:10 technique (Horwitz and Latimer, 2000). To the dried and weighted plates, two grams (2 g) of well-homogenised sorghum flour samples were added. Samples that contained dishes were placed in the drying oven for one hour at 130°C, or until the sample's weight remained constant. The dried ground sorghum samples were then taken out of the drying oven, allowed to cool to room temperature in a desiccator, and then reweighed.

$$\textbf{Moisture (\%)} = \frac{(W_1 - W_2) * 100}{S_W}$$

where W_1 is the weight of the cap and fresh sample,

W_2 is the weight of the dry sample and cap,

and S_W is the sample weight.

4.4.2 Ash determination

Ash determination was done using the AOAC 923:03 procedures, as follows: Four (4) grams of well-homogenized sorghum flour were weighed out and added to a sanitized, pre-weighed crucible. The crucible containing the sample was heated to 550°C in a muffle furnace. After the samples were lit until a light grey colour was achieved, they were taken out, allowed to cool to ambient temperature in a desiccator, and then weighed" (Horwitz and Latimer, 2000).

$$\textbf{Ash (\%)} = \frac{(W_1 - W_2) * 100}{S_W}$$

where W_1 is the weight of ash plus crucible after ashing,

W_2 is the weight of the empty crucible,

and S_W is the sample weight.

4.4.3 Fat content determination

Fat content determination was carried out using the Soxhlet extraction method, which is outlined in AOAC 945:16 (Robinson, 2006). An extraction thimble that had been previously prepared was filled with two grams of sorghum flour. Cotton wool that was absorbent and fat-free was used to plug the thimble that held the sample. Before the sample's fat was extracted, the Soxhlet extraction equipment was put together and the petroleum ether spirit was poured into it to half its volume. After that, the extraction went on for four hours. The extracted fat was taken out once the allotted time had passed, and flasks holding oil or fat were connected to a rotating evaporator to evaporate the majority of the solvent. After 30 minutes at 103°C the flasks were dried in an oven to remove any remaining solvent, cooled in a desiccator and reweighed.

$$\textbf{Fat content (\%)} = \frac{(W_f - W) * 100}{S_W}$$

where W_f is the weight of the receiver flask and fat deposit,

W is the weight of the empty receiver flask only,

and *S*W is the weight of the sample used.

4.4.4 Crude fibre determination

The AOAC techniques were followed in determining the crude fibre content (Horwitz an Latimer, 2000). After transferring two grams of pre-defatted material into a one-litre beaker, the sample was digested in a hot plate for one hour using a solution of equal parts 2.5M sulphuric acid (H_2SO_4) and 2.5M sodium hydroxide. The filtering process was then carried out by moistening it with a tiny amount of ethanol. After the filtrate was dried at 100°C in an oven, a consistent weight was achieved (W_1). The samples that had been oven-dried were next burned

for three hours at 600°C in a muffle furnace. Subsequently, the burnt sample defrosted at ambient temperature and was weighed again (W_2).

Crude fibre (%) = $$\frac{(W_1 - W_2) * 100}{Sw}$$

where W_1 is the weight of the porcelain crucible and sample before ashing,

W_2 is the weight of the porcelain crucible containing ash,

and W is the weight of the sample.

4.4.5 Protein determination

The Kjeldahl method was used to conduct the protein determination test (Horwitz and Latimer, 2000). A 50 ml Kjeldahl flask containing 0.5 g of sorghum flour sample was weighed, and 2 g of a catalyst mixture consisting of copper and potassium sulphate were added along with 8 ml of concentrated H2S04. Samples were broken down until only a colourless, pure solution was visible. The digested samples were then distilled using a Kjeldahl distiller, and 25 ml of a mixture of 2% boric acid combined indicator of bromocresol green + methyl red was used to collect the distilled steam gas (ammonia). Next, 0.1N HCl was used to titrate the distilled sample until the first signs of pink hue appeared.

Crude protein (%) = $$\frac{(a * b * 14 * 6{:}25) * 100}{W}$$

where a is the normality of the acid;

b is the volume of standard acid used (ml), corrected for the blank (i.e., the sample minus the blank); W is the sample weight (g);

and 6.25 is the conversion factor for protein from % nitrogen.

4.4.6 Carbohydrates determination

Carbohydrate content was determined as a total carbohydrate by subtracting measured protein, fat, ash, and moisture from 100% (Pearson, 1976).

Total carbohydrate (%) = 100 – (Moisture (%) + Protein (%) + Fat (%) + Ash (%)).

4.5 DATA ANALYSIS

All data were subjected to descriptive statistics and analysis of variance (ANOVA) using the computer program SAS (XLSTAT 2020.5.1.1046). For the statistical analysis, one-way analysis of variance (ANOVA) was used, as well as least significant differences of means (5% level). Principal component analysis (PCA) of agronomic traits was conducted, Pearson's correlation coefficient was also determined, heatmap analysis was carried out and Agglomerative Hierarchical Clustering (AHC) was also conducted using the Ward method. Neighbor-joining was also used to observe the distance relationships of different *Striga**cultivar interactions.

4.6 RESULTS

4.6.1 Analysis of variance

One way Analysis of variance was carried out to analyse the effects of three levels of *Striga* on the levels of phenolic compounds in the sorghum seed of 74 genotypes. There was a significant difference ($p<0.01$) in *Striga**genotype interaction for protocatechuic acid, ferulic acid, p-coumaric acid, syringic acid, gallic acid, cinnamic acid and hydroxybenzoic acid, as shown in **Table 4**. Most lines and genotypes which showed tolerance to *Striga* damage showed an increase in the amount of these phenolic compounds.

Highly susceptible lines and genotypes did not vary from the control in the amounts of phenolic compounds they produced. There were significant differences ($p<0.001$) for ash content, fat content, protein content, and fibre content for the sorghum genotypes for *Striga**genotype interaction. Moisture content was not significantly different across the 74 genotypes.

4.6.2 Pearson's correlation matrix

Most of the interactions showed that there were positive correlations among the phenolic compounds. However, there were some interactions which exhibited high correlations for certain phenolic compounds. Hydroxybenzoic acids and protocatechuic acid showed a very strong correlation (0.756) at $p<0.05$. P-coumaric acid and vanillic acid (0.738), P-coumaric acid and caffeic acid (0.701) and P-coumaric acid and cinnamic acid (0.777) showed very strong correlations as well in this research. Caffeic acid and vanillic acid (0.931) and vanillic

acid and cinnamic acid (0.734) showed very strong correlations as well. Cinnamic acid and caffeic acid (0.702) exhibited strong correlations.

Table 4: Summary of ANOVA of sorghum genotypes nutritional attributes to *Striga*

Source	DF	Moisture Percent	Ash Percent	Fat Percent	Fiber Percent	Protein Percent	Carbohydrate Percent
Striga	2	0.469	0.263	0.005	0.013	<0.001	0.508
Genotype	74	0.947	0.922	<0.001	<0.001	<0.001	0.884
Striga **x Genotypes**	148	0.659	<0.001	<0.001	<0.001	<0.001	<0.001
Error	222	0.4357	0.12838	0.1782	0.4793	0.3698	5.888

Table 5: Summary of ANOVA of sorghum genotypes secondary metabolites to *Striga*

Source	DF	Protocatechuic Acid	Ferulic Acid	Pcoumaric Acid	Syringic Acid	Vanillic Acid	Gallic acid	Caffeic._Acid	Cinnamic._Acid	Hydroxybenzoic Acids
Striga	2	0.026	0.390	0.002	0.003	<0.001	0.174	0.004	<0.001	0.024
Genotype	74	<0.001	<0.001	<0.001	<0.001	0.079	<0.001	<0.001	<0.001	<0.001
Striga **x genotype**	148	<0.001	<0.001	<0.001	<0.001	1.000	<0.001	0.063	<0.001	<0.001
Error	222	9.731	16.09	4.513	0.6360	1.619	2.735	1.5612	1.0907	3.210

Table 6: Summary of correlation matrix for nutrition composition of 74 sorghum genotypes after *Striga* infestation

Variables	Moisture Percent	Ash Percent	Fat Percent	Fibre Percent	Protein Percent	Carbohydrate Percent
Moisture Percent	**1**					
Ash Percent	0.039	**1**				
Fat Percent	0.029	**0.563**	**1**			
Fibre Percent	0.034	**0.581**	**0.791****	**1**		
Protein Percent	0.003	**0.583**	**0.823****	**0.895****	**1**	
Carbohydrate Percent	-0.008	0.017	0.002	0.000	0.014	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table 7: Summary of correlation matrix of phenolic compounds for 74 sorghum genotypes after *Striga* infestation

Variables	Mean	Standard deviation	1	2	3	4	5	6	7	8	9
1. Protocatechuic _Acid	**202.978**	**55.037**									
2. Ferulic _Acid	**186.129**	29.411	0.198								
3. p-coumaric _Acid	**94.212**	**34.056**	0.369	0.056							
4. Syringic _Acid	**20.755**	**4.721**	**0.466**	0.048	0.521						
5. Vanillic _Acid	**31.536**	**10.962**	**0.207**	**0.033**	0.738	0.692					
6. Gallic _Acid	**23.865**	**5.945**	**0.422**	**0.190**	**0.197**	0.248	0.128				
7. Caffeic _Acid	**25.743**	**8.560**	**0.229**	**0.025**	**0.701**	**0.657**	0.931	0.125			
8. Cinnamic _Acid	**18.781**	**6.867**	**0.325**	0.034	**0.777**	**0.483**	**0.734**	**0.076**	0.702		
9. Hydroxybenzoic _Acids	**20.385**	**9.514**	**0.756**	**0.208**	**0.341**	**0.518**	**0.223**	**0.428**	**0.235**	0.277	

*Values in bold are different from 0 with a significance level alpha=0.05, ** highly correlated*

A positive correlation was observed on ash percentage and fat percentage, fibre percentage and protein percentage (0.563; 0.581; 0.583) at $p<0.05$, respectively. Fibre percentage and fat percentage had very strong correlation (0.791) at $p<0.05$, protein percentage and fat percentage (0.823); $p<0.05$ and protein percentage and fibre percentage (0.895) at $p<0.05$.

4.6.3 Principal component analysis

The first principal component explained approximately 47.8% of the total variation (measured by the inertia). The contribution of variables to the first principal axis (PC1) in **Figure 6** was due mostly to the protocatechuic_acid, ferulic_acid, syringic_acid, gallic_acid and hydroxybenzoic_acids. The second principal axis (PC2) with 20% contribution to the variation was mainly contributed by p-coumaric_acid, vanillic_acid, caffeic_acid and cinnamic_acid. The two principal components accounted for 67.8% of the variation. Nutrition composition PC1 explains 52.3% of the variation while the PC2 explains 16.8% of the variation (**Figure 7**). The first two principal components explain 69.1% of the variation. PCA results for nutritional composition showed that there are three groups: Group 1: carbohydrates percentage; Group 2: protein percentage, fibre percentage and fat percentage, and Group 3: moisture percentage. Moisture percentage was the only one located on the negative axis while all others were on the positive axis. It is very clear from the results that the nutritional components play different roles in the plant's natural defence mechanisms to *Striga hermonthica* infection.

Carbohydrates, however, merely act as a source of nutrition to the obligate parasite while the second group (protein percentage, fibre percentage) is responsible for the natural defence system.

The neighbor-joining results for secondary metabolites showed that the control where *Striga* was not applied grouped very close to each other, showing no variations in their production of secondary metabolites. However, genotypes showed varied response to *Striga* infestation as shown in the dendrogram in **Figure 8**. For nutritional composition, the exposure to *Striga* had varied impacts on the nutritional composition of sorghum genotypes. Negative impacts were seen with the 0.5 mg exposure group while there were some genotypes which still showed tolerance to *Striga* infestation, as shown in **Figure 9**.

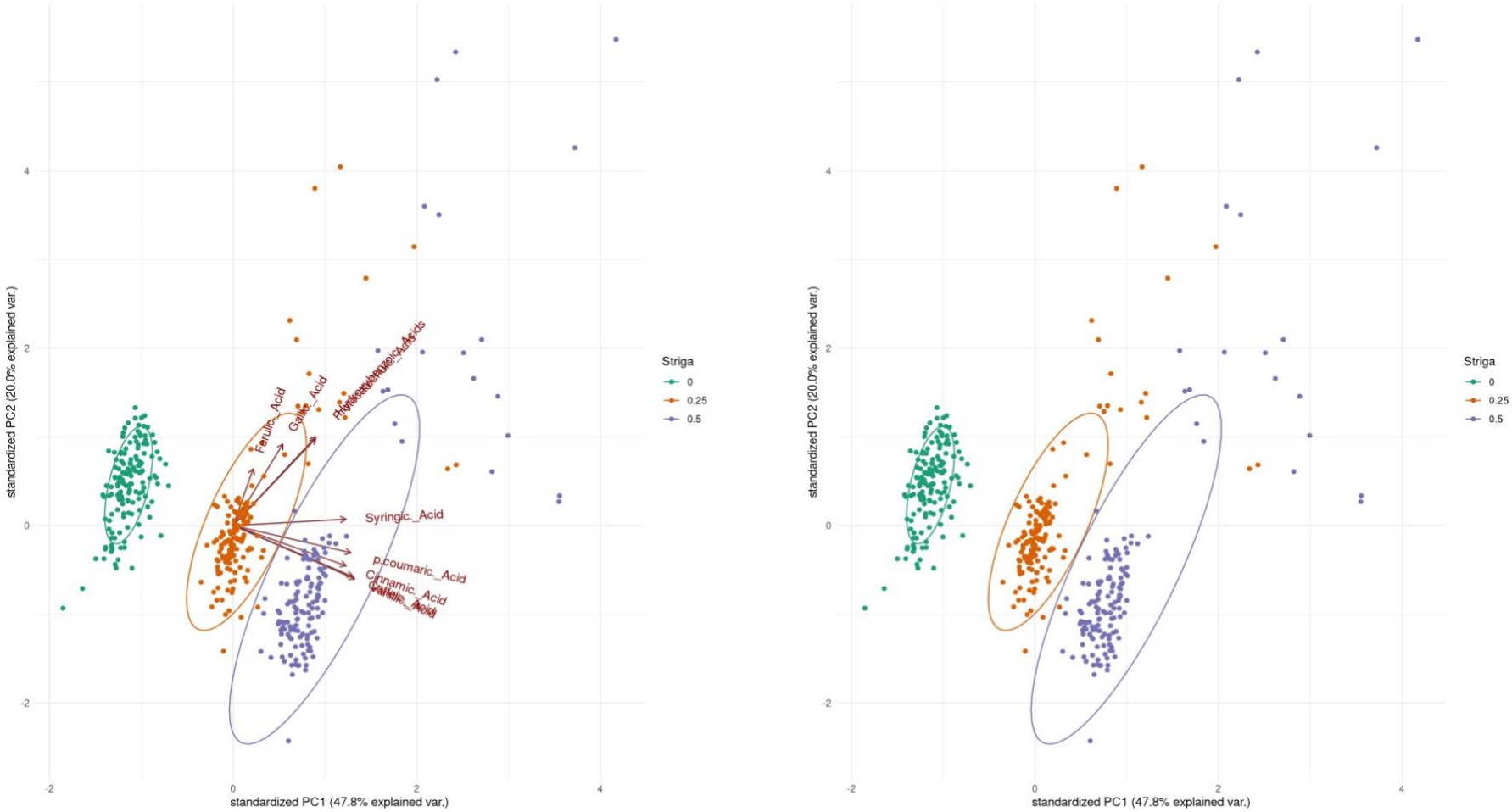

Figure 6: Observations and box plot for secondary metabolites of 74 sorghum genotypes exposed to *Striga hermonthica*

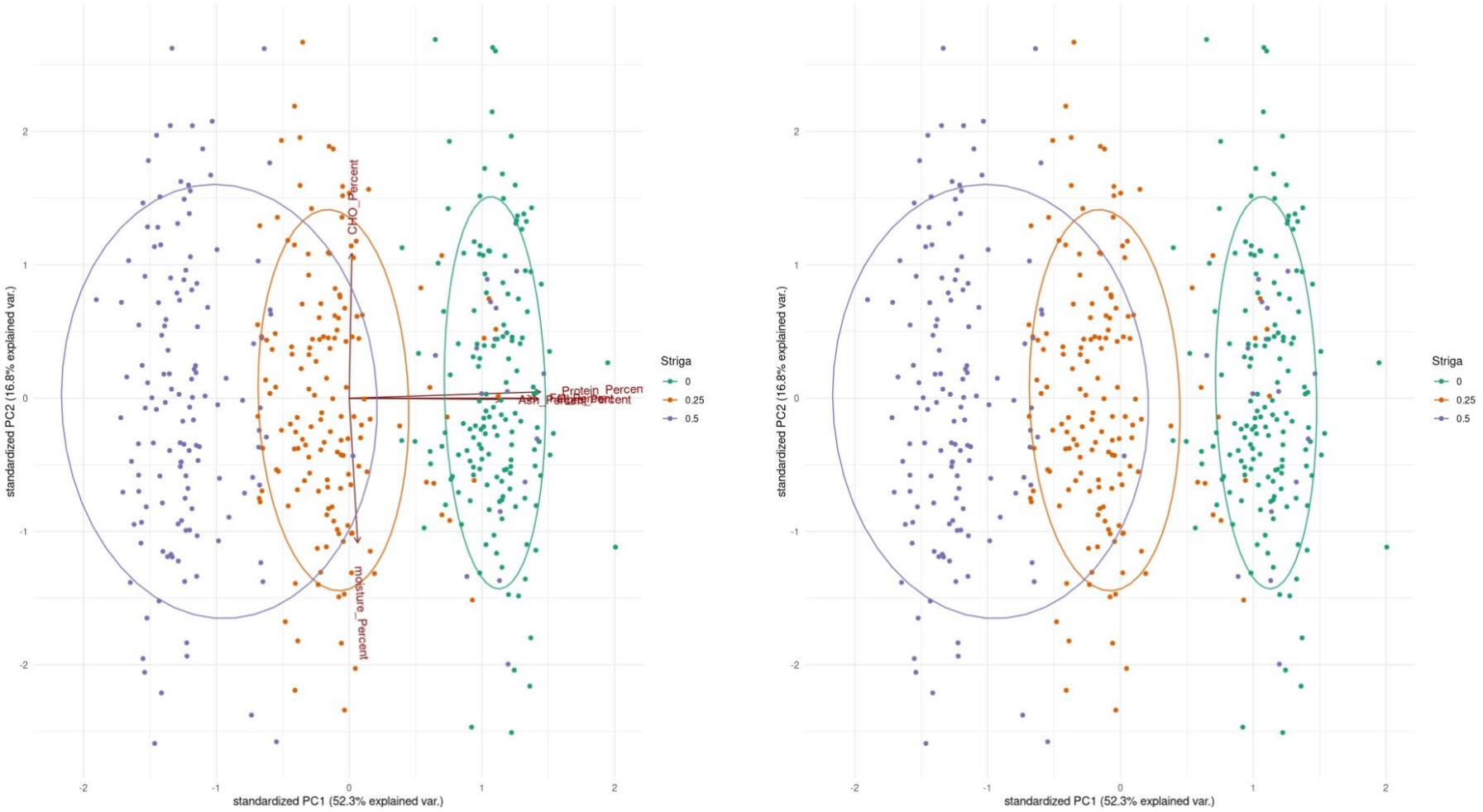

Figure 7: Observations and box plot for nutritional components in of 74 sorghum genotypes exposed to *Striga hermonthica*

