

MAKERERE UNIVERSITY

GENOMIC REGIONS ASSOCIATED WITH BRUCHID RESISTANCE IN A COWPEA MAGIC POPULATION

BY

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DECLARATION

The work presented in this thesis is my own research and has not been presented for the award of degree or diploma in any other University.

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DEDICATION

I dedicate this work to my wife, Mrs. Semira Mohamed and our children, Juliana, Simona, Elhana and Demyana for their unceasing prayers, love, support and patience during my entire study. To my parents Mr. Isaac Ghebrendrias and Mrs. Letebrhan Woldu and my aunt Mrs. Zfan Ghebrendrias, though you are no longer here, your spirit lives on everything I do. Your unwavering support, boundless love, and unshakable faith in me have been the foundation of all my achievements. "I extend my heartfelt gratitude to all my brothers, especially my elder brother Mussie for their unwavering emotional support and constant inspiration. Their encouragement has played a significant role in shaping my academic and professional journey."

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism	Min	Minimum
ANH	Average Number of holes	MR	Medium Resistance
ANOVA	Analysis of Variance	MUARIK	Makerere University Agricultural Research Institute, Kabanyalo
bp	Base pairs	NaSARRI	National Semi-Arid Resources Research Institute
BR	Bruchid Resistance	NE	Number of Eggs Laid
CV (%)	Percentage Coefficient of Variation	NEI	Number of Bruchid Emergence
Df	Degree of Freedom	%NEI	Percentage of Adult Insects Emergence
DSI	Dobie Susceptibility Index	PWL	Percent of Weight Loss
GI	Growth Index	QTL	Quantitative Trait Loci
GWAS	Genome-Wide Association Study	QTN	Quantitative Trait Nuecleotide
H^2	Heritability	RSW	Residual Seed Weight
ISSR	Inter- Simple Sequence Repeat	R	Resistant
ISW	Initial Seed Weight	RILs	Recombinant Inbred Lines
WTL	Weight Loss	SNP	Simple Nuecleotide Polymorphism
LSD	Least Significant Difference	S.E.M	Standard Error of Mean
LIS	Legume Information System	S.E.D	Standard Error of Difference
MAF	Minor Allele Frequency	SS	Sum of Squares
MAGIC	Multi-parental Advanced Generation Inter-Cross	SSA	Sub-Sahra Africa
MaRCCI	Makerere Research Centre for Crop Improvement Institute	SOV	Source of Variation
Max	Maximum	SD	Standard Deviation
Mb	Megabase	TASSEL	Trait Analysis by Association, Evaluation and Linkage
MDP	Median Development Period	USA	United States of America

ABSTRACT

Cowpea (*Vigna unguiculata)*, is an important legume crop for its nutritional value and economic significance. However, the bruchid (*Callosobruchus maculatus*) is a major pest that causes significant losses to cowpea grain and seed during storage. Bruchid damage results in loss of grain quality, as well as changes in nutritional value and aesthetic appeal. The study aimed to address the challenges by identifying resistant cowpea genotypes, map genomic regions, and candidate genes associated to bruchid resistance within Makerere University sourced (MaRCCI) cowpea MAGIC populations. A total of 210 cowpea genotypes were artificially infested with 0 to 1 day-old C. *maculatus* adults under controlled laboratory conditions of 27 ± 2°C ambient temperature and 70 ± 5% relative humidity. The experiments were carried out in two batches, using alpha-lattice design with three replications at the MaRCCI Biotechnology Laboratory. Data was collected on different cowpea traits associated with bruchid resistance including initial seed weight (ISW), number of eggs laid (NE), number of emerged insects (NEI), percentage of adult insect/bruchid emergence (%NIE), number of exit holes on seeds (ANH), bruchid growth index (GI), median development period (MDP), residual seed weight (RSW), seed weight loss (WTL), percentage seed weight loss (PWL), Average number of exit holes (ANH), and Dobie susceptibility index (DSI); and analyzed using R software.

Significant variation was observed among the genotypes for all the analyzed parameters. Correlation analysis also showed that several bruchid-resistance traits are strongly correlated, suggesting they can be simultaneously targeted during selection. Hence, four cowpea genotypes namely WC 10, MAGIC251, MAGIC060, and MAGIC113 have been identified as resistant to bruchids.

In addition, genomic analysis identified ten significant SNP associations for bruchid resistance related traits (SWLP, SWL%, NEB, GI, DSI, and ANH) across chromosomes *Vu03, Vu04, Vu05, Vu09,* and *Vu10,* using multiple multi-locus GWAS models that explained 3.5–13.30% of the phenotypic variance. Notably, chromosomes *Vu03, Vu04, Vu05*, and *Vu09* have been consistently reported as key regions associated with bruchid resistance, with chromosome *Vu09* repeatedly identified across independent studies. These consistencies reinforce the robustness and reliability of the detected loci.