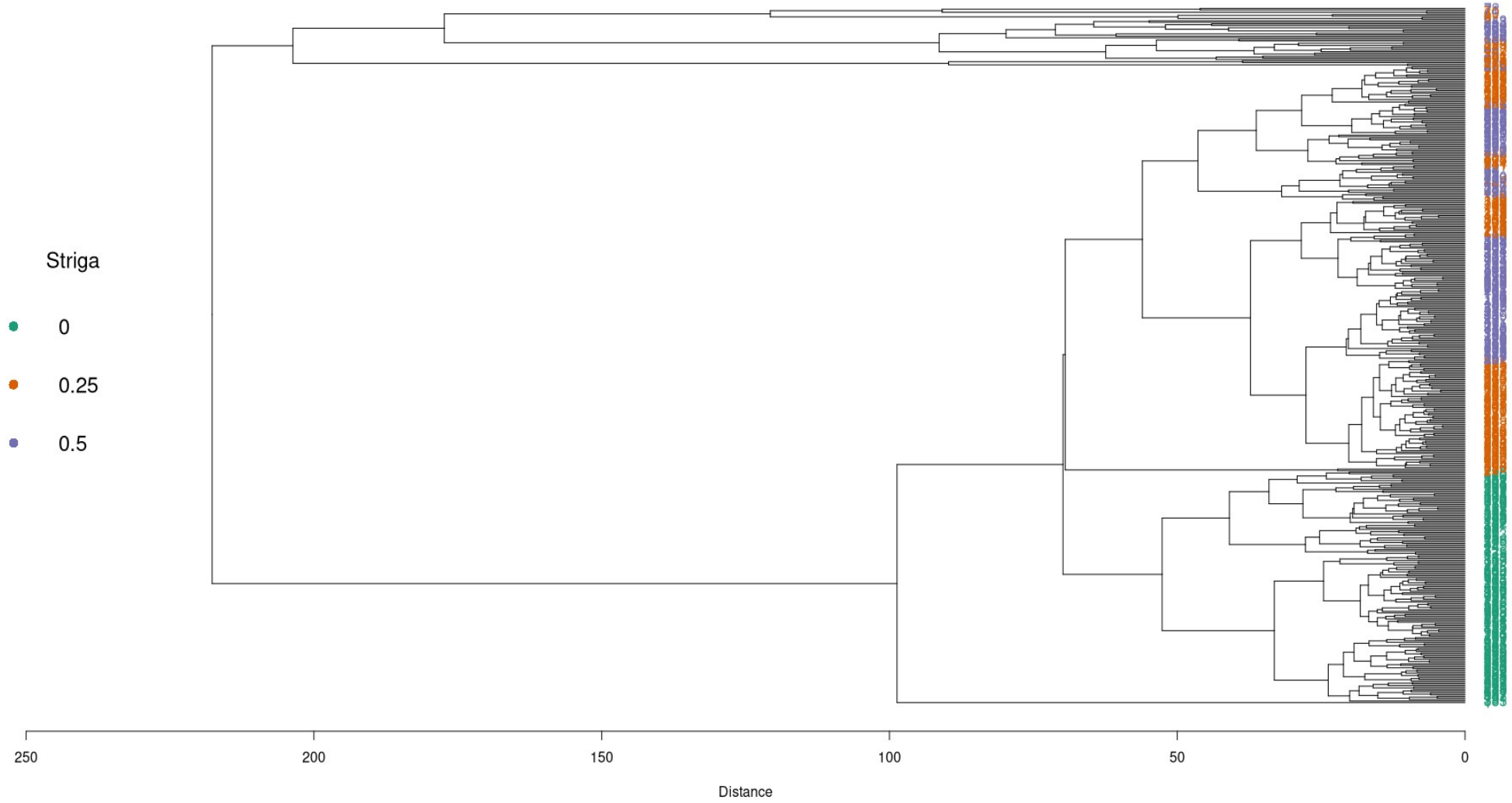

Figure 8: Dendrogram showing secondary metabolites groups of 74 sorghum genotypes

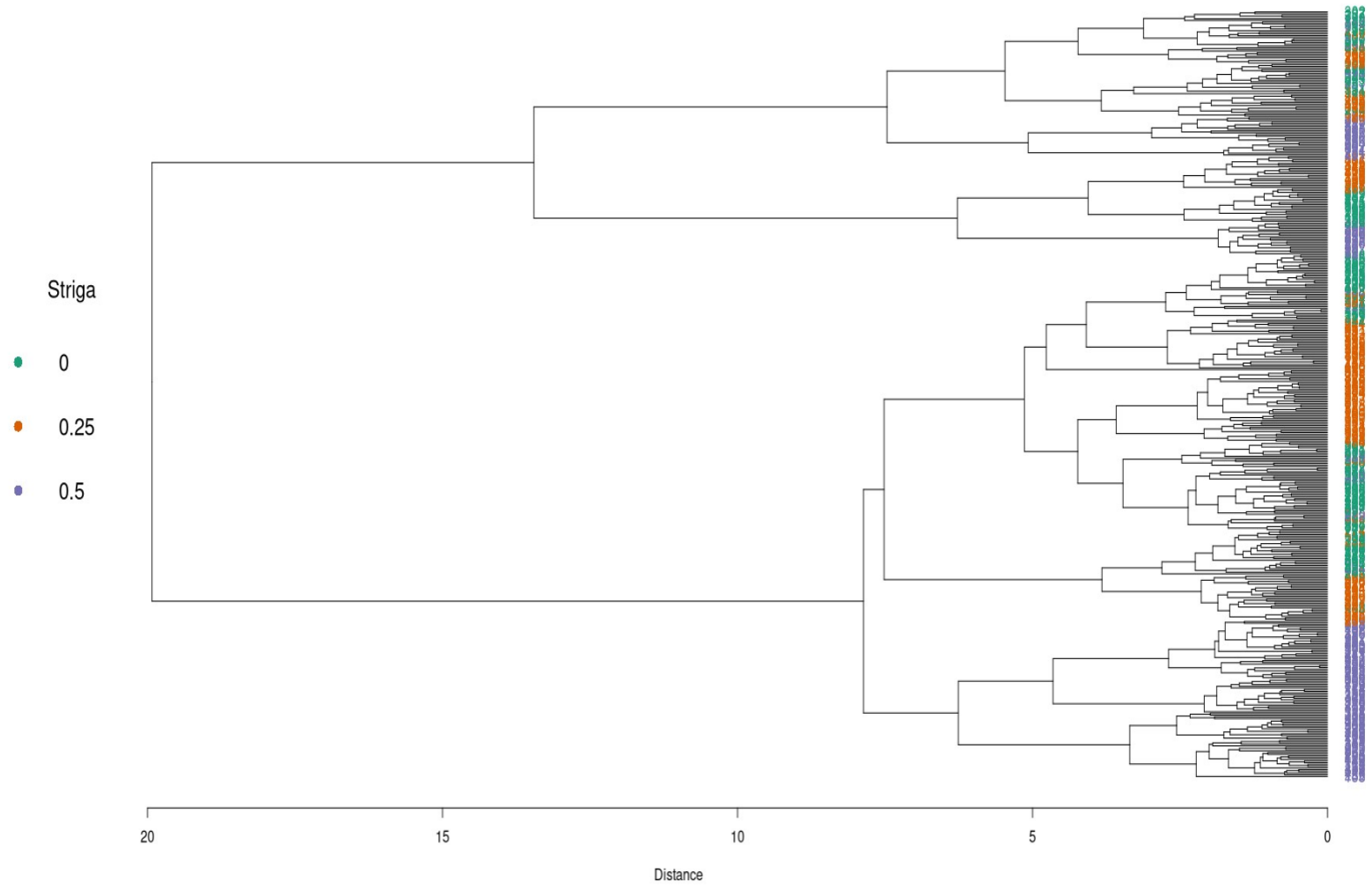

Figure 9: Dendrogram showing nutritional composition groups for 74 sorghum genotypes

4.7 DISCUSSION

Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress (Gebremedhin *et al*., 2020). The functions of phenolic compounds in plant physiology and interactions with biotic and abiotic environments cannot be overstated. Phenolics play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Importantly, phenolic phytoalexins, secreted by wounded or otherwise agitated plants, repel or kill many microorganisms, and some pathogens can counteract or nullify these defences or even subvert them to their own advantage (Johnson *et al*., 2021). The study investigated the effects of *Striga* on levels of phenolic compounds and nutrition composition of different sorghum genotypes.

Analysis of variance results showed that there was a significant difference ($p<0.01$) on *Striga**genotype interaction for protocatechuic acid, ferulic acid, p-coumaric acid, syringic acid, gallic acid, cinnamic acid and hydroxybenzoic acid. There were significant differences ($p<0.001$) for ash content, fat content, protein content, and fibre content for the sorghum genotypes. The total concentration of phenolic compounds in all the sorghum lines and genotypes examined increased with *Striga hermonthica* infection, except for caffeic acid and vanillic acid. This is because caffeic acid is actively involved in plant physiology and in the mechanism of stress tolerance that is primarily utilised by plants for the synthesis of lignin which ultimately thickens cell walls so plants become resistant to ion toxicity, sodium, and heavy metal stress. It also reconciles the absorption of high energy radiations in mesophyll cells under drought stress, and this mechanism involves the production of ferulic acid through the methylation of caffeic acid catalysed by O-methyltransferase (Johnson *et al*., 2021).

Similarly, vanillic acid is associated with salt tolerance as reported in other studies. It is also responsible for the alleviation of osmotic, ionic, and oxidative stresses leading to improved plant growth and chlorophyll synthesis in stressed tomato seedlings (Ayaz *et al.,* 2005; El-Akkad, Hassan and Ali, 2002; Matern and Kneusel, 1988). Therefore, vanillic acid significantly improves salinity tolerance and plant growth performance by involving the actions of plant antioxidant defence and glyoxalase systems. The level of fatty acids or lipids contributes to self defence mechanisms in plants, which is also in line with observations made in this study. There has been a huge shift in the levels of fatty acids in genotypes which are tolerant to *Striga* as compared to the most susceptible ones.

Phytotoxic substances (categorised as a complex of peptides, fatty acid esters, and lipopolysaccharides) are other biochemicals produced that inhibit seed germination and radicle elongation of *Striga* which were also observed. In similar research, it was reported that inhibition of *Striga* seed germination and radicle elongation have also been ascribed to breakdown and chemical modification of the germination stimulant GR24, as well as the production of Strigolactone-like hormones that inhibit radicle elongation, or produce GR24 inhibitors (Mounde, Anteyi & Rasche, 2020). It is evident from this research that much of the variability can be explained as the self-defence and natural mechanisms employed by different genotypes as they respond to *Striga* infestation. The ash content decreased for the susceptible genotypes and remained unchanged for the tolerant ones. There was a notable decrease in the amount of carbohydrates for the genotypes that had a high germinating percentage of *Striga* compared to the low germination genotypes. It is therefore evident that *Striga hermonthica* depends on nutrition from its host for its survival. The capability of the parasite to pull water and nutrients from the host undoubtedly depends on the fluidity of sap across the xylem bridge (Robertson, 2012).

Correlation matrix for phenolic compounds showed that hydroxybenzoic acids and protocatechuic acid have a very strong correlation (0.756) at $p<0.05$. P-coumaric acid and vanillic acid (0.738), P-coumaric acid and caffeic acid (0.701) and P-coumaric acid and cinnamic acid (0.777) showed very strong correlations as well. Caffeic acid and vanillic acid (0.931) and vanillic acid and cinnamic acid (0.734) showed very strong correlations. Cinnamic acid and caffeic acid (0.702) exhibited strong correlations. In this research, most of the genotypes were susceptible to *Striga hermonthica* attack, as seen by the low concentrations of ferulic acid and p-coumaric acid they produced. Infection enhances conjugation of *p*-coumaric acid to phenolic polyamine (PH) conjugates, whereas caffeic acid appears in the soluble hydroxycinnamate (SH) fraction in the leaf, root, and stem (Dicko *et al.,* 2006; Matern and Kneusel, 1988). However, ferulic acid appears to be the main hydroxycinnamic acid derivative in the shoots. Chlorogenic acid is associated with the SH fraction from the stem of healthy plants, although this changes to free phenolics after infection (Punia *et al*., 2021; Xu, Wang and Zhao, 2021). In similar research into the faba bean (*Vicia faba*), it was discovered that chlorogenic acid was evidently predominant at all stages of shoot growth (except in the 74-day-old plants) (Dicko *et al.,* 2006). Caffeic acid was the most abundant among the remainder of the phenolic acids. For an unknown reason, p-coumaric acid occurred in 65-day-old plants

(instead of chlorogenic acid), then disappeared afterwards and was replaced by chlorogenic acid (Johnson *et al.,* 2021).

The temporary occurrence of p-coumaric acid at this stage (65-day-old host) and concomitant disappearance of chlorogenic acid might be in relation with corresponding establishment of broomrape with faba bean (El-Akkad *et al*., 2002). These results support evidence in this research that there are recurring challenges in regulation of phenolic compounds depending on stage of infection and crop type. Vanillic acid is mainly associated with abiotic stress response, specifically salt stress. In this study, sorghum was watered with salt-free water with little evidence of biotic stress exposure, hence the medium levels recorded.

Correlation matrix for nutritional composition showed that fibre percentage and fat percentage had a very strong correlation (0.791) at $p<0.05$, protein percentage and fat percentage (0.823) at $p<0.05$, and protein percentage and fibre percentage (0.895) at $p<0.05$. Proteins, fatty acids, and fibre play a vital role in the pathogen/parasite-host interaction. The parasite or pathogen introduces immunity-suppressing effector proteins into the host plant, which interfere with the host's immune response. This leads to effector-triggered susceptibility, a compatible interaction that facilitates the establishment of the pathogen or parasite within the host. By suppressing the host's defenses, the effector proteins enable the pathogen or parasite to bypass the immune system and cause disease. This process highlights the molecular manipulation by pathogens, where they exploit the host's vulnerabilities to successfully infect and proliferate.

The immune response of host plants to parasitic plants is multi-faceted as well. A number of genes have been observed to be up regulated during resistance responses of different hosts to *Striga* occurrence, and it is becoming increasingly clear that the salicylic acid (SA) signalling pathway and, to a smaller extent, the jasmonic acid (JA) signalling pathway play vital roles in the activation of resistance to parasitic plants (Ali & Baek, 2020; Ghorbel *et al*., 2021). This up regulation is evidenced by the changes in protein content because of changes in enzymatic regulation and production. In a similar investigation, it was shown that during the parasitism of rice (*O. sativa*) roots by *S. hermonthica,* both JA and SA pathways were induced, but the induction of the JA pathway preceded that of the SA pathway (Li, Sun & Liu, 2022; Peng *et al*., 2021).

Principal component results reveal that two principal axes (PC1 and PC2) were selected, which explained approximately 67.8% of the total variation for phenolic compounds while, for nutritional composition, it explained around 69.1% of the total variation. These results indicate

the participation of at least three steps in development of resistance in sorghum plants: the main step is linked to a partial inhibition of development of the young haustorium; the subsequent step could play a role in the physiological events that decrease nutrient translocation towards the haustoria; and the last step seems to be linked with the accumulation of a coloured phenolic-like material (Shan, 2023).

In another study, it was observed that the content of 3-deoxyanthocyanidins (3-DAs) in sorghum grain was not merely a display of resistance to mould, but also to sooty stripe, anthracnose, sorghum midge, and *Striga* (Fakrudin *et al*., 2021; Lam *et al*., 2023). Neighbouring-joining clustering results showed four groups for the NJ for phenolic compounds of the 74 sorghum genotypes analysed. Groups 1, 2 and 3's interactions represent the interactions for lines and genotypes which managed to produce phenolic compounds in response to *Striga* attack. Group 4 reflects those lines and genotypes which produced very low amounts of phenolic compounds and, hence, lines and genotypes in this group are likely to exhibit high susceptibility to *Striga* damage. These lines and genotypes produced very low levels of phenolic compounds which are associated with natural plant defence mechanisms. Neighbouring-joining clustering results showed that there were five groups for the interaction of *Striga**genotype on nutritional composition of 74 sorghum genotypes. Group 2 showed interactions for genotypes which were highly affected with *Striga* attack. The genotypes in this group exhibited high reductions in the nutritional compositions because of *Striga* damage. There were no interactions involving the control in these groups. It could also be seen that there is natural variability in the nutritional composition of sorghum genotypes, even before *Striga* attack, as shown by the presence of 0 mg interactions in Groups 1, 3 and 4.

4.8 CONCLUSION

It can be concluded that *Striga* can have devastating effects on most of the genotypes for nutritional components. It is also clear that all susceptible genotypes showed a reduction in nutritional composition. Susceptible genotypes were not able to use the natural defence mechanism as evidenced by their failure to produce adequate amounts of phenolic compounds to fight against the parasitic weed *Striga*. It is therefore clear that any intervention to increase natural defence mechanisms will help in reducing the effects of *Striga* damage in sorghum. Reducing *Striga* damage through the manipulation of the germination signalling pathway therefore remains a promising potential method of reducing the devastating effects of *Striga* in sorghum.

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CHAPTER 5

GENETIC DIVERSITY ANALYSIS OF SORGHUM [SORGHUM BICOLOR] GENOTYPES USING DIVERSITY ARRAYS TECHNOLOGY SEQUENCING (DArTSeq) TECHNIQUE

ABSTRACT

Evaluating the genetic diversity of *Sorghum bicolor* is essential for informed selection and the success of breeding programs. This study evaluated the genetic diversity and population structure of 74 breeding lines and released varieties from South Africa and Zimbabwe. Sorghum is becoming an important crop in response to climate change. However, sources of genetic diversity need to be explored for use in other breeding programs, such as breeding for drought and disease resistance. In this study, 27,870 single-nucleotide polymorphisms (SNPs) were used to analyse 74 sorghum genotypes. Only 23.5% of the SNPs in this study met the selection criteria and exhibited polymorphism across the 74 genotypes. The polymorphism information content (PIC) values obtained from the Diversity Arrays Technology (DArT) SNP markers ranged from 0.1 to 0.27, on average, indicating moderate genetic diversity. The genotypes displayed significant genetic diversity, with an average genetic diversity of 0.3. Molecular variance analysis revealed low variation within individuals, but substantial variation among individual genotypes. The genotypes showed low levels of differentiation among individuals within the same subpopulation, but differed significantly ($p < 0.001$). Chromosomes with the highest SNP density (> 163 SNPs/10 megabase pairs (Mb)) were Chr1, Chr2, Chr3, Chr4, Chr6, and Chr9. The average SNP density for chromosomes Chr5 and Chr8 was less than 91 SNPs per 10 Mb. The genotypes were divided into nine groups using cluster analysis of the 74 genotypes produced using the unweighted pair group method with the arithmetic mean (UPGMA) approach. Distantly related sorghum accessions, including SCSHYB017085, SA2490, SA1796, SCSHYB015024, SA2527, SCSHYB015022, SCSHYB015026, and SCSHYB015018, were found in the study. For South African and Zimbabwean sorghum breeding efforts, the extensive genetic diversity of these accessions will help create new gene pools and genotypes. Low variation among individuals suggests the necessity for a more extensive analysis of the genetic material of sorghum.

Key words: population diversity, SNP density, genetic mapping

5.1 INTRODUCTION

Sorghum is the fifth most significant cereal crop in the world (Mudaki *et al*., 2023). It exhibitsa remarkable diversity, with five distinct races, their intermediates, and multiple crop forms categorised as varieties of grain, fodder, sweet sorghum, and broomcorn (Chakrabarty *et al*., 2022). Grain type, plant type, adaptability, productive potential, and underdeveloped genetic potential are just a few of the remarkable unrealised variations in sorghum (Allan *et al*., 2020; Mengistu *et al*., 2020; Yahaya *et al*., 2023). Due to its extensive tolerance to heat and drought, sorghum is predicted to become more significant as the world's climate changes and the amount of marginal land used for agriculture continues to increase (Mufumbo *et al*., 2023).

Morphological markers are useful for diversity studies, particularly for attributes such as height, yield, maturity, grain colour, and resistance to insects and diseases (Yahaya *et al*., 2023). However, depending on only morphological markers is unreliable because they are limited in number, susceptible to environmental factors, and influenced by plant growth stages (Yin *et al*., 2021). Therefore, molecular markers have become indispensable in genetic research, particularly in assessing genetic diversity (Bi *et al*., 2021). Molecular markers enable the rapid and accurate identification of varieties, germplasm characterisation, collection, and management. Earlier molecular markers including random amplified polymorphic deoxyribonucleic acid (DNA) (RAPD) (Bi *et al*., 2021; Kim *et al*., 2021; Nasution *et al*., 2021; Raza *et al*., 2020; Yin *et al*., 2021), inter-simple sequence repeat (ISSR) (Felix *et al*., 2020; Kim *et al*., 2021; Ma *et al*., 2021; Mir *et al*., 2021), sequence-related simplified polymorphism (SRAP) (Al-Ghamedi *et al*., 2023; Almarri *et al*., 2023; Feng *et al*., 2020; Zagorcheva *et al*., 2020), amplified fragment length polymorphism (AFLP) (Dementieva *et al*., 2022; Rahimi *et al*., 2021; Trevisan *et al*., 2021), and inter simple sequence repeat (ISSR) (Felix *et al*., 2020; Kim *et al*., 2021; Ma *et al*., 2021; Mir *et al*., 2021), have been widely used to fingerprint and characterise varieties and germplasm genotypes of different crop species.

Despite the potential benefits of these molecular markers in plant breeding, their usefulness is limited due to their reliance on prior sequence information, which can be costly and time-consuming to obtain (Allan *et al*., 2020). Genotyping-by-sequencing (GBS) techniques such as diversity array technology sequencing (DArTSeq) is a low-cost and rapid genotyping method that enables the screening of hundreds of highly polymorphic markers without previous sequence information for the detection of loci (Adomako *et al*., 2024; Kimwemwe *et al*., 2023; Mudaki *et al*., 2023).

DArTseq further reduces genome representation and provides abundant markers, thus improving the rate of genotype calling and the ability to sequence more samples at a lower cost. DArTseq produces both dominant (SilicoDArT) and co-dominant (SNP) markers that have successfully been applied for genetic diversity and population structure analysis in several crops, such as cassava, taro, rice, rye, sorghum, and wheat (Adu *et al*., 2024; Kimwemwe *et al*., 2023; Mudaki *et al*., 2023; Odoi *et al*., 2023). SilicoDArT markers are dominant markers that indicate the presence or absence of specific DNA fragments in the genome. They do not distinguish between homozygous and heterozygous states. SNP markers (Single Nucleotide Polymorphisms) are co-dominant markers that detect single base-pair variations in DNA and

can differentiate between homozygous and heterozygous genotypes, providing more detailed genetic information

The use of DArTseq markers enables a comprehensive analysis of genetic diversity, complete genome profiling, and high-density mapping of complex traits, all crucial for marker-based breeding. These molecular markers have the potential to be very valuable in plant breeding, but their applicability is restricted as they depend on previous sequence information, which can be expensive and time-consuming to gather (Adu *et al*., 2024). Genotyping-by-sequencing (GBS) approaches such as DArTseq, offer a quick and inexpensive genotyping method. In addition to reducing genome representation, DArTseq provides a large number of markers, promoting genotype calling efficiency and lowering the cost of sequencing a greater number of samples (Adu *et al*., 2024).

The DArTseq method has been used to evaluate population diversity for sorghum in different countries. This method was used for phenotypic and genotypic characterisation of 108 distinct landraces and wild genotypes, including 36 genotypes from the ICRISAT gene bank that were conserved for pigeon pea, pearl millet, and sorghum. Approximately 15–25 individuals were genotyped for each accession, totalling 1,980 (Allan *et al*., 2020). This resulted in high-quality SNPs in pearl millet, sorghum, and pigeon pea, with 45,249, 19,052 and 8,211 (Allan *et al*., 2020; Mudaki *et al*., 2023). The diversity of 169 sorghum accessions in Kenya was assessed using 6,977 SNP markers. The markers had a polymorphism information content of 0.31, which was considered moderately high. Population analysis using the ADMIXTURE tool through structural analysis identified ten sub-populations (Adomako *et al*., 2024). In a similar study that evaluated population structure and genetic variation in a diverse population of sorghum genotypes from different parts of Ethiopia, a total of 342 sorghum genotypes were genotyped using 5,060 SNPs with >5% minor allele frequency generated from sequences utilising diversity array technique (Yahaya *et al*., 2023).

In a related study, researchers found that the average Ho value of 0.15 was rather low, which is common for autogamous crop species like sorghum. Anticipated heterozygosity values ranged from 0.10 to 0.50, with an average of 0.32 (Yahaya *et al*., 2023). Moderate polymorphic information content (PIC) values were found, with a mean of 0.26, indicating the informativeness of the selected SNP markers. The extensive genetic variety of accessions in West African sorghum breeding efforts will help create new gene pools and genotypes. Similarly, SNP analysis revealed very little genomic variation among East African sorghum

accessions. Genotype variations, sorghum's autogamous nature, the build-up of local diversity over time, and an increase in recombination events that disrupt links between neighbouring and adaptable loci are all possible explanations for the fluctuations in genomic variations. Despite the use of reliable marker systems like SNPs, there is a lack of knowledge regarding population genetic architecture and familial relatedness among sorghum accessions in Southern Africa. Therefore, this study aimed to evaluate the genetic diversity and infer the population structure of 74 sorghum accessions from South Africa and Zimbabwe.

5.2 MATERIALS AND METHODS

5.2.1 Plant material

Refer to section 3.3

5.2.2 DNA extraction, sequencing, and SNP calling

Seventy-five 20 cm diameter pots were filled with clean sand and topsoil mixture (1:3) 14 days before planting. At the Rattray Arnold Research Station in Harare, Zimbabwe, seeds of various sorghum genotypes were sown in 20 cm diameter pots filled with a 1:3 mix of topsoil and sand in a greenhouse. Young, succulent and fresh leaves were taken from each genotype during the five-leaf stage. The removed leaf samples were oven-dried for 24 hours at 35 degrees Celsius. For sequencing and SNP analysis, leaf samples were delivered to the SEQART Africa, Hosted Institution at the International Livestock Research Institute (ILRI), Old Naivasha Road, Nairobi, Kenya. The samples were wrapped in a resealable bag and labelled with the relevant genotypic code. Furthermore, 74 genotypes of sorghum collected from Zimbabwe and South Africa were analysed for genetic diversity using the DArTSeq method. The DArTSeq methodology (Diversity Arrays Technology Pty Ltd., Canberra, Australia) was used for DNA extractions and sequencing (Mudaki *et al*., 2023). Approximately 1 g of young (fresh?) leaf tissue was used for DNA extraction. Genomic DNA was extracted from oven-dried leaves using a modified cetyltrimethylammonium bromide (CTAB)/chloroform/isoamyl alcohol technique (Doyle, 1991). It was subsequently milled and mixed into 2% pre-warmed (60°C) CTAB isolation buffer (Sigma, Saint Louis, USA), had 1.4 M NaCl, 100 mM Tris (pH 8.0), and 20 mM EDTA. The mixture was then placed into a 2 ml microcentrifuge tube and allowed to sit at 60°C for an hour. Chloroform-isoamyl alcohol (Chl/IAA; 24:1) (Sigma, Saint Louis, USA) was used to extract DNA once, and two volumes of isopropanol were used to precipitate

the extract. After being cleaned with 70% EtOH, the pellet was dried and dissolved in 100 µl of TE buffer containing 50 µg/ml of RNase A (Sigma, Saint Louis, USA). Using 0.8% agarose gel electrophoresis, the isolated DNA was measured and normalised to 50 ng/µl for SNP genotyping and DArT using 0.8% agarose gel electrophoresis.

5.2.2.1 DArT analysis

In line with Kilian *et al*. (2012), DNA was processed in digestion/ligation procedures by substituting two separate adaptors that matched two different restriction enzymes (RE; PstI and SphI)-compatible adaptors for a single PstI-compatible adaptor (Kilian *et al*., 2012). An Illumina flow cell attachment sequence with staggered sequences of different length barcode regions – similar to the sequence published by (Elshire *et al*., 2011) – was used in the construction of the PstI-compatible adapter (Elshire *et al*., 2011). The average adapter contained a flow cell attachment area with an overhang sequence compatible with SphI. In 30 rounds of PCR, only mixed fragments (PstI–SphI) were successfully amplified. The reaction conditions were as follows: 94°C for 1 minute, followed by 30 cycles of 94°C for 20 seconds, 58°C for 30 seconds, 72°C for 45 seconds, and 72°C for 7 minutes. Following PCR, each sample from the 96-well microtiter plate was amplified in equimolar levels, bulked, and subjected to c Bot (Illumina) bridge PCR. This was followed by sequencing using the Illumina Hiseq 2500 system (Illumina, San Diego, California, USA). At this point, 77 rounds of single-read sequencing were conducted. The proprietary DArT analytical pipelines (PLs) were used to process the sequences generated from each lane. In the initial step of the data analysis pipeline, we removed sequence fragments of low quality. These fragments had a reproducibility below 90% and read depths lower than 3.5 for SNPs or 5 for presence-absence markers. The barcode region was subjected to more stringent selection criteria than the rest of the sequences; the sequences were assigned to specific samples carried within the barcode splitting step with high reliability; no samples were dropped due to low coverage across loci; however, individual sequences were removed if they did not meet the above criteria. For marker calling, almost 2.5 million sequences were found for each barcode and sample. For reference alleles and SNP alleles, the average browsing depth across loci was 9.2 reads per individual and 6.5 for each locus, respectively. Lastly, identical sequences were compacted into files called fastqcoll. Using DArT PL's proprietary technique, we utilised collapsed tags with numerous members as a template to process the fastqcoll files. This approach corrects low-quality bases from singleton tags into correct bases.

Using DArTsoft14 (Diversity Arrays Technology Pty Ltd., Canberra, Australia), the secondary pipeline for DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation; PA markers) calling algorithms utilised the groomed fastqcoll files. The C++ algorithm software developed by DArT PL, was employed for SNP calling on every tag from each library included in the DArTsoft14 analysis and clustered at a three-blink distance. The balance of read counts for the allelic pairing method was used to categorise the clusters into distinct SNP loci. The algorithm program was refined with additional selection criteria based on assessing approximately one thousand controlled cross-populations. Tests were conducted in these populations to identify variations from the Hardy-Weinberg equilibrium of alleles, making it easier to determine technical parameters needed to differentiate genuine allelic variants from paralogous sequencing.

Moreover, several samples were processed from DNA to allelic calling as technical duplicates, with the primary criterion for selecting high-quality/low-error rate markers being scoring consistency. A high average browsing depth per locus (averaging over 30 reads per locus across all markers) ensured calling quality. For GBS analysis, 50 ng/µl of DNA was utilised.

5.3 DATA ANALYSIS

All analyses in this research were carried out using the R statistical software version 4.3.0. SNP quality analysis was carried out using the snpReady package. SNPs were filtered based on MAF = 0.05 and call rate = 0.95. SNPs that had missing alleles for more than 5% of the genotypes, monomorphic SNPs, null alleles (those that failed to amplify any segment), and minor allele frequencies of less than 5% were all filtered out.

5.3.1 Population structure and diversity studies

The SNP density plot was analysed and plotted using the *rMVP* package. Population structure was assessed in the LEA package. The optimum number of clusters/subpopulations (K) was determined using the *snmf* function and visualised through the cross-entropy criterion of the LEA package. A Q matrix was created to plot admixture proportions. The identified clusters were visualised by a dendrogram plotted using the *adegenet*, *dendextend*, *NAM*, and *circlize* packages. Analysis of molecular variance (AMOVA) was conducted using the *poppr* package. Population diversity analyses were performed using LEA. Analyses of fixation index (FST) and inbreeding coefficient (FIS) among populations were conducted using the *dartR* package. The 74 sorghum genotypes underwent cluster analysis using the Jaccard dissimilarity matrix

index. The unweighted pair group method using arithmetic average (UPGMA) in *DARwin* 5.0 software was utilised for cluster analysis based on a neighbour-joining technique. Subsequently, a dendrogram was generated using the dissimilarity matrix. Genetic distances between each pair of individual accessions were computed to create a dendrogram, which was used to examine the genetic relationships between accessions. Ten thousand bootstrap values were used in the bootstrap analysis for node creation.

5.4 RESULTS

5.4.1 Genetic parameters

Table 8 displays summary data for 6,562 SNP markers, with an average score of 0.33 and gene diversity (GD) values ranging from 0.10 to 0.50, indicating significant diversity in the sample. The Ho values ranged from 0.01 to 0.46, with an average value of 0.21.

An average inbreeding coefficient (FIS) of 0.53 was observed. The PIC from the DArT SNP markers had an average of 0.27 and varied from 0.1 to 0.38. The minor allele frequency (MAF) measurements had a mean of 0.24 and ranged from 0.05 to 0.50. The main allele frequency (F) ranged from -0.38 to 0.96 with an average of 0.36.

Table 8: Summary of population diversity statistics for 6,562 SNP markers of 74 sorghum genotypes

Parameters	GD	PIC	MAF	Ho	F
Mean	0.33	0.27	0.24	0.21	0.36
Lower	0.1	0.1	0.05	0.01	-0.38
Upper	0.5	0.38	0.5	0.46	0.96

[1]GD = gene diversity, Ho = observed heterozygosity, PIC = polymorphic information content, MAF = minor allele frequency, F = major allele frequency Tables may have a footer.

5.4.2 Population structure and genetic relationships

As shown in **Figure 10**, the demographic pattern of the 74 sorghum genotypes indicated nine different subpopulations. The cross-entropy criterion helps determine the number of ancestral populations or the best run for a given value of K. A lower cross-entropy value indicates higher prediction ability. The ideal K values suggest nine groupings (Populations 1 through 9) with

the highest likelihood of population clustering. Members within the same group were found to be closer than those in separate groups based on the pairwise genetic distances of the nine subpopulations found in STRUCTURE (**Figure 11**). The distribution of single nucleotide polymorphisms from the 6,562 remaining SNPs after quality control was plotted throughout the *Sorghum bicolor* genome in 10 Mb windows (**Figure 12**). There was variation in the distribution of variants among the chromosomes. Chromosomes Chr1, Chr2, Chr3, Chr4, Chr6, and Chr9 showed the highest SNP density (> 163 SNPs/10 Mb) while chromosomes Chr5 and Chr8 had the lowest average SNP density (less than 91 SNPs per 10 Mb). The SNP density plot provided genome-wide coverage across all 10 sorghum chromosomes (**Figure 12**).

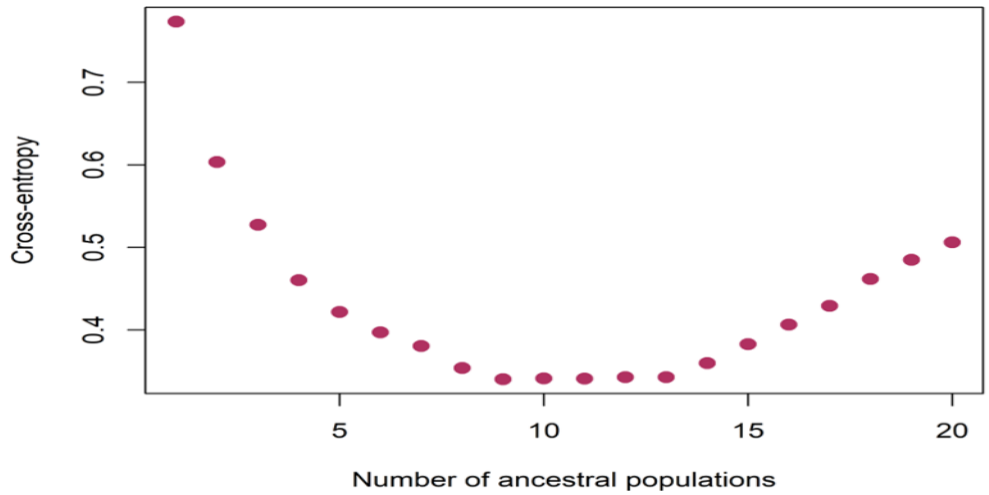

Figure 10: Determination of number of sub-populations using cross entropy value method (Chan & Kroese, 2012)

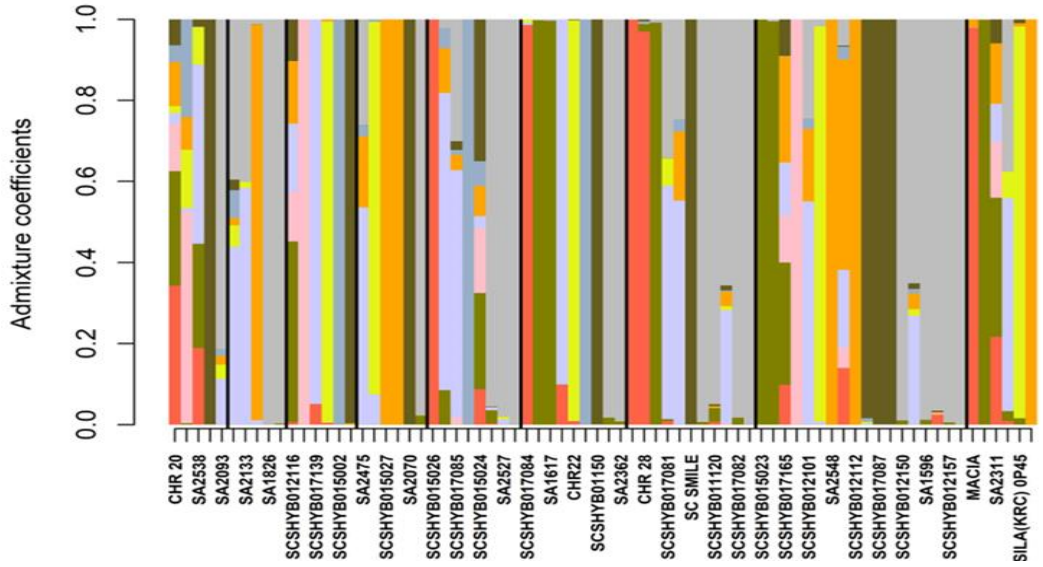

Figure 11: Admixture proportions for 6,562 SNP markers of 74 sorghum genotypes

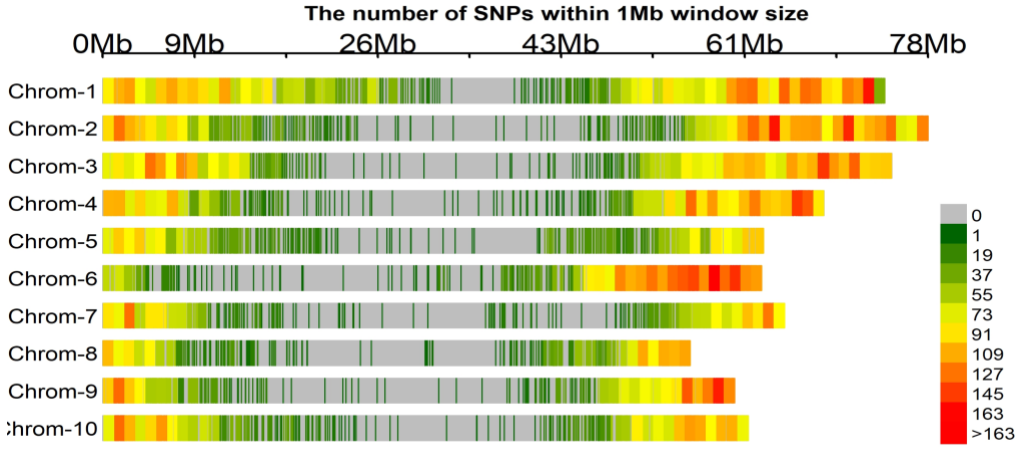

Figure 12: SNP density plot chromosome wise representing number of SNPs within 1 Mb window size. The horizontal axis represents the chromosome length in Mb. Different colours correspond to SNP density

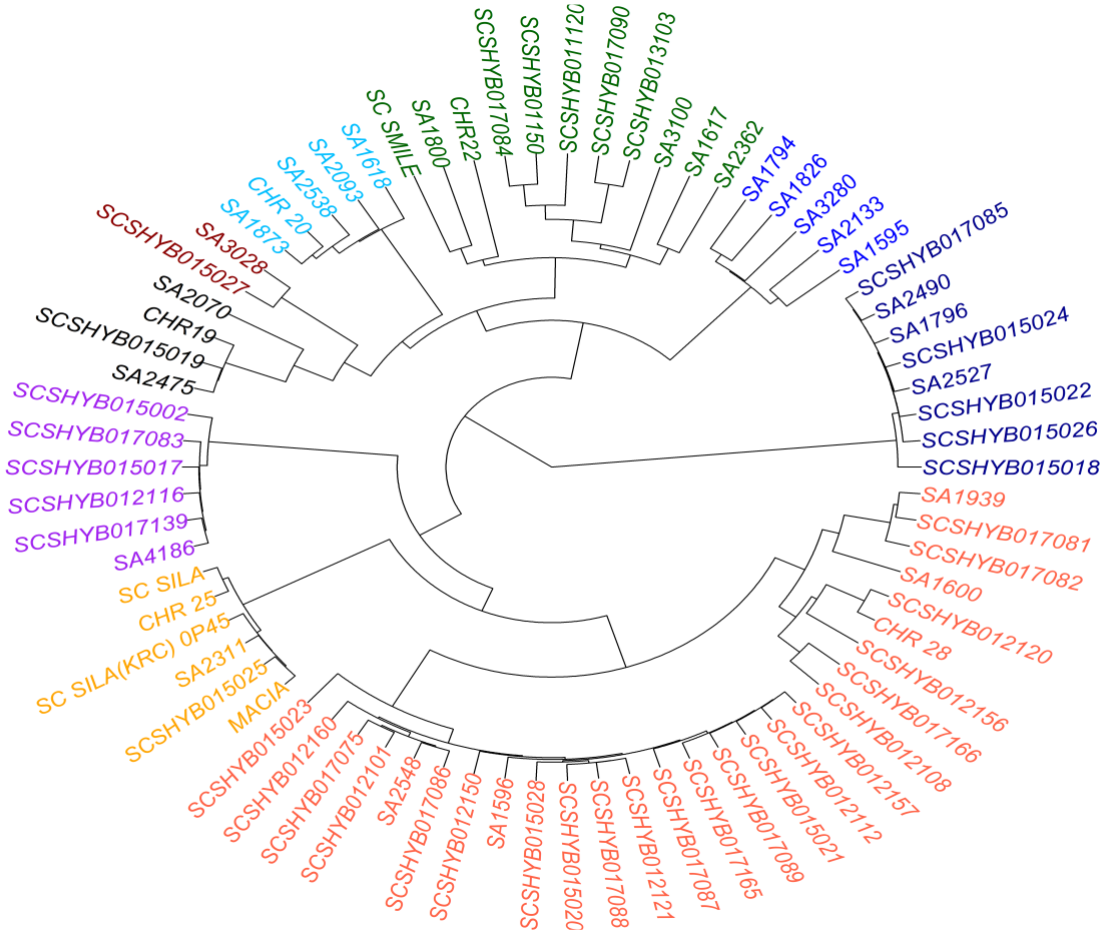

Figure 13: SNP density plot chromosome-wise representing the number of SNPs within 1 Mb window size. The horizontal axis represents the chromosome length in Mb. Different colours correspond to SNP density.

The neighbour-joining phylogenetic tree (**Figure 13**) illustrates the genetic distance within the population. The 74 genotypes were categorised into nine distinct groups by the neighbour-joining phylogenetic tree, aligning with the results of STRUCTURE (**Figure 13**), indicating a significant level of mixing among genotypes. There is no evidence of clustering according to origin as both South African and Zimbabwean genotypes mixed in the clusters.

5.4.3 Genetic diversity and SNP characterisation

The rare alleles (less than 2%), high-missing ratios (greater than 20%), and monomorphic alleles were eliminated to screen the SNPs. Only 6,562 SNP markers (23.5%) of the 27,870 SNPs analysed were polymorphic across the 74 genotypes and met the selection criteria. **Table 9** displays the genetic diversity metrics determined from 74 sorghum genotypes after genetic analysis of the chosen SNPs. In population 3, there were 22,177 polymorphic SNPs per chromosome, compared to 17,318 in population 5. Population 3 had the highest polymorphic loci content (74%), while population 5 had the lowest with 58%. The Ho ranged from 0.441114 to 0.442511 per population cluster. Similarly, gene diversity (He) ranged from 0.243821 to 0.259213, with a mean of 0.254079. Populations 7 and 9 had the highest and lowest Ho and He values (0.441113639, 0.258233) and (0.442511072, 0.253997) respectively, while populations 5 and 4 revealed the highest and lowest Ho and He values. The observed mean fixation rate (FIS) was -59.9%. Population 8 had the highest FIS value at -0.52583, while population 3 had the lowest FIS value at -0.67905.

Table 9: Genetic diversity within and among 74 sorghum genotypes based on 6,562 SNPs markers

Population group	Number of loci	Polymorphic Loci	Monomorphic Loci	Ho	He	FIS
1	30035	20423	9633	0.44147451	0.254964	-0.608901347
2	29993	19501	10555	0.441699174	0.253358	-0.576525268
3	30053	22177	7879	0.441341749	0.254526	-0.679047196
4	30008	20600	9456	0.441214565	0.259213	-0.540252655
5	29935	17318	12738	0.441773287	0.243821	-0.613872508
6	30049	20862	9194	0.441815327	0.251986	-0.663378444
7	30051	21455	8601	0.441113639	0.258233	-0.600661445

8	30018	19505	10551	0.44170109	0.256613	-0.525828278
9	30031	19384	10672	0.442511072	0.253997	-078084411

Ho = observed gene diversity within genotypes; He = average gene diversity within genotypes; FIS = inbreeding coefficients

5.4.4 Analysis of Molecular Variance (AMOVA)

R studio software was used to analyse the AMOVA and fixation index (FST) for the nine subpopulations identified in STRUCTURE (**Table 10**). The AMOVA results showed that 36% of the variance was among individuals, while 64% was due to differences between structure groups.

Table 10: Analysis of molecular variance (AMOVA) among 74 sorghum genotypes, using 6,562 SNP markers

Source	df	SS	MS	Est. Var.	Per. Var.	F-Statistics
Among populations	8	139387.8	17423.48	2036.057	64%	F_{ST} ($p \leq 0.001$)
Within individuals	65	75564.58	1162.53	1162.532	36%	F_{IT} ($p \leq 0.001$)
Total	73	214952.4	2944.553	3198.589	100%	

Pairwise population F_{ST} values between different groups of sorghum population clusters are presented in **Table 10**. From the results in **Table 11**, the structure groups that had the highest F_{ST} values were population 4 and 5 (0.022026) while population 3 and 5 had the lowest (-0.00033). The highest F_{ST} values ranged from 0.010042 to 0.022026.

Table 11: Summary F_{ST} matrix for 74 sorghum genotypes using 6,562 SNP markers

Population	1	2	3	4	5	6	7	8	9
1	-								
2	0.002076	-							
3	0.000153	0.001484	-						
4	0.004065	-0.004115	0.008535	-					
5	-0.007821	0.010722	-0.000325	0.022026	-				
6	-0.00464	0.003406	-0.00198	0.016198	-0.00438	-			
7	-0.007332	-0.01006	-0.004317	-0.007309	0.000774	-0.005062	-		
8	-0.001205	-0.005361	-0.001119	-0.012724	0.010042	0.002258	-0.009677	-	
9	-0.001751	-0.007839	-0.008272	-0.004606	-0.00084	0.000131	-0.008203	-0.009733	-

5.5 DISCUSSION

Access to fresh genetic varieties is crucial for improving crops. Landraces or wild relatives of cultivated crop species are the most significant sources of genetic material. In Africa, smallholder farmers primarily grow sorghum crops using unimproved landraces, which enhances their resistance to changing climatic conditions. Due to their advantageous genetic features, local farmers value landraces for their ability to adapt to various environmental conditions (Adomako *et al.*, 2024; Odoi *et al.*, 2023). Therefore, a comprehensive understanding and characterisation of indigenous germplasms is necessary for effective use. Studying the genetic diversity and population structure of sorghum landraces is essential for establishing heterotic groups, breeding populations, genotypes, and their release. The polymorphic information content (PIC)of a marker provides an estimate of its information content, with the maximum PIC value in this study being 0.38, indicating that alleles were present in approximately 14.1% of the population. The average PIC value of 0.27 in this study is comparable to the results of previous research that used SNP markers.

The results suggest that the SNP markers used in this study provided valuable data for assessing the genetic variation among the 74 sorghum genotypes (Afolayan *et al.*, 2020; Odoi *et al.*,

2023). The Ho value of 0.22 in this study aligns with findings from other studies, although it outperforms earlier research by Afolayan *et al*. (2020). Contrasting results reported moderately high PIC values of 0.48 and 0.31, respectively, as well as varying Ho values (Mamo *et al*., 2023; Mudaki *et al*., 2023).

The maximum PIC value found in this investigation was 0.38, meaning that alleles were present in about 14.1% of the population. The results of Afolayan *et al.* (2020) and Enyew *et al.* (2022), who examined sorghum germplasm collections using SNP markers, are comparable to the average PIC value of 0.27. The results of this investigation indicate that the SNP markers used were adequate in offering useful data for determining the degree of genetic variation among the 74 sorghum genotypes under investigation. The results of Afolayan *et al*. (2020) and Odoi *et al*. (2023), who employed SNP markers for sorghum analysis, are consistent with the average observed heterozygosity (Ho) value of 0.21 obtained in this study (Ho = 0.22). It does, however, greatly outperform the findings of earlier research employing SNP markers by Enyew *et al*. (2022). Contrasting results have been reported by (Mamo *et al*., 2023; Mudaki *et al*., 2023)), showing the PIC values of 0.48 and 0.31 which are considered moderately high, respectively. They also reported varying Ho values of 0.53 and 0.05, respectively (Mamo *et al*., 2023; Mudaki *et al*., 2023).

Heterozygosity is a fundamental measure of genetic variation within a population. A locus's expected He or gene diversity describes the anticipated proportion of heterozygous genotypes under Hardy-Weinberg equilibrium (Medraoui *et al*., 2024). In this study, the gene diversity of the SNP markers ranged from 0.1 to 0.50 across all genotypes, with an average of 0.33, indicating a high level of diversity. These markers are valuable for genotyping populations in genetic diversity studies, as noted by (Yahaya *et al*., 2023). Additionally, the significant difference between the average Ho (0.21) and expected heterozygosity (0.33) values, along with the relatively high number of pairwise individuals with low genetic distance observed in this study, suggests limited genetic variation among the sorghum genotypes. The population structure analysis provides insights into the genetic diversity among sorghum genotypes and is useful in controlling false-positive associations between marker loci and traits of interest (Yahaya *et al*., 2023).

The STRUCTURE analysis indicated a genetic structure consisting of nine sub-populations of the sorghum genotypes under study. Population 1, 2, 3, 4, 5, 7, 8 and 9 constituted 10.8%, 8.1%, 24.3%, 8.1%, 6.8%, 14.9%, 12.2%, 6.8% and 8%, respectively. Population 5 was the

only cluster that included only South African genotypes, while the rest of the clusters showed a mix of genotypes from Zimbabwe and South Africa in varying proportions. SNP density plots for Subpopulations 2 and 5 (0.022026) showed the highest FST value. These two populations comprised 80% Zimbabwean genotypes and 20% South African genotypes for population 2, while population 5 consisted entirely of South African genotypes. Despite their diverse origins, the grouping of genotypes from South African and Zimbabwean collections indicates a strong genetic association. However, Subpopulation 5 proved to be purely of South African origin.

The polymorphic genus Sorghum's primary centre is in Africa, according to (Aleksandrov *et al*., 2023). Improved pure-line varieties and hybrids were released in the region due to subsequent pedigree breeding programs that involved local and exotic crosses. As a result, the subpopulations had a combination of South African and Zimbabwean genotypes in varying proportions. A dendrogram analysis revealed nine clusters, consistent with the population structure analysis, indicating significant genetic variation among the 74 sorghum genotypes studied. This study's analysis of molecular variance showed that the genetic variation within populations (36%) was lower than among populations (64%). In self-pollinating species like sorghum, it is normal to maintain genetic variation within populations, while genetic variation tends to be lower among populations.

A recent genetic diversity study utilising SNP marker on sorghum genotypes from Ethiopia revealed that 64.5% of the total variation was attributed to variation among genotypes, while 35.5% was attributed to variation within genotypes (Mamo *et al*., 2023). Similarly, in a study by Adomako *et al*. (2024) involving 22 sorghum genotypes, a substantial genetic variation of 66.9% was observed among the genotypes, with within-accession variation accounting for 23.6% of the total variation. Low genetic variation within the genotypes is expected in self-pollinating crops like sorghum, as reported by Latta *et al*. (2022). Furthermore, the high genetic variation within the population could be attributed to the preservation of sorghum landraces by farmers in Africa and suggested differences in adaptation and parentage.

Genetic differentiation (FST) quantifies the extent of genetic diversity resulting from allele frequency variations among populations, reflecting population structure. Values above 0.15 are considered significant in distinguishing populations, while values below 0.05 indicate a lack of substantial genetic structuring. In this study, the highest FST value (0.022026) was observed between subpopulations 4 and 5, indicating poor genetic differentiation between these two subpopulations. Furthermore, the FST value for subpopulations 3 and 5 (-0.00033) suggests

very low genetic variability. From the FST index, 36% of the combinations had a positive FST value while 64% were negative. Negative values may indicate that sample sizes are insufficient relative to the total number of alleles. Therefore, more sampling may reveal positive values and eliminate negative values, changing potential findings. Most programs calculate FST using the computations given by Weir (2012), which allow for negative FST values. Negative values generally indicate that there is more variety within than between populations.

5.6 CONCLUSIONS

This study utilised SNP markers obtained from DArTseq to conduct genetic diversity analysis and population structure assessment on a panel of 74 sorghum genotypes from South Africa and Zimbabwe. The aim was to establish a foundation for future breeding programs. The study demonstrated the effectiveness of SNP markers from DArTseq in evaluating relatedness and genetic diversity in sorghum. Populations 1–9 represented 10.8%, 8.1%, 24.3%, 8.1%, 6.8%, 14.9%, 12.2%, 6.8%, and 8% of the total population. Population 5 consisted only of South African genotypes (SA1595, SA2133, SA3280, SA1826, and SA1794). However, further research is necessary to enhance the genetic basis of the crop. Accessions SCSHYB017085, SA2490, SA1796, SCSHYB015024, SA2527, SCSHYB015022, SCSHYB015026, and SCSHYB015018 should be further investigated and utilised as breeding stock since they are distantly related to all other genotypes. There was no evidence of clustering based on origin, indicating potential genetic material overlap between the two countries. Morphological data should complement molecular data to provide clusters with biological significance. These findings are valuable for identifying SNP markers associated with desirable agronomic traits, including enhanced stress tolerance to biotic and abiotic factors.

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CHAPTER 6

ASSOCIATION MAPPING FOR *STRIGA* TOLERANCE AND YIELD-RELATED TRAITS IN SORGHUM GENOTYPES

ABSTRACT

A substantial portion of arable land used for cereal production in sub-Saharan Africa is heavily infested with *Striga* species, creating a major constraint to agricultural productivity across the region. In this study, association mapping was done on plant height, panicle height, number of leaves per plant, field fresh grain weight, dry grain weight, and chlorophyll with 6,094,317 single nucleotide polymorphism (SNP) markers for *Striga* resistance genes in diverse sorghum breeding lines and varieties released for resistance breeding. Chromosomes containing significant SNPs in FASTmrMLM and FarmCPU models were identified and computed. Chromosomes 1, 2, 3, 4 and 6 harboured SNPs significant for *Striga* tolerance in sorghum for agronomic-related traits. Agronomic traits measured revealed significant SNP counts as follows: plant height (4), panicle height (3), leaves per plant (2), field fresh grain weight (8), dry grain weight (2), and chlorophyll content (3). After successful validation, these 22 newly identified SNP markers linked to *Striga* resistance could be used for trait introgression and marker-assisted selection to increase *Striga* resistance in sorghum.Aslo detected were 12 SNPs using the FASTmrMLM model without adjusting the threshold level. However, no significant SNPs were detected with FarmCPU before the threshold was adjusted. Also, identified were 95 significant SNPs upon lowering the Bonferroni threshold value to $p<0.001$. The parent materials for the intraspecific cross that produced the currently accessible molecular map were selected from the gene pool of cultivated sorghum. This map is invaluable for real-world breeding applications. Subsequent crosses among cultivated sorghum genotypes of interest to breeders will likely produce polymorphic segregating Diversity Array Technology (DArTSeq) markers within the cultivated gene pool.

Keywords: DArTSeq (Diversity Array Technology), sub-Saharan Africa, *Striga hermonthica*, single nucleotide polymorphism, *Sorghum bicolor*

6.1 INTRODUCTION

Parasitism by *Striga* spp. is one of the most significant threats in efforts to increase agricultural productivity in sub-Saharan Africa. *Striga* spp. are endemic to a vast proportion of arable land available for cereal cultivation in sub-Saharan Africa (Abdullahi and Musa, 2024; Yonli *et al*., 2024). Numerous biotic variables, such as parasitic weeds, infectious diseases, viruses, and insect pests, pose hazards to sorghum crops. Diseases, particularly those caused by biotic factors, inflict significant financial losses in sorghum production every year (Aslam *et al*., 2024). *Striga* spp. are among the predominant invasive and the most pervasive weeds, limiting sorghum productivity worldwide, especially in Africa. In severely infested soils, *Striga* spp. can cause up to 100% yield losses (Rouamba *et al*., 2024). Overall, deploying contemporary

genetic modification techniques to facilitate the identification and cloning of candidate genes associated with essential agronomic traits, including high yield and biotic stress resistance, will enhance sorghum yield and resistance performance (Mukherjee *et al*., 2024).

Quantitative trait locus (QTL) mapping is an essential tool that can facilitate the identification of parasitic weed resistance genes in sorghum. Using single sequence repeat (SSR) markers in conjunction with marker assisted selection (MAS), Afolayan *et al*. (2004) identified six resistance quantitative trait loci (QTL) genes in sorghum that confer resistance against the *Striga* on Chromosomes A, J1, B, I, and J2. It is effective to use resistant QTL and MAS-based studies to help produce resistant genotypes" (Afolayan *et al*., 2020, Kavuluko *et al*., 2021). Understanding host plant resistance to *Striga*, including its genetic underpinnings, is a crucial prerequisite for breeding initiatives. Additionally, the effectiveness of the discovery and introgression of *Striga* resistance genes in highly adapted germplasms of maize and sorghum were recently validated through genome-wide association (GWAS) and marker-assisted selection studies (Afolayan *et al*., 2020; Kavuluko *et al*., 2021; Mudaki *et al*., 2023). For instance, Pfunye *et al.* (2021) discovered markers linked to all *S. asiatica* emerging plants on chromosomes 5, 6 and 7 (Pfunye *et al*., 2021). These markers could be verified and applied in future breeding initiatives. According to Pfunye *et al.* (2021), the three single nucleotide polymorphism (SNP) markers discovered in all emerging *Striga* plants indicate their potential for successful genomic selection (Pfunye *et al*., 2021).

GWAS examines the whole genome for correlations between behavioural changes and genetic markers, typically SNPs (Abdellaoui *et al*., 2023; Beck *et al*., 2023; Wu *et al*., 2023). The main objective of a GWAS is to separate spurious relationships that may emerge from confounding factors, such as kinship and population structure, from true marker-trait connections derived from actual genetic effects (Beck *et al*., 2023). Previous studies have shown that selecting suitable and optimum-performance GWAS models to support GWAS-based analysis requires an extensive investigation (Wu *et al*., 2023). GWAS models have effectively identified genetic areas linked to traits of significant agronomic interest in sorghum (Li *et al*., 2022; Zhang *et al*., 2023). Molecular markers linked to desirable qualities are crucial for identifying genetic loci or alleles that cause phenotypic variations based on the linkage disequilibrium (Lee *et al*., 2023). In this study, performed association mapping was done on plant height, panicle height, number of leaves per plant, field fresh grain weight, dry grain weight, and chlorophyll with 6,094,317 SNP markers for *Striga* resistance genes in diverse sorghum breeding lines and varieties released for resistance breeding

6.2 MATERIALS AND METHODS

6.2.1 Phenotype data

A total of 74 sorghum genotypes from breeding lines and released varieties were collected in South Africa and Zimbabwe as shown in section 3.3. All the phenotyped populations were grown under greenhouse conditions. The sorghum genotypes were planted in pots with topsoil mixed with compost (3:1) at the Rattray Arnold Research Station (RARS) in March 2022. A triplicate 3 × 25 alpha-lattice design was used for the experiments. After screening for Striga resistance, mature panicles were harvested and dried for storage (<15% moisture).

6.2.2 DNA extraction, sequencing, and SNP calling

The procedure is as detailed in Chapter 5, Section 5.2.2.

6.2.3 Diversity Array Technology analysis

The procedure is as detailed in Chapter 5, Section 5.2.2.1.

6.3 DATA ANALYSIS

6.3.1 Population structure

The procedure is as detailed in Chapter 5, Section 5.3.1.

6.3.2 Association analysis

Phenotypic traits with a standard deviation (SD) of 0 were excluded from genome-wide association studies (GWAS). GWAS was performed using the FarmCPU model in the GAPIT package. Furthermore, a multi-locus random GWAS analysis was performed on agronomic traits using the FASTmrMLM model in the mrMLM package to identify significant SNPs without adjusting the threshold. Manhattan and Quantile-Quantile (QQ) plots were generated using the *qqman* package.

6.4 RESULTS

6.4.1 Summary of traits and chromosomes with significant SNPs identified via FASTmrMLM and FarmCPU

Chromosomes harbouring significant SNPs identified in both FASTmrMLM and FarmCPU models were characterised. Chromosomes 1, 2, 3, 4 and 6 contained SNPs significant for *Striga* tolerance in sorghum, specifically for agronomic-related traits. Chromosomes 1(1;3), 2 (5;4), 3 (2;1), 4 (2;1), and 6(1;2) contained SNPs with varying levels of significance for FarmCPU and FASTmrMLM (**Figure 14**). The agronomic traits measured using FASTmrMLM and FarmCPU showed that the SPNs for plant height (3;1), panicle height (1;2), number of leaves per plant (1;1), field fresh grain weight (3;5), dry grain weight (1;1), and chlorophyll content (2;1) were significant (**Figure 15**). Fresh field weight contained the highest number of significant SNPs (8). In contrast, the number of leaves per plant and dry grain weight contained the lowest number of SNPs (two) (**Figure 15**).

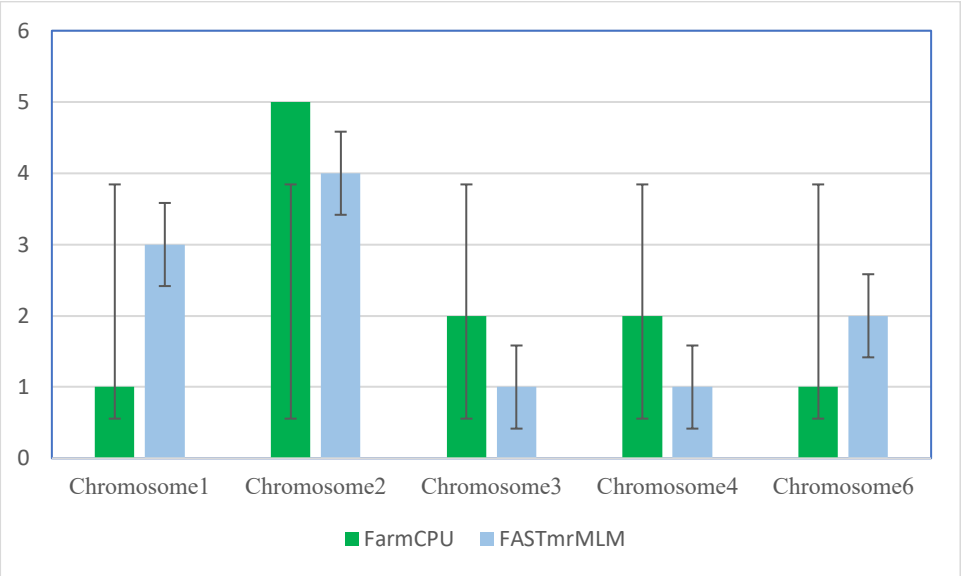

Figure 14: Number of SNPs per chromosome for FarmCPU and FASTmrML analysis method

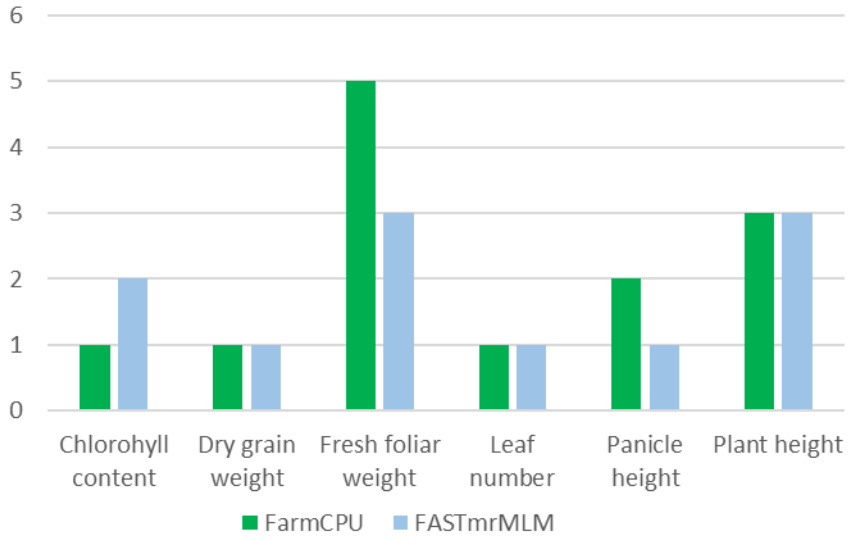

Figure 15: Number of SNPs for each trait for FarmCPU and FASTmrMLM analysis method

6.4.2 Association mapping of agronomic and yield-related traits of sorghum in response to *Striga* infestation

Association mapping was performed using the FASTmrMLM model and FarmCPU model to identify loci linked to the evaluated traits. For the FarmCPU model, no significant SNPs were identified at $p<0.005$; therefore, the Bonferroni threshold was reduced to $p<0.001$. Multi-locus random GWAS analysis was performed on agronomic traits using the FASTmrMLM model in the mrMLM package. Association analyses of *Striga* resistance and agronomic traits were performed (**Figure 16)**. The fresh field weight showed that all 10 chromosomes contained significant SNPs above the threshold level using the FarmCPU model (**Figure 16a**), with chromosomes 2, 3, and 5 containing significant SNPs. The QQ plot followed the expected distribution of SNPs (**Figure 16b**). Analysis using the FASTmrMLM model revealed that chromosomes 2 (2) and 10 (1) contain significant SNPs above the threshold level (**Figure 16c**). The QQ plots deviated positively from the expected line of the SNPs, with three SNPs at the tail (**Figure 16d**). The dry grain weight showed that all 10 chromosomes harboured significant SNPs above the threshold level when using the FarmCPU model, with chromosome 9 containing a significant SNP. The QQ plot was below the expected distribution of the SNPs (**Figure 16f**). Analysis using the FASTmrMLM model revealed that chromosomes 2 (1), 3(1), and 5 (1) contained significant SNPs above the threshold level (**Figure 16g)**. The QQ plots deviated positively from the expected line of the SNPs, with three SNPs at the tail (**Figure 16h**).

In the FarmCPU model, the number of leaves per plant showed that chromosomes 1, 2, 3, 4, 6, 9 and 10 contain significant SNPs above the threshold level, with chromosome 9 containing a highly significant SNP. The QQ plot was below the expected distribution of the SNPs (**Figure 16j**). Analysis using FASTmrMLM revealed that chromosome 6 (2) contains significant SNPs above the threshold level (**Figure 16k**). The QQ plots deviated positively from the expected line of the SNPs, with two SNPs at the tail (**Figure 16l**). Association analysis using the FarmCPU model for *Striga* resistance and the number of leaves per plant showed that chromosomes 1, 2, 4, 5, 9 and 10 harbour significant SNPs above the threshold level (**Figure 16m**) with chromosome 5 containing a significant SNP. The QQ plot was below the expected distribution of the SNPs (**Figure 16n**).

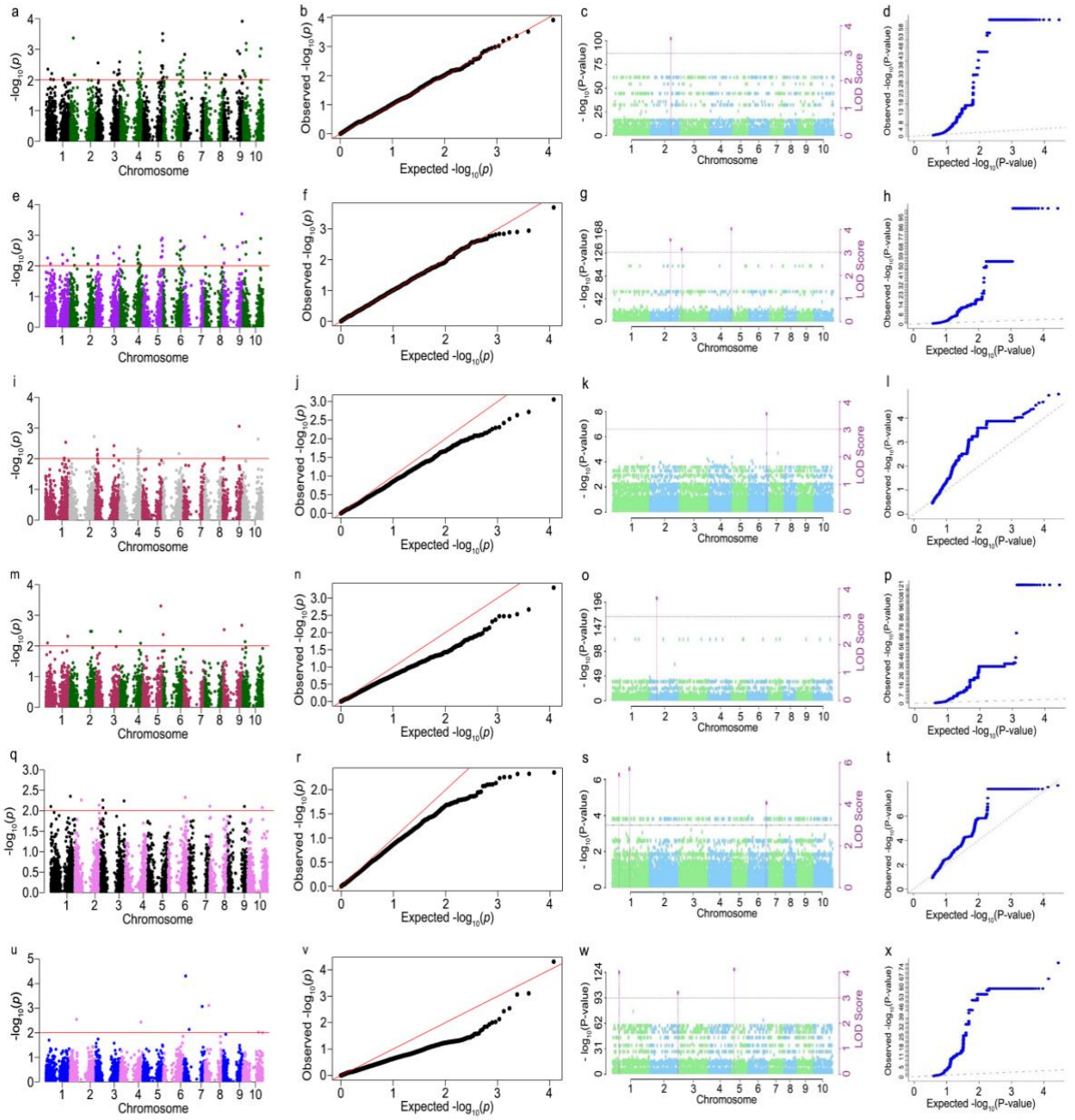

Figure 16: Association analysis results for *Striga* resistance and agronomic traits of 74 sorghum genotypes using the FarmCPU and FASTmrMLM models

(a) Fresh field weight Manhattan plots from association mapping using FarmCPU. **(b)** Q-Q plot for Fresh field weight using FarmCPU. **(c)** Fresh field weight Manhattan plots using FASTmrMLM. **(d)** Q-Q plot for fresh field weight using FASTmrMLM. **(e)** Dry grain weight Manhattan plots using FarmCPU. **(f)** Q-Q plot for dry grain weight using FarmCPU. **(g)** Dry grain weight Manhattan using FASTmrMLM. **(h)** Q-Q plot for dry grain weight using FASTmrMLM. **(i)** Number of leaves Manhattan plot using FASTmrMLM. **(j)** Q-Q plot for number of leaves using FarmCPU. **(k)** Number of leaves Manhattan plots from association mapping using FarmCPU. **(l)** Q-Q plot for number of leaves using FASTmrMLM. **(m)** Panicle height Manhattan plot using FASTmrMLM. **(n)** Q-Q plot for panicle height using FarmCPU. **(o)** Panicle height Manhattan plot using FarmCPU. **(p)** Q-Q plot for panicle height using FASTmrMLM. **(q)** Plant height Manhattan pl using FASTmrMLM. **(r)** Q-Q plot for plant

height using FarmCPU. **(s)** Plant height Manhattan plot using FASTmrMLM. **(t)** Q-Q plot for plant height using FASTmrMLM. **(u)** Chlorophyll content Manhattan plot using FASTmrMLM. **(v)** Q-Q plot for chlorophyll content using FarmCPU. **(w)** Chlorophyll content Manhattan plot using FASTmrMLM. **(x)** Q-Q plot for chlorophyll content using FASTmrMLM. *The red or solid line denotes the expected distribution of the SNPs.

FASTmrMLM analysis revealed that chromosome 2 (2) contains significant SNPs above the threshold level (**Figure 16o**). The QQ plots deviated positively from the expected line of the SNPs, with three SNPs at the tail (**Figure 16p**). Using FarmCPU, plant height analysis showed that chromosomes 1, 2, 3, 6, 8, 9 and 10 harbour significant SNPs above the threshold level, with chromosome 1 containing SNPs with high significance (**Figure 16q**). The QQ plot was below the expected distribution of the SNPs (**Figure 16r**). Analysis using FASTmrMLM revealed that chromosomes 1 (2) and 6 (1) contain significant SNPs above the threshold level (**Figure 16s**). The QQ plots deviated positively from the expected line of the SNPs, with three SNPs at the tail (**Figure 3t**). Chlorophyll content analysis using FarmCPU showed that chromosomes 2, 4 and 7 contain significant SNPs above the threshold level (**Figure 16u**) with chromosome 7 harbouring highly significant SNPs. The QQ plot was below the expected distribution of the SNPs (**Figure 16v**). Analysis using FASTmrMLM revealed that chromosomes 1 (1), 2 (1), and 5 (1) contain significant SNPs above the threshold level (**Figure 16w**). The QQ plots deviated positively from the expected line of the SNPs, with three SNPs at the tail (**Figure 16x**).

6.5 DISCUSSION

Although population structure can influence the results of GWAS and increase false connections, GWAS are effective tools for identifying genetic variations related to diverse phenotypes (Abdellaoui *et al*., 2023). These GWAS models use a kinship matrix to lessen false positives when germplasm accessions have a common ancestor (Wu *et al*., 2023). Numerous genes conferring essential agronomic traits and *Striga* resistance were identified in the 10 sorghum chromosomes. Additionally, we demonstrated that five of these chromosomes harbour important markers according to results from FASTmrMLM and FarmCPU analyses. A total of 22 significant SNPs linked to *Striga* resistance were identified in chromosomes 1 (4), 2 (9), 3 (4), 4 (2) and 6 (3) (**Figure 16**). After successful validation, these SNP markers could be used for trait introgression and marker-assisted selection to increase *Striga* resistance in sorghum. Varying numbers of SNPs for agronomic traits corresponding to plant height (4),

panicle height (3), number of leaves per plant (2), fresh field grain weight (8), dried grain weight (2), and chlorophyll content (3) were identified (**Figure 15**).

The greatest number of significant SNPs for *Striga* tolerance was found in fresh field grain weight. A study conducted in Kenya using DArT sequencing classified the genotypes into three primary clusters; all resistance checks, except for N13, fell into the same cluster (Muchira, 2022). The present findings support previous research (Muchira, 2022) that identified SNPs linked to *Striga* resistance genes and this can significantly accelerate the prospects of future breeding projects for *Striga* resistance.

Numerous studies have examined *Striga* resistance in pearl millet and maize (Badu-Apraku *et al*., 2016; Belay, 2022; Irondi *et al*., 2022). Laboratory research in Eritrea identified one to three *Striga* resistance QTL in eight sorghum genotypes using SSR markers (Tadesse, 2022). Similarly, 28 important SNP markers associated with *Striga* emergence were found on chromosomes 1, 2, 3, 4, 6 and 7 in pearl millet, indicating its potential for trait introgression and marker-assisted selection to enhance *Striga* resistance (Rouamba *et al*., 2024). Notably, no SNP overlap was detected in maize between the host plant's *Striga* damage syndrome rating and the number of *Striga*-emerged plants. However, five SNPs on chromosome 3 showed that the number of *Striga*-emerged plants overlapped with four QTL found for the host plant's *Striga* damage syndrome rating in previous studies (Gowda *et al*., 2021; Stanley *et al*., 2021; Yacoubou *et al*., 2021), suggesting that this region may carry a significant gene or genes associated with *Striga* resistance.

Furthermore, according to Muchira (2022), the most consistently *Striga* resistant genotypes across both field and potted artificially infested trials were the F4 generation of F6YQ212 × B35 cross and GBK 045827 wild relative, though not necessarily high yielding as indicated by their high grain weightvalues in both trials. In this study, association mapping was conducted using both the FASTmrMLM and FarmCPU models to identify SNPs linked to the traits of interest. The FASTmrMLM model proved more effective, detecting 12 significant SNPs without the need to adjust the significance threshold. In contrast, the FarmCPU model failed to identify any significant associations under the same conditions. This suggests that FASTmrMLM offers greater sensitivity and is the more suitable model for detecting marker-trait associations in this context.

Ninety-five SNPs were found to be significant when the Bonferroni threshold was lowered to $p<0.001$ to identify significant genetic connections (Rosoff *et al*., 2021). To identify SNPs for

follow-up investigations, Duggal *et al*. (2008) proposed that a suggestive association threshold should be used, and both the significant and highly significant associations should be considered as regions of more likely of association. These authors concluded that even if a region does not naturally match these levels, researchers should still consider it significant if it has good candidate genes or biological plausibility because these thresholds are merely recommendations that consider the interdependency of SNPs.

The use of molecular markers in plant breeding is a potential new tool. Under field conditions, they enable the identification and mapping of genes for QTL implicated in polygenic and quantitative resistance, as well as individual and monogenic resistance mechanisms (such as the low stimulant locus). All linkage groups had a high percentage of DArT markers, suggesting that DArT markers are more common than SSRs. According to previous reports (Adu *et al*., 2024; Li *et al*., 2022; Mudaki *et al*., 2023), DArT markers are more likely to map to gene-rich regions than genomic SSR and AFLP markers (Ali *et al*., 2016). Analyses of marker-trait associations have shown that relationships between specific phenotypes and genotypes within a genome may help identify genes that control these traits.

To identify novel sources of *Striga* resistance, Kavuluko *et al*. (2021) used rhizotrons in a laboratory assay to screen a worldwide sorghum diversity panel and GWAS to uncover the mechanisms of resistance and clarify the genetic loci underlying resistance. They observed QTL overlaps on chromosomes 1, 2, 9 and 10 in both mapped populations. Interestingly, even though the genotype was employed as a donor for pre-germination resistance, QTL from the IS9830 RIL corresponded to significant SNPs in our GWAS research (Kavuluko *et al*., 2021). Twenty-four markers were identified at the threshold of -log (p) = 4, number of emerged *Striga* plants, number of ears per plant, ear aspect, and grain yield under *Striga* infestation, which increases the level of resistance to *Striga* in the available early maturing tropical maize germplasm. These loci were found on chromosomes 1, 2, 3, 4 and 6. In the present study, 23 SNPs were identified on chromosomes 1, 2, 3, 4 and 6.

In this study, the parent materials for the intraspecific cross that produced the currently accessible molecular map were selected from the cultivated sorghum gene pool. This molecular map is particularly valuable for real-world breeding applications, as it provides essential insights into improving sorghum traits. This is because subsequent crossings between the genotypes of cultivated sorghum that are of interest to breeders are likely to have polymorphic segregating DArTseq markers found within the cultivated gene pool. Sorghum (Haussmann *et*

al., 2002) and several other crops (Al-Ghamedi *et al*., 2023; Ali *et al*., 2016; El-Rawy and Hassan, 2021; Ren *et al*., 2003) have exhibited marker clustering with distorted segregation. Most sorghum genetic maps are constructed using wide crosses. A disadvantage of these maps is that the identified loci may only be polymorphic between divergent genotypes. Molecular maps based on crosses involving wild progenitors also have few direct applications in breeding programs, which usually exploit intraspecific variations within cultivated forms. This study offers valuable insights because the population used involves inbred lines and released varieties, which can provide meaningful intraspecific variations among genotypes.

6.6 CONCLUSIONS

Marker trait association is essential to identify genomic areas linked to *Striga* resistance for marker-assisted breeding in sorghum improvement initiatives. Twenty-two new markers associated with *Striga* resistance were identified. In the future, parental selection, quantitative trait loci analysis, trait introgression, gene pyramiding, and marker-assisted selection of *Striga* resistance in sorghum breeding programs in Zimbabwe and South Africa or related agro-ecologies will benefit from these novel genetic markers found in current sorghum populations. However, further research is required to validate the markers identified in this study.

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CHAPTER 7

GENETIC WIDE ASSOCIATION MAPPING FOR *STRIGA* RESISTANCE, SECONDARY METABOLITES AND NUTRITIONAL COMPOSITION RELATED TRAITS IN SORGHUM

ABSTRACT

Africa faces challenges in growing sorghum because of the crop's biotic and abiotic elements that vary depending on the region. Still, *Striga* (*Striga hermonthica*) and drought are the continent's two main problems. This study analysed the nutritional composition and association between *Striga* resistance for secondary metabolites and sorghum breeding lines and released varieties in Zimbabwe and South Africa. Every chromosome included unique SNPs for sorghum resistance to *Striga*, according to analysis of secondary metabolites. Chromosome 1 had the highest number of SNPs discovered, whereas chromosome 7 had the lowest number. The hydroxybenzoic acid (6), gallic acid (5), ferulic acid (74), cinnamic acid (11), hydroxybenzoic acid (6), p-coumaric (9), and caffeic acid (2) SNPs were identified. On chromosomes 1 and 9 of the Manhattan plot for caffeic acid significant SNPs were discovered. Protocatechuic acid detected significant SNPs on chromosome 2, 3, 4, 5 and 10, and syringic acid detected significant SNPs on chromosome 3, 5, 8, 9 and 10. Gallic acid revealed significant SNPs on chromosomes 1, 2, 3 and 10, hydroxybenzoic acid found significant SNPs on chromosomes 1, 2, 3, 5 and 9, and p-coumaric acid found significant SNPs on chromosomes 1, 2, 5, 9 and 10. Significant SNPs for *Striga* resistance were found on chromosomes 1, 3, 4, 8 and 9 in relation to dietary content. For each of the 74 sorghum genotypes that were sequenced, different numbers of SNPs were found for varying nutritional parameters, including ash content (1), carbohydrates (3), fat content (1), fibre content (2), and protein content (1). The Manhattan plot for traits related to nutritional composition for 74 genotypes of sorghum showed that significant SNPs were found on chromosomes 8 and 9 for carbohydrates, on chromosomes 4 for ash content, on chromosome 3 for fat content, on chromosomes 1 and 3 for fibre content, and on chromosome 3 for protein content. A total of 119 SNPs were identified for the following: syringic acid, protocatechuic acid, p-coumaric, hydroxybenzoic acid, gallic acid, ferulic acid, cinnamic acid, and caffeic acid. Regarding food composition, significant SNPs for *Striga* resistance was discovered on chromosomes 1, 3, 4, 8 and 9. For the nutritional characteristics of ash content, carbohydrates, fat content, fibre content, and protein content, a total of eight SNPs were discovered. This study identified critical SNPs that can be used to breed sorghum for nutritional components that are resistant to *Striga* as well as agronomic features. Identification of more genes linked to the discovered markers is therefore crucial for further research.

Keywords: DArTSeq, phenolic compounds, *Striga* resistance, single nucleotide polymorphism, *Sorghum bicolor*

7.1 INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the Poaceace family and it is one of the globally important cereal crops (Kundu *et al*., 2023. It is extensively cultivated in the semi-arid tropical regions, which frequently face droughts (Kundu *et al*., 2023). However, biotic and abiotic constraints are endangering sorghum's potential output. Included among the abiotic factors are salt, drought, and low soil fertility. Insects, panicle diseases, stem borers, and the hemi-parasitic weed *Striga* are among the significant biotic restrictions on agriculture (Yacoubou *et al*., 2021; Yonli *et al*., 2024). The difficulties in producing sorghum in Africa are due to both biotic and abiotic factors, which differ according on the location. The two biggest issues facing the continent, nevertheless, are drought and *Striga* (*Striga hermonthica*). "With over 30,000 accessions available in global genetic collections, sorghum is a cereal with remarkable genetic variability" (Upadhyaya *et al*., 2014). The physical traits vary depending on the growth environment and genetics. As most tropical sorghum plants have short days, their ability to adjust to the duration of the day is a crucial adaptation.

The genome wide association (GWAS) is an effective technique for determining the inheritance pattern of complex agronomical traits because it uses a single nucleotide polymorphism (SNP) biomarker, which has been successfully utilised in the investigation of the genetic basis of complicated agronomical variables (Beck *et al*., 2023). Using GWAS, significant genomic areas and genes that control important complex traits in sorghum have been identified. Morris *et al*. (2013) created the most well-known panel and genotyped it using genotyping-by-sequencing (GBS), yielding a total of 265,487 SNPs. The genetic basis of yield and related variables, including plant height, inflorescence morphology, grain quality attributes, stalk rot, and resistance to the anthracnose (*Colletotrichum sublineolum*) disease, were next examined using this panel. Strigolactones (SLs) are secondary plant metabolites generated from carotenoids that are important in the interaction between parasitic root weeds and host plants, in addition to their significance in other aspects of plant growth (Afolayan *et al*., 2020). The management of parasitic weeds may be greatly aided by such strategies as suicidal germination with synthetic SLs, breeding for genotypes with low SL production and exudation, and altering the SL signalling in host plants (Beck *et al*., 2023). The biosynthesis, structural diversity, production, function in the host-parasite relationship, and possible application for parasitic weed control of SLs are very important factors to consider (Mandumbu *et al*., 2019).

The phenylpropanoid pathway, which plays a key role in lignin biosynthesis, was investigated. Within this pathway, one phenylalanine ammonia-lyase (PAL), ferulate 5-hydroxylase (F5H), caffeic acid O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), and five peroxidase genes were identified in gene modules (light blue steel and coral1) that were enriched for lignin production (Tak & Kumar, 2020. In both the early and late stages of infection, IS2814, N13, and IS9830 showed considerable induction of all these genes. The induction in IS2814 was more significant than in N13 and IS9830. The hormone signalling pathway with particular attention to the salicylic acid (SA) and jasmonic acid (JA) signalling pathways were also studied (Morris *et al*., 2013).

Jasmonate zim-domain (JAZ) proteins, which act as transcriptional repressors, are degraded in response to jasmonic acid (JA), thereby releasing downstream transcription factors that trigger defense responses, including programmed cell death. This study observed a 4- to 8-fold reduction in the expression of nine JAZ genes in genotypes IS2814, IS14963, and IS41724, aligning with JA-mediated defense activation and indicating suppression of JAZ expression as part of the response mechanism (Fakrudin *et al*., 2021; Mutinda *et al*., 2023). The JAZ levels in the other two genotypes, IS9830 and N13, did not change. Based on these findings, it was concluded that genotypes IS2814, IS14963, and IS41724 exhibit genotype-specific *Striga* resistance responses, which are mediated by JA. Additionally, all genotypes experience a *Striga*-induced SAR (Mutinda *et al*., 2023).

Public and private research institutions, including the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), have begun cultivating sorghum to address the dietary needs of vulnerable groups, such as mothers and children (Ashok Kumar *et al*., 2011). Sorghum genotypes with low anti-nutritional characteristics, including phytate levels and high iron and zinc concentrations, were recently issued by ICRISAT. Nutrient contents and other quality parameters are largely influenced by additive genetic regulation and are highly heritable.

Sorghum exhibits lower protein digestibility compared to other cereal grains due to disulfide cross-linking in the β- and γ-kafirin proteins. Two major QTLs on linkage group 1 influence this trait: one linked to marker Xtxp11 negatively affects digestibility, while another, located approximately 20 cM away and associated with marker Xtxp88, has a positive impact on protein digestibility. (Winn *et al*., 2009). SNPs in multiple genes were found by association mapping for grain quality in a broad sorghum collection (Ayalew *et al*., 2022; Kimani *et al*., 2020; Zhang *et al*., 2023).

An SNP located on the candidate gene SSIIa on linkage group 10 was associated with kernel hardness, explaining 8% of the trait's variation. Additionally, SNPs SB00214.1 and SB00214.2 were also linked to kernel hardness, collectively accounting for 10% of the observed variation in this trait (Ayalew *et al*., 2022). SNPs linked to kernel hardness (SB00214.1 and SB00214.2) were found at the locus pSB1700 on LG 3 and accounted for 10% of the variation in the trait. The calcium content-related SNPs SB00156.1 and SB00054.1 were found in LG 3 at 50 and 59 cm, respectively (Zhang *et al*., 2023). "The locus pSB0289, where SB00156.1 was found, was anticipated to generate serine/threonine protein kinase. 10% of the variance in the characteristic was explained by a single nucleotide polymorphism (SNP) on LG 4's starch synthase IIb (SSIIb) gene, which was found to be significantly correlated with starch content. The identification of genetic loci influencing features relevant to renewable energy in sorghum has advanced significantly" (Punia *et al*., 2021. The juicy (sugar-rich) stem of sweet sorghum is one of its main characteristics. A substantial link was observed by Burks *et al*. (2015) in an association analysis of 42,926 SNPs associated to sugar yield, sugar volume, and midrib colour at a distance of 51.8 Mb (on chromosome 6), which correlates to a region that contains a dry midrib locus (Burks *et al*., 2015).

The objective of this study was to evaluate the association among *Striga* tolerance, production of secondary metabolites and nutritional composition of breeding lines and released varieties of sorghum from South Africa and Zimbabwe using the DArTseq method.

7.2 PLANT MATERIAL

Refer to section 3.3

7.3 SECONDARY METABOLITES DETERMINATION

7.3.1 Lipids

Extraction of solid material is achieved through solid liquid extraction or leaching. For lipids, hexane was used as the extraction solvent. Mass spectrometry was used for fatty acids analysis. Isolation of lipids of intermediate polarity was analysed using the Folch method (Bligh and Dyer, 1959).

7.3.2 Total phenolic content

The Folin-Ciocalteu colorimetric technique was utilised to ascertain the total phenolic content in the samples/extracts, with gallic acid serving as the standard (Luo *et al*., 2018).

7.3.3 Antioxidant assays

The evaluation of antioxidant capacity may vary according to the methods used. In this research, it was determined using the trolox equivalent antioxidant capacity (TEAC) assay using 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid radical) (Luo *et al*., 2018).

7.3.4 Total flavonoid content

The concentration of all flavonoids was measured at 500 nm using a colorimetric method and a chromogenic system including aluminium. Using the DPPH radical scavenging technique and ABTS, the antioxidant activity was measured (Luo *et al*., 2018).

7.3.5 Anthocyanins

Ultrasound-assisted extraction (UAE) was used to extract the anthocyanins from pericarp and lipids from the seeds of sorghum, as described by Luo and colleagues in 2018 (Luo *et al*., 2018).

7.4 NUTRITIONAL COMPOSITION TRAIT DETERMINATION

7.4.1 Moisture determination

Moisture content is ascertained using the AOAC 925:10 technique (Horwitz & Latimer, 2000). To the dried and weighted plates, two grams (2 g) of well-homogenised sorghum flour samples were added. Samples that contained dishes were put in the drying oven and left there for one hour at 130°C, or until the sample's weight remained constant. The dried samples were then taken out of the drying oven, allowed to cool to room temperature in a desiccator, and then reweighed.

$$\textbf{Moisture (\%)} = \frac{(W_1 - W_2) * 100}{S_W}$$

where W_1 is the weight of the cap and fresh sample,

W_2 is the weight of the dry sample and cap,

and S_W is the sample weight.

7.4.2 Ash determination

Ash Determination was done using the AOAC 923:03 procedures. “Four (4) grams of well-homogenized sorghum flour were weighed out and added to a sanitized, pre-weighed crucible. The crucible containing the sample was heated to 550°C in a muffle furnace. After the samples were lit until a light grey colour was achieved, they were taken out, allowed to cool to ambient temperature in a desiccator, and then weighed” (Horwitz & Latimer, 2000).

$$\textbf{Ash (\%)} = \frac{(W_1 - W_2) * 100}{S_W}$$

where W_1 is the weight of ash plus crucible after ashing,

W_2 is the weight of the empty crucible,

and S_W is the sample weight.

7.4.3 Fat content determination

Fat content determination was carried out using the Soxhlet extraction method as outlined in AOAC 945:16 (Horwitz & Latimer, 2000). An extraction thimble that had been previously prepared was filled with 2 g of sorghum flour. Cotton wool that was absorbent and fat-free was used to plug the thimble that held the sample. Before the sample's fat was extracted, the Soxhlet extraction equipment was put together and the petroleum ether spirit was poured into it to half its volume. After that, the extraction went on for four hours. The extracted fat was taken out once the allotted time had passed, and flasks holding oil or fat were connected to a rotating evaporator to evaporate most of the solvent. After 30 minutes at 103°C in a dry oven to remove any remaining solvent, the flasks were dried and cooled in a desiccator and reweighed.

$$\textbf{Fat content (\%)} = \frac{(W_f - W) * 100}{S_W}$$

where W_f is the weight of the receiver flask and fat deposit,

W is the weight of the empty receiver flask only,

and *S*w is the weight of the sample used.

7.4.4 Crude fibre determination

The AOAC techniques were followed in determining the crude fibre content (Horwitz & Latimer, 2000). After transferring two grams of pre-defatted material into a one-litre beaker, the sample was digested on a hot plate for one hour using a solution of equal parts 2.5M sulphuric acid (H_2SO_4) and 2.5M sodium hydroxide. The filtering process was then carried out by moistening it with a tiny amount of ethanol. After the filtrate was dried at 100°C in an oven, a consistent weight was achieved (W_1). The samples that had been oven-dried were next burned for three hours at 600°C in a muffle furnace. Subsequently, the burnt sample defrosted at ambient temperature and was weighed again (W_2).

$$\textbf{Crude fibre (\%)} = \frac{(W_1 - W_2) * 100}{Sw}$$

where W_1 is the weight of the porcelain crucible and sample before ashing,

W_2 is the weight of the porcelain crucible containing ash, and

W is the weight of the sample.

7.4.5 Protein determination

The Kjeldahl method was used to conduct the protein determination test (Horwitz & Latimer, 2000). A 50 ml Kjeldahl flask containing 0.5 g of sorghum flour sample was weighed and 2 g of a catalyst mixture consisting of copper and potassium sulphate was added along with 8 ml of concentrated H2S04. Samples were broken down until only a colourless, pure solution was visible. The digested samples were then distilled using a Kjeldahl distiller, and 25 ml of a mixture of 2% boric acid, combined indicator of bromocresol green + methyl red was used to collect the distilled steam gas (ammonia). Next, 0.1N HCl was used to titrate the distilled sample until the first signs of pink hue appeared.

$$\textbf{Crude protein (\%)} = \frac{(a \times b \times 14 \times 6.25) \times 100}{W}$$

where *a* is the normality of the acid;

b is the volume of standard acid used (ml), corrected for the blank (i.e., the sample minus the blank);

W is the sample weight (g); and

6.25 is the conversion factor for protein from % nitrogen.

7.4.6 Carbohydrates determination

Carbohydrate content is determined as a total carbohydrate by subtracting measured protein, fat, ash, and moisture from 100% (Pearson, 1976).

Total carbohydrate (%) = 100 – (Moisture %)+ Protein %+ Fat % + Ash %).

7.5 DNA EXTRACTION, SEQUENCING, AND SNP CALLING

The procedure is detailed in Chapter 5, Section 5.2.2.

7.6 DART ANALYSIS

The procedure is detailed in Chapter 5, Section 5.2.2.1.

7.7 DATA ANALYSIS

7.7.1 Population structure analysis

The procedure is detailed in Chapter 5, Section 5.3.1.

7.7.2 Association analysis

The procedure is detailed in Chapter 6, Section 6.3.2.

7.8 RESULTS

7.8.1 SNPs with significant P values for secondary metabolites and nutritional composition-related traits

Secondary metabolite analysis revealed that all the chromosomes showed different SNPs detected for *Striga* resistance in sorghum. Chromosomes 1, 2, 3, 4,5, 6, 7, 8, 9, and 10 resulted in 20, 10, 12, 17, 11, 18, 1, 5, 12, and 12 SNPs detected, respectively, as shown in **Figure 17**. Chromosome 1 had the highest number, while chromosome 7 showed the lowest number of

SNPs detected. The different traits showed a varied number of SNPs detected for secondary metabolites. Syringic acid (6), protocatechuic acid (6), p-coumaric (9), hydroxybenzoic acid (6), gallic acid (5), ferulic acid (74), cinnamic acid 11, and caffeic acid (2) SNPs were detected for the 74 sorghum genotypes sequenced, as shown in **Figure 18**. For nutritional composition, chromosome 1, 3, 4, 8 and 9 had 1, 3, 1, 1 and 2 SNPs, respectively, detected for *Striga* resistance, as shown in **Figure 19**. The nutritional traits had a varying number of SNPs detected with ash content (1), carbohydrates (3), fat content (1), fibre content (2) and protein content (1) SNPs detected for *Striga* resistance for the 74 sorghum genotypes sequenced, as shown in **Figure 20**.

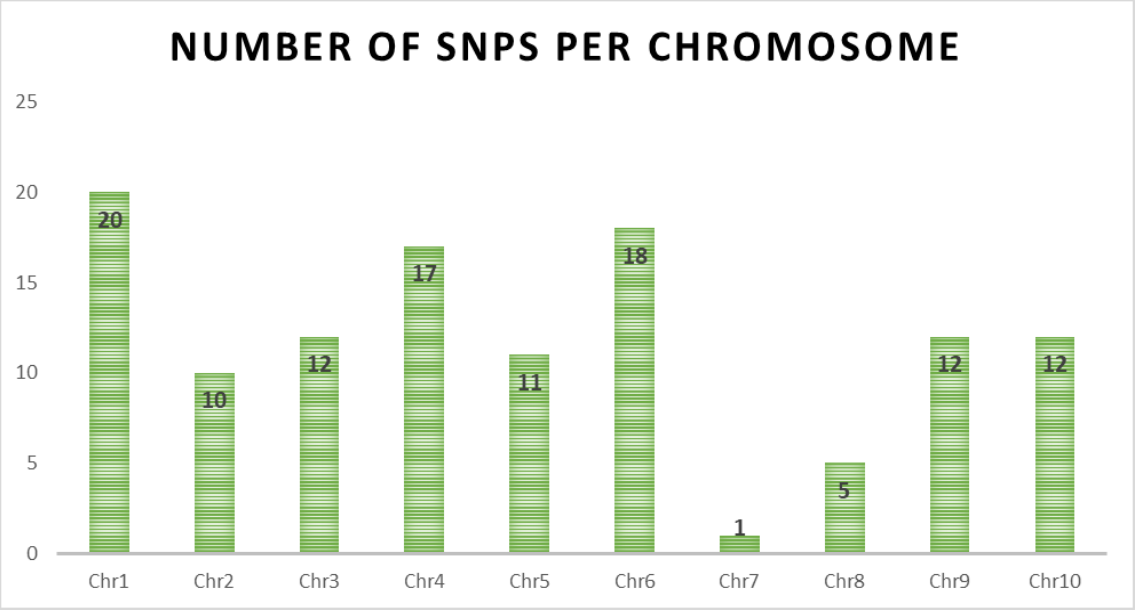

Figure 17: Number of SNPs detected for each chromosome using 6,562 SNP markers for 74 sorghum genotypes

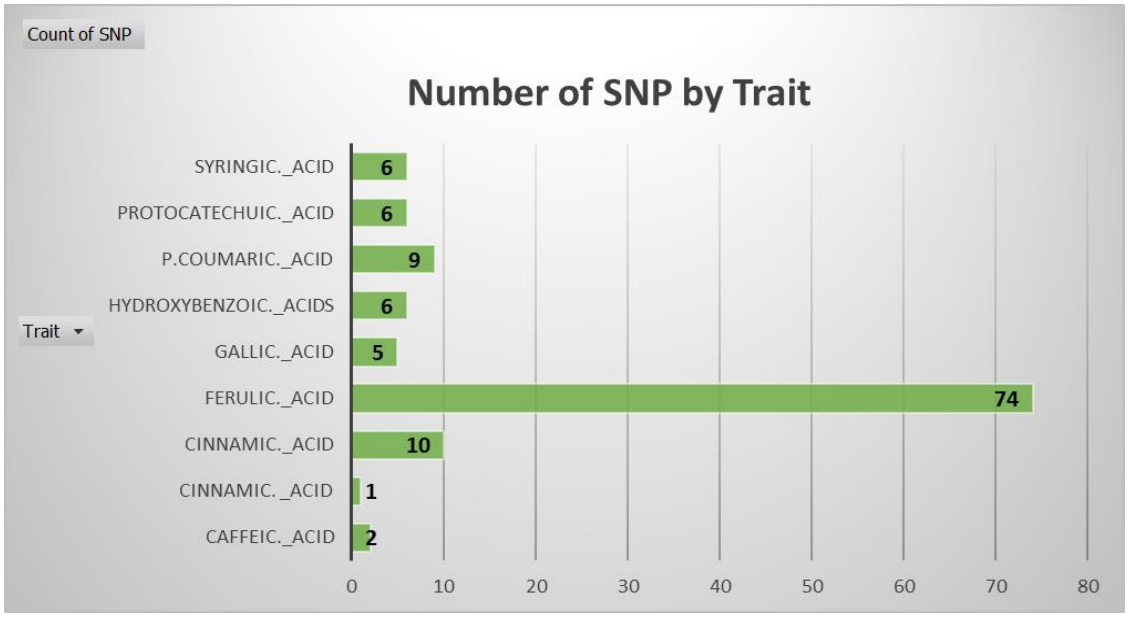

Figure 18: Number of SNPs for each secondary metabolite related trait using 6,562 SNP markers for 74 sorghum genotypes

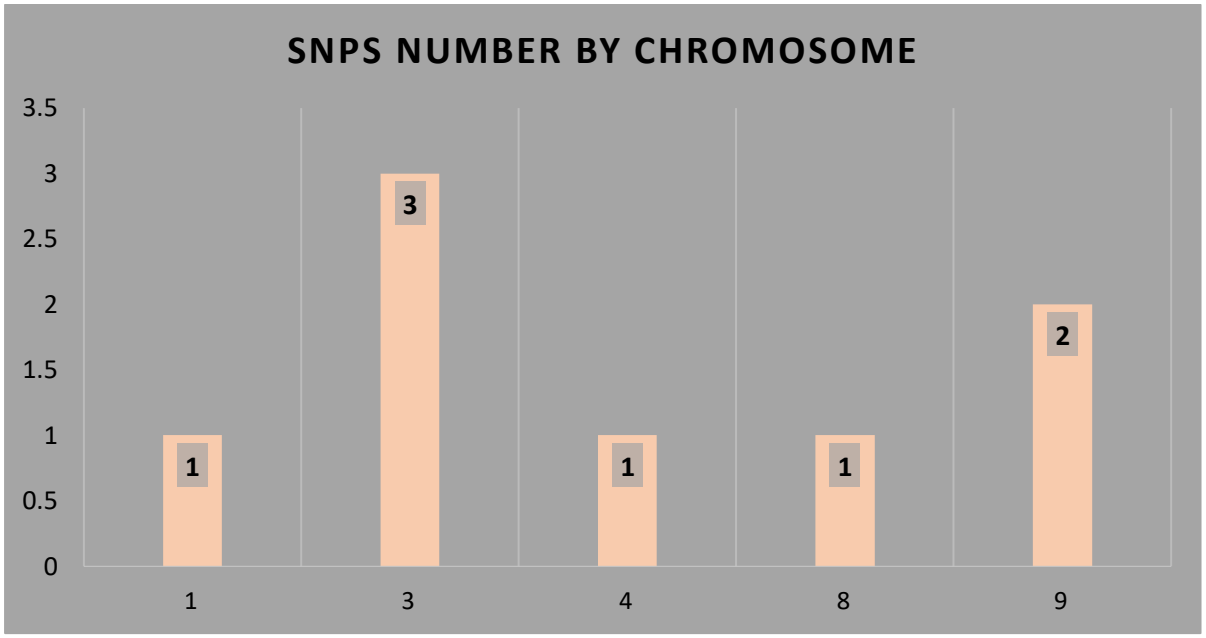

Figure 19: Number of SNPs per chromosome for nutritional composition related traits using 6,562 SNP markers for 74 sorghum genotypes

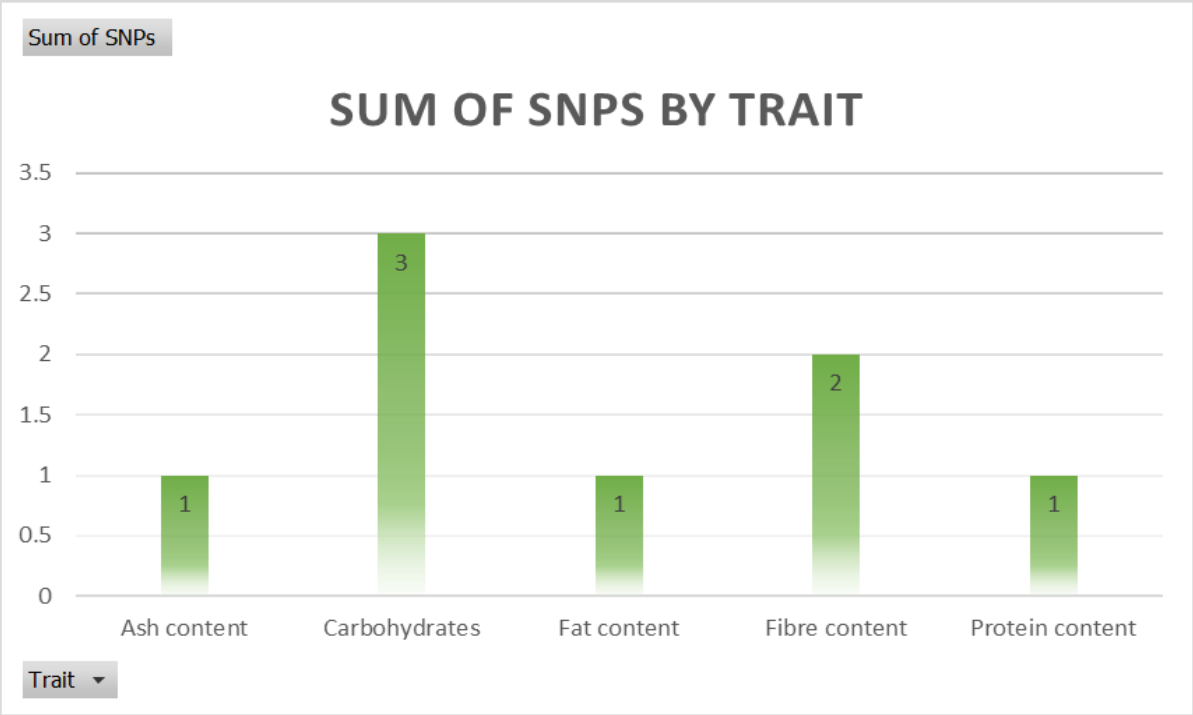

Figure 20: Number of SNPs by nutritional related trait using 6,562 SNP markers for 74 sorghum genotypes

7.8.2 Association analysis for *Striga* resistance and secondary metabolites using 6,562 SNP markers for 74 sorghum genotypes

Manhattan plot for caffeic acid shows that chromosomes 1 and 9 had significant SNPs. The Q-Q plot for caffeic acid showed a very good tail deviating for expected normal plot as shown in **Figure 21 (a)** and **(b)**, respectively. Cinnamic acid Manhattan plot revealed that chromosomes 1, 2, 4, 6, 8 and 9 detected significant SNPs with chromosomes 2 and 6 showing the most significant SNPs, as shown in **Figure 21 (c)**. QQ plot for cinnamic acid showed a perfect tail, as shown in **Figure 21 (d)**. The ferulic acid Manhattan plot detected significant SNPs on all ten chromosomes. However, chromosome 4 detected the most significant SNP. The QQ plot for ferulic acid also revealed one important SNP located at the top of the plot, as shown in **Figure 21 (e)** and **(f)**, respectively. Gallic acid Manhattan plot detected significant SNPs on chromosomes 2, 5 and 10, as shown in **Figure 21 (g)**. The QQ plot for gallic acid showed a perfect tail, as shown in **Figure 21 (h)**. Hydroxybenzoic acid Manhattan plot revealed significant SNPs on chromosomes 1, 2,3 and 10 with most significant SNP on chromosome 1, as shown **Figure 21 (i)**.

The Q-Q plot for hydroxybenzoic acid showed a perfect tail with one SNP at the end of tail, as shown in **Figure 21 (j)**. P-coumaric acid Manhattan plot detected significant SNPs on

chromosomes 1, 3, 5 and 9, while the QQ plot had a positive deviation as shown in **Figure 21 (k)** and **(l)**, respectively. Protocatechuic acid Manhattan plot detected significant SNPs on chromosomes 2, 3, 4, 5 and 10. The most significant SNPs were located on chromosome 5, while the QQ plot showed three SNPs at the end of the tail, as shown in **Figure 21 (m)** and **(n)**, respectively. Gallic acid Manhattan plot detected significant SNPs on chromosomes 3, 5, 8, 9 and 10, while the QQ plot had a perfect tail with three significant SNPs, as shown in **Figure 21 (o)** and **(p)**, respectively.

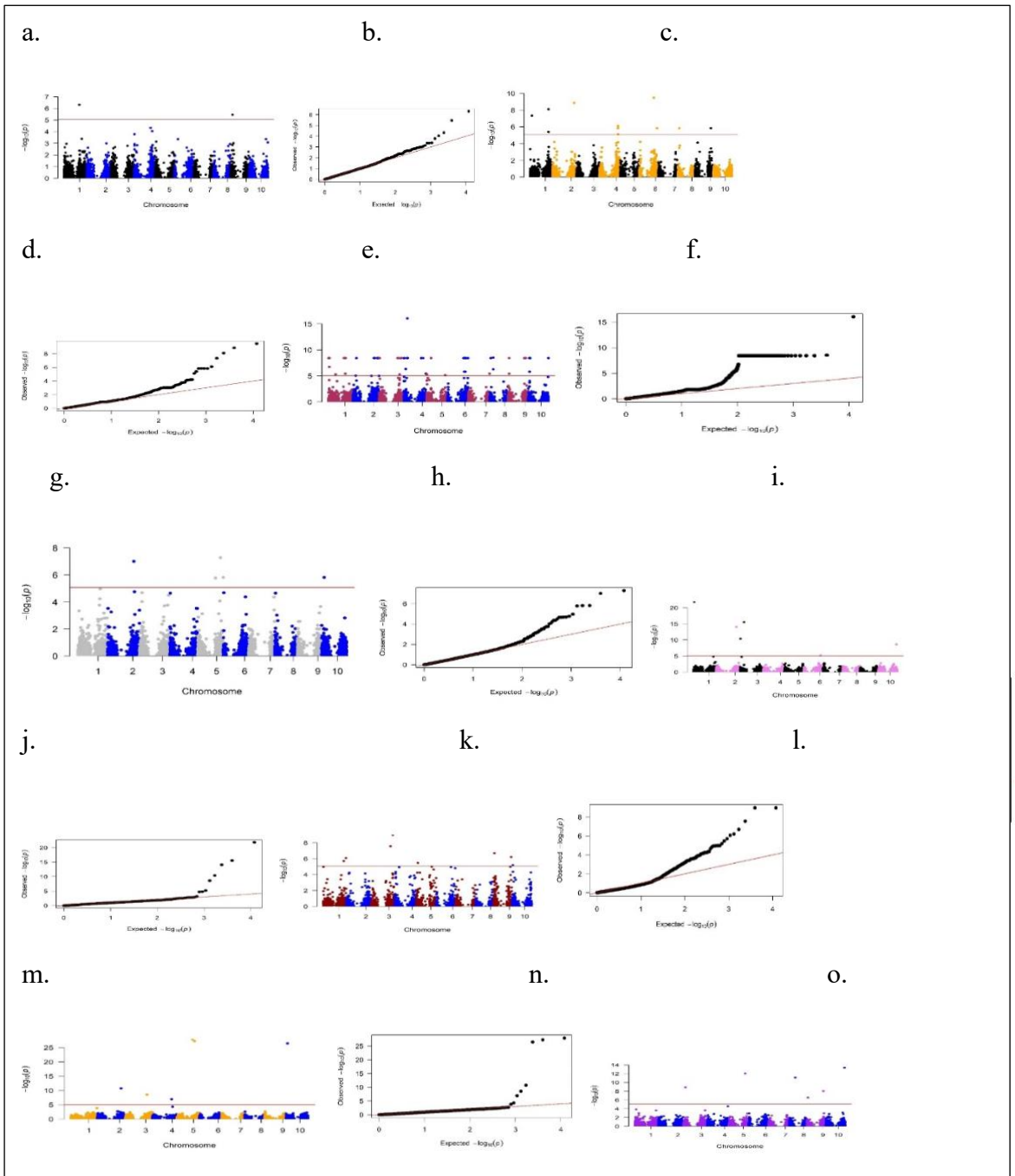

Figure 21: Manhattan plot and Q-Q plots for secondary metabolites for 74 sorghum genotypes.

(a) Caffeic acid Manhattan plot. **(b)** Q-Q plot for caffeic acid. **(c)** Cinnamic acid Manhattan plot. **(d)** Q-Q plot for cinnamic acid. **(e)** Ferulic acid Manhattan plot. **(f)** Q-Q plot for ferulic acid. **(g)** Gallic acid Manhattan plot. **(h)** Q-Q plot for Gallic acid. **(i)** Hydroxybenzoic acid Manhattan plot. **(j)** Q-Q plot for hydroxybenzoic acid. **(k)** P-coumaric acid Manhattan plot. **(l)** Q-Q plot for P-coumaric acid. **(m)** Protocatechuic acid Manhattan plot. **(n)** Q-Q plot for protocatechuic acid.

7.8.3 Association analysis for *Striga* resistance and nutrition composition of 74 sorghum genotypes using 6,562 SNP markers

Manhattan plot and Q-Q plots for nutritional composition related traits for 74 sorghum genotypes are as shown in **Figure 22**. Carbohydrates Manhattan plot detected significant SNPs on chromosomes 8 and 9, as shown in **Figure 22 (a)**. The QQ plot is below the expected red line of SNP plot, as shown in **Figure 22 (b)**. Ash content Manhattan plot revealed only chromosome 4 had a significant SNP, while the QQ plot had a positive deviation, as shown in **Figure 22 (c)** and **(d)**, respectively. Fat content Manhattan plot detected significant SNPs on chromosome 3 only while the QQ was below the expected SNP plot with only one SNP appearing on the positive side, as shown in **Figure 22 (e)** and **(f)**, respectively. Fibre content Manhattan plot chromosomes 1 and 3 detected significant SNPs while the QQ plot followed the expected normal plot as shown in **Figure 22 (g**) and **(h)**, respectively. In the protein content Manhattan plot, only chromosome 3 had a significant SNP and the QQ plot followed the expected plot, as shown in **Figure 22 (i)** and **(j)**, respectively.

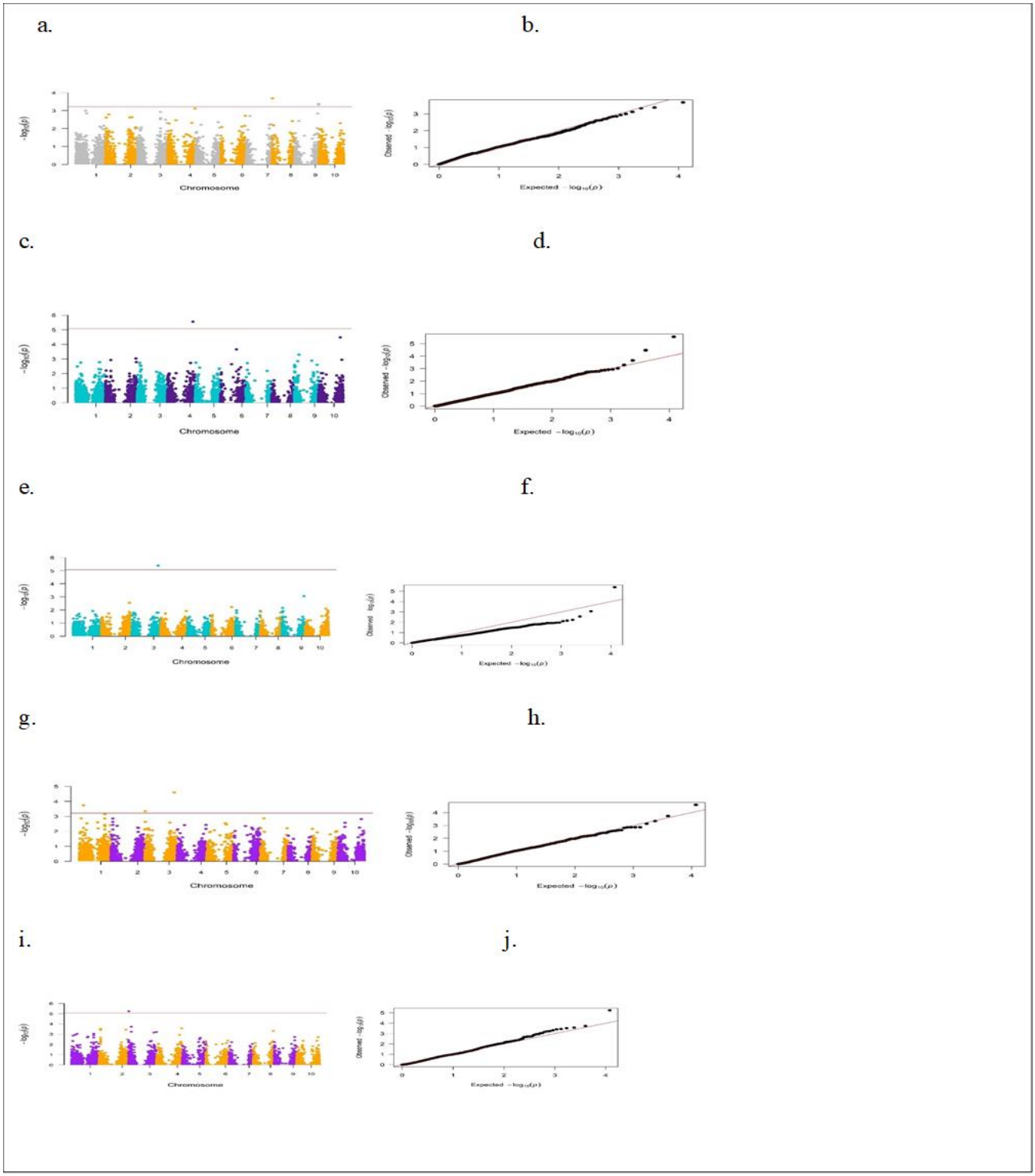

Figure 22: Manhattan plot and Q-Q plots for nutritional composition related traits for 75 sorghum genotypes

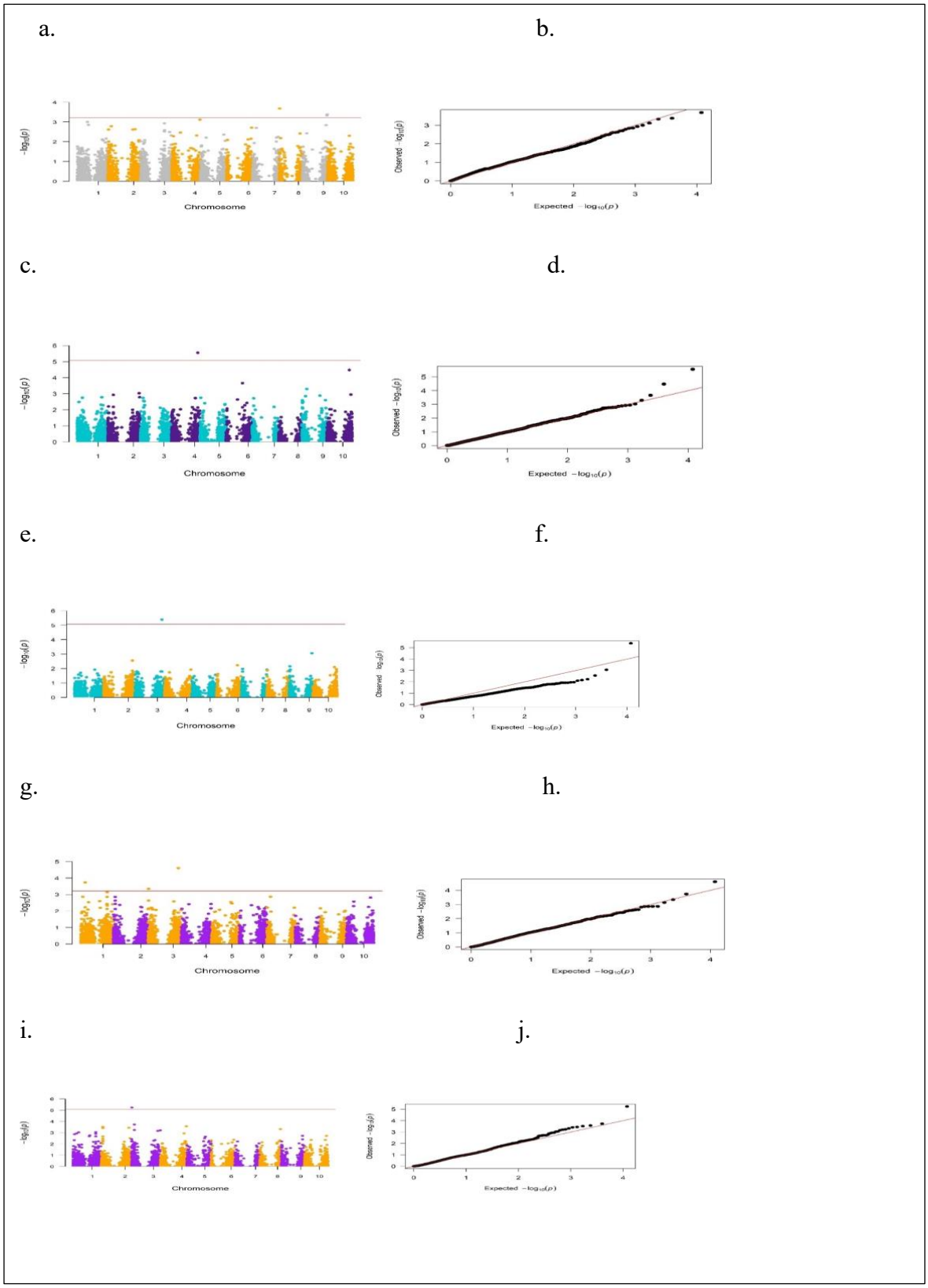

Figure 23: Manhattan plot and Q-Q plots for nutritional composition related traits for 74 sorghum genotypes

(a) Carbohydrates Manhattan plot. **(b)** Q-Q plot for carbohydrates. **(c)** Ash content Manhattan plot. **(d)** Q-Q plot for Ash content. **(e)** Fat content Manhattan plot. **(f)** Q-Q plot for fat content. **(g)** Fibre content Manhattan plot. **(h)** Q-Q plot for fibre content. **(i)** Protein content Manhattan plot. **(j)** Q-Q plot for protein content.

7.9 DISCUSSION

Plant phenolics, also known as polyphenols, are secondary metabolites with a variety of uses, including for reducing biotic (bacterial, fungal, viral, insect, or weed) and abiotic (heat, drought, cold, salt, or heavy metal) stressors. Common phenolic compounds produced by the phenylpropanoid pathway serve as signalling molecules and have potential applications as plant shielding agents. Phenolic compounds, by virtue of their antibacterial effect, serve as protective agents against disease-causing organisms, such as bacteria, nematodes, weeds, and other pathogens (Tak and Kumar, 2020). Important vitamins, minerals, proteins, and micronutrients required for optimum health, development, and growth are found in sorghum. Evaluation of sorghum varieties' nutritional content would directly affect sorghum's improvement for high-quality breeding and food product development (Tasie and Gebreyes, 2020). Assessing the nutritional properties of various sorghum varieties is therefore crucial for creating and designing goods with better nutritional value, guaranteeing food quality, and assisting sorghum breeders in improving future varieties based on nutrition. It is yet unknown, however, whether *Striga* has a detrimental effect on the nutritional makeup of sorghum.

7.9.1 Secondary metabolites association analysis in sorghum

Analysis of secondary metabolites demonstrated that distinct SNPs for sorghum resistance to *Striga* were found on every chromosome. The greatest number of SNPs found was on chromosome 1, and the lowest number was found on chromosome 7. The number of SNPs that were found was as follows: hydroxybenzoic acid (6), gallic acid (5), ferulic acid (74), cinnamic acid (11), hydroxybenzoic acid (6), p-coumaric (9), and caffeic acid (2). Significant SNPs were found on chromosomes 1 and 9 of the Manhattan plot for caffeine. Significant SNPs were found on chromosomes 1, 2, 4, 6, 8 and 9 by cinnamic acid; on all ten chromosomes by ferulic acid; on chromosomes 1, 2, 3 and 10 by gallic acid; on chromosomes 1, 2, 3 and 10 by hydroxybenzoic acid; on chromosomes 1, 3, 5 and 9 by p-coumaric acid; protocatechuic acid detected significant SNPs on chromosomes 2, 3, 4, 5 and 10, and syringic acid detected significant SNPs on chromosomes 3, 5, 8, 9 and 10.

Significant amounts of secondary metabolites are present in the stem, leaves, and roots of sorghum. Significant concentrations of phenolic acids, particularly p-coumaric acid, as well as ferulic, syringic, vanillic, and p-hydroxybenzoic acids were found in a study on the chemical composition of sorghum residues (Hussain *et al*., 2021). This validates the findings of this study, which also found that the same chemicals are critical to *Striga* defense mechanisms. Hussain *et al*. (2021) verified that various phenolic compounds were detected in sorghum, with p-coumaric acid, m-hydroxybenzoic acid, and protocatechuic acid being the primary inhibitors in the plant's roots. Meanwhile, sorgoleone and dhurrin were found to be more significant allelochemicals in the plant's shoots (Hussain *et al*., 2021). These outcomes agree with the findings of this investigation. Austrian sorghum varieties were discovered to include protocatechuic, vanillic, caffeic, syringic, p-coumaric, and ferulic acids in free, conjugated, and bound forms; the latter being the most common (Speranza *et al.*, 2021). Comparably, ferulic acid has the highest number of SNPs (74) discovered in this investigation. A common hydroxycinnamic acid in plant cell walls, ferulic acid is primarily esterified to form polysaccharides. Along with a host of other qualities like antibacterial, anti-inflammatory, and neuroprotective benefits, it is well known for having a substantial antioxidant activity (Pratyusha, 2022). Similar findings were reported by Aoki *et al*. in 2022, wherein prehaustorium was discovered to be induced in *S. hermonthica* response by cell wall-related phenolics, such as ferulic acid, syringic acid, acetosyringone, and vanillic acid (Aoki *et al*., 2022). Moreover, AS9-9 genotypes in maize demonstrated resistance to *Striga* and had the greatest levels of total phenolics and tannins (Elemosho *et al*., 2021).

As previously discovered in maize, where significant loci on chromosomes 9 and 10 were found to be closely linked to ZmCCD1 and amt5 genes, which are related to plant defence mechanisms against *Striga* parasitism (Adewale *et al*., 2020), the study presents the confirmation of a modified signalling system and cell wall thickening to reduce the chances of *Striga* penetration. Genes related to secondary metabolism and defence were found to be elevated in the resistant maize genotype and down regulated in the susceptible genotype by Unachukwu (2019). The findings of this investigation are consistent with previous studies in that red sorghum was found to contain phenolic chemicals, including caffeic acid, ferulic acid, vanillic acid, gallic acid, p-hydroxybenzoic acid, coumaric acid, and cinnamic acid (Bhukya *et al*., 2020). Only ferulic acid was found to be more concentrated than the other phenolic compounds in the red grain sorghum genotypes (Bhukya *et al*., 2020). Other phenolic compounds that were found to be present were protocatechuic acid, protocatechuic aldehyde,

caffeic acid, and p-coumaric acid. According to Unachukwu's (2019) report, genes related to secondary metabolism and defence were found in sorghum in response to *Striga*. Benzoxazineless 1 and chalcone synthase 2 genes were found to be increased in the resistant genotypes. Additionally, in vulnerable types, the genes for secondary metabolism and defence, chalcone synthase and cellulose synthase, were downregulated (Unachukwu, 2019). These findings suggest that genes that are connected to the markers found in this study for *Striga* resistance breeding may exist.

7.9.2 Nutritional composition association analysis in sorghum

For nutritional composition, chromosomes 1, 3, 4, 8 and 9 showed significant SNPs for *Striga* resistance. The nutritional traits showed a varying number of SNPs detected with ash content (1), carbohydrates (3), fat content (1), fibre content (2) and protein content (1) SNPs detected for *Striga* resistance for the 74 sorghum genotypes sequenced. Manhattan plot for nutritional composition related traits for 74 sorghum genotypes showed that, on carbohydrates, significant SNPs were detected on chromosomes 8 and 9, ash content on chromosome 4 had a significant SNP, fat content detected significant SNPs on chromosome 3 only, fibre content on chromosome 1 and 3 detected significant SNPs, and for protein content, only chromosome 3 had a significant SNP. The results of genomic areas on chromosomes 1, 2, 4, 8, 9 and 10 that were linked to characteristics related to drought tolerance, nutrition, and thousand-kernel weight on chromosomes 1, 4 and 9 were verified (Kamal *et al*., 2023).

Similar findings were found in sorghum grain starch content studies, which identified three related genomic regions: two new associations in chr-8 and one in chromosome (chr) 1. The Manhattan plot for carbohydrates also revealed the presence of chromosomes 8 and 9, supporting the earlier findings. Significant SNPs for *Striga* resistance were found on chromosomes 1, 3, 4, 8 and 9 in this investigation. Luteinidin diglucoside was identified by Lee *et al*. in 2023. It was found on chromosome 4 in SbRio.04G259800 (56,584,076–56,588,610). A crucial stage of cellular respiration that takes place in the mitochondria of eukaryotic cells involves the protein PDHA1 (Lee *et al*., 2023). The findings validate the conclusions drawn from this study. After conducting a GWAS analysis on 390 different types of sorghum, including biomass and sweet varieties, Elango *et al*. (2020) found that a vacuolar transporter and cellulase enzymes were linked to non-fibrous carbohydrate buildup. A GWAS of 206 forage sorghum accessions also revealed nine QTLs, including 184 genes related to lignin concentration (Elango *et al*., 2020). The genetic development of sorghum as a biofuel

and fodder crop in the future will benefit greatly from the knowledge gained from these studies (Lee *et al*., 2023). On chromosome 1, a significant peak for TGMR at 51.8 Mbp was found in another study (Nida *et al*., 2021). Furthermore, two of these loci, which are large peaks on chromosomes 1 and 3 at 51.8 and 15.6 Mbp, respectively, were consistently found in all contexts (Nida *et al.*, 2021). The outcomes of this study are supported by these earlier investigations, and the SNPs that were found will be important for breeding *Striga* resistance.

Three SNPs related to carbohydrates were found on chromosomes 8 and 9 in this investigation. Parallel to this, in the study conducted by Kurmar *et al*.(2024) for non-functional carbohydrates GWAS, 15 significant associations (88 SNPs) on Chr1, Chr3, Chr5, Chr6, Chr8, and Chr9 were found. The most prominent genomic area (~ 65 kb) exhibited a large peak and strong relationships (75 SNPs) for NFC on Chr8 (5-6 Mb). On Chr1 (4 Mb) and Chr9 (0.5 Mb), one additional extremely important locus was found (Kumar *et al*., 2024). Furthermore, the GWAS discovered five SNPs in total that exceeded the Bonferroni significance threshold using the compressed mixed linear model (CMLM) from GAPIT 2, which correlated to four distinct regions – two on chromosome 4 and two on chromosome 8 (Brenton *et al*., 2020). On chromosome 3, fat content found only one significant SNP. Corroborating data revealed that, in a related study, the Chr03 and Chr05 areas had a substantial enrichment of SNPs for the fatty acid biosynthesis (Elango *et al*., 2020).

7.9.3 Conclusion

Plant phenolics, also known as polyphenols, are secondary metabolites with a variety of uses, including for reducing biotic (bacterial, fungal, viral, insect, or weed) and abiotic (heat, drought, cold, salt, or heavy metal) stressors. Important vitamins, minerals, proteins, and micronutrients required for optimum health, development, and growth are found in sorghum. Analysis of secondary metabolites demonstrated that distinct SNPs for sorghum resistance to *Striga* were found on every chromosome. For syringic acid, protocatechuic acid, p-coumaric, hydroxybenzoic acid, gallic acid, ferulic acid, cinnamic acid, and caffeic acid, a total of 119 SNPs were found. Significant SNPs for *Striga* resistance was found on chromosomes 1, 3, 4, 8 and 9 in relation to dietary content. Eight SNPs in all were found for the nutritional attributes of ash content, carbs, fat content, fibre content, and protein content. Critical SNPs that can be exploited to breed sorghum for both agronomic traits and nutritional components that are resistant to *Striga* were found in this study. For more investigation, it is essential to identify additional genes associated with the found markers.

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CHAPTER 8

SUMMARY DISCUSSIONS, CONCLUSIONS, AND RECOMMENDATIONS

8.1 DISCUSSION

8.1.1 Evaluation of sorghum genotypes for *Striga* tolerance

The study revealed substantial genetic variation among sorghum genotypes in their response to *Striga hermonthica* infestation. Some genotypes demonstrated enhanced tolerance, maintaining higher chlorophyll content, plant height, and yield under infestation such as SCSHYB012150, CHR 20, SCSHYB017166, SCSHYB015022, SCSHYB015023, SCSHYB015025, SCSHYB015027, SCSHYB017082, SA2133, SA1617, SA1800, SA1796, SA2538, SA3028, SA2311, SA2070, SA4186, and SA1794. The principal component analysis (PCA) revealed that 74.45% of the total variation in response to *Striga* could be attributed to two key principal components, reinforcing the genetic diversity present in sorghum. The findings suggest that resistance is controlled by multiple genetic factors, and breeding programs can capitalise on this diversity to develop *Striga*-resistant sorghum varieties.

8.1.2 Effects of *Striga* infestation on secondary metabolites and nutritional composition

Striga infestation significantly altered the biochemical profile of sorghum. Secondary metabolites such as flavonoids, tannins, and phenolic acids increased in resistant genotypes, indicating their role in biochemical defence mechanisms. However, susceptible genotypes exhibited significant reductions in protein and carbohydrate content, negatively affecting grain quality such as SCSHYB013103, SC SILA, SA1595, CHR19, MACIA, SC SMILE, SCSHYB011120, CHR 28, SA1596, SCSHYB017089, SCSHYB017087, SCSHYB017088, SCSHYB012156, SCSHYB012120, SC SILA(KRC) 0P45, SCSHYB012157, SCSHYB012155, SCSHYB012160, SCSHYB017081, SCSHYB017139, SCSHYB017165, SCSHYB017086, CHR 25, SCSHYB012101, SCSHYB017075, SCSHYB012116, SCSHYB012112, SCSHYB015002, SCSHYB017090, SCSHYB017084, SCSHYB017083, SCSHYB015017, SCSHYB015018, SCSHYB015021, SCSHYB015024, SCSHYB015028, SA2527, SA2490, SA3100, SA2475, and SA2093. This suggests that biochemical resistance mechanisms are crucial in mitigating *Striga*'s impact on sorghum and should be prioritised in breeding programs.

8.1.3 Genetic diversity analysis using Diversity Arrays Technology Sequencing (DArTSeq)

Using 27,870 SNP markers, the study revealed moderate genetic diversity among the 74 sorghum genotypes analysed. Analysis of Molecular Variance (AMOVA) confirmed significant variation within and among populations, emphasising the need to explore underutilised landraces and wild relatives as sources of resistance. The findings suggest that integrating diverse genetic materials can improve *Striga* resistance and enhance the overall adaptability of sorghum.

8.1.4 Association mapping for *Striga* tolerance and yield-related traits

Genome-wide association studies (GWAS) identified significant SNP markers on chromosomes 1, 2, 3, 4 and 6 linked to agronomic traits such as plant height, panicle height, chlorophyll content, and grain yield under *Striga* infestation. These findings provide a valuable molecular framework for marker-assisted selection (MAS), which can accelerate the breeding of high-yielding and *Striga*-resistant genotypes.

8.1.5 Genetic wide association mapping for *Striga* resistance and nutritional traits

The GWAS analysis also mapped SNP markers associated with both *Striga* resistance and nutritional traits. Notably, chromosome 1 contained the highest number of SNPs related to *Striga* resistance, while nutritional traits such as protein, fibre, and carbohydrate content were mapped on chromosomes 2 and 5. This indicates the feasibility of breeding sorghum varieties that are both *Striga*-resistant and nutritionally superior, addressing food security and dietary needs simultaneously.

8.2 CONCLUSIONS

This study provides critical insights into the genetic, biochemical, and agronomic responses of sorghum to *Striga hermonthica* infestation. There is significant genetic variability among sorghum genotypes, with some demonstrating enhanced *Striga* tolerance. Biochemical defence mechanisms play a crucial role in *Striga* resistance, with secondary metabolites serving as indicators of tolerance. Genetic diversity analysis confirmed the presence of unique alleles that can be harnessed to improve resistance and adaptability. GWAS identified key SNP markers associated with *Striga* resistance and agronomic traits, facilitating marker-assisted selection

(MAS). Breeding strategies should therefore integrate both *Striga* resistance and nutritional quality to ensure improved food security and sorghum marketability.

8.3 RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

8.3.1 Breeding and genetic improvement

- Utilise marker-assisted selection (MAS) to develop *Striga*-resistant sorghum genotypes.
- Incorporate wild sorghum relatives and landraces into breeding programs to increase genetic diversity.
- Focus on breeding genotypes that maintain high nutritional quality under *Striga* infestation.

8.3.2 Agronomic and biochemical strategies

- Promote the use of biochemical markers (e.g., flavonoids and tannins) as indicators of *Striga* resistance.
- Explore integrated *Striga* management (ISM) strategies, combining genetic resistance with cultural and agronomic control measures.
- Investigate soil amendments and microbial inoculants that can enhance *Striga* resistance and crop productivity.

8.3.3 Future research directions

- Conduct functional genomics studies to validate the identified SNP markers and their role in *Striga* resistance.
- Investigate gene-editing approaches (e.g., CRISPR-Cas9) to enhance resistance mechanisms in sorghum.
- Expand multi-location field trials to evaluate the stability of resistant genotypes under diverse environmental conditions.

8.3.4 Policy and farmer adoption

- Promote farmer awareness programs on *Striga*-resistant sorghum varieties.
- Encourage government and private sector investment in sorghum breeding and seed distribution.

- Develop subsidy programs for *Striga*-resistant seed varieties to enhance adoption among smallholder farmers.

8.4 FINAL REMARKS

This research has provided a strong foundation for sustainable *Striga* management in sorghum through genetic improvement, biochemical analysis, and molecular mapping. The integration of molecular breeding, agronomic strategies, and farmer adoption will be essential in enhancing sorghum productivity, resilience, and food security in *Striga*-infested regions. Further research should focus on validating genetic markers, improving resistance mechanisms, and ensuring the economic viability of improved sorghum varieties.