Markers located on chromosomes *Vu03* and *Vu10* were repeatedly detected across multiple traits and models, underscoring their central role in the genetic control of bruchid resistance. Among these, SNPs 2_14396, 2_23649, and 2_49489 represent priority candidates for further validation and potential deployment in marker-assisted breeding programs. Overall, the results indicate a polygenic architecture underlying bruchid resistance, involving both adequately demonated and minor effect loci, with several SNPs consistently detected across multiple models, highlighting their potential utility for marker-assisted selection in cowpea breeding programs. The findings provide valuable insights for breeding programs aimed at enhancing cowpea resilience to bruchid infestations. The identification of key genomic regions and candidate genes offers a clear pathway for more targeted genetic improvement, facilitating the development of durable bruchid resistant cowpea varieties.

CHAPTER ONE: INTRODUCTION

1.1 Background

1.1.1 Origin and distribution of cowpea

Cowpea (*Vigna unguiculata (L.)* Walp.) is a herbaceous annual crop that is primarily farmed in arid agro-ecological zones of the tropics. It is a significant member of the *Fabaceae* family, tribe *Phaseoleae*, subtribe *Phaseolinae,* family *Fabaceae,* subfamily *Faboideae*, and genus *Vigna* (Saied and Gaafar, 2020). Cowpea is predominantly a self-pollinating diploid species and has an estimated genome size of 620 Mb and a chromosome number of 2n = 22 (Chen et al., 2011; Matos, 2019). Compared to other warm-climate legumes, particularly the common bean (*Phaseolus vulgaris* L.), its genome is highly collinear (Muñoz *et al.*, 2017). The specific origin of cowpea has been a source of speculation and debate for many years (Singh, 2005). Early observations revealed that cowpeas in Asia were diversified and physically distinct from those in Africa, leading to the assumption that both Asia and Africa were independent places of origin for cowpea (Nielsen, 2001 & Steele, 2009). Nonetheless, it has lately been questioned why there aren't any wild cowpeas in Asia, which are thought to be the ancestors of an Asian center of origin (Fuller & Boivin, 2009). Even though cowpeas are widely distributed throughout Asia, all available evidence suggests that they originated in Africa (Sylla Ba *et al.*, 2003; Singh, 2005). However, it has not been possible to locate the exact location of the crop's initial domestication in Africa. Nonetheless, West and Central Africa have the greatest diversity of landraces and farmed cowpeas (Boukar *et al.*, 2019a).

1.1.2 Importance of cowpea

Cowpea is a highly adaptable warm-climate legume widely cultivated by smallholder farmers across Sub-Saharan Africa, Asia, and parts of America due to its multiple agronomic and socioeconomic benefits (Sindhu *et al.*, 2019). It is a crucial source of dietary protein, iron, and other essential nutrients, contributing significantly to food and nutritional security in low-resource communities. In Sub-Saharan Africa, cowpea is a staple food and a critical protein source, consumed in various forms including fresh or dry grains, leaves, and fodder (Ayalew and Yoseph, 2022). In Eastern Africa, it complements starchy staples like maize, millet, and cassava, and provides a rich nutritional profile of approximately 64% carbohydrates, 25% protein, along with essential vitamins and minerals such as iron, calcium, thiamin, and folic acid (Mutuku & Malaba, 2003; Boukar & Belko *et al.*, 2019a). In addition to its dietary value,

cowpea plays an important ecological role. It enriches soil fertility through nitrogen fixation, thereby reducing reliance on synthetic fertilizers (Brisibe *et al.*, 2011), and serves as an effective cover crop in sustainable farming systems. Cowpea's resilience to harsh conditions such as heat, drought, and nutrient-poor soils makes it particularly valuable in areas affected by climate change. It can thrive in regions that have as little as 300 mm of annual rainfall, including parts of the Sahara Desert (Horn *et al.*, 2022), and often outperforms other legumes under such stresses (Bisikwa *et al.*, 2014; Boukar & Togola *et al.*, 2019). Recent advances in cowpea breeding, such as the development of Multiparent Advanced Generation Inter-Cross (MAGIC) populations, offer powerful tools for improving traits like drought tolerance, pest resistance, yield potential, and nutritional quality. These genetic resources are instrumental in enhancing the crop's performance and resilience under changing environmental conditions.

1.1.3 Cowpea production

Cowpea's global yearly production is roughly 5.8 million tons (Xiong *et al*., 2016) at a minimum of 11 million hectares. Over the last three decades, global cowpea production grew at an average rate of 5%, with 3.5% annual growth in area and 1.5% growth in yield; and the area expansion accounts for 70% of the total growth during this period (Fatokun *et al*., 2012). Nigeria produces most quantity of cowpea grains annually at approximately 2.14 million metric tons (Boukar, *et al.*, 2019; Horn and Shimelis, 2020), followed by Niger Republic and Burkina Faso with an average of 1.59 and 0.57 million metric tons, respectively. Uganda is among the ten leading producers of cowpea, ranked 8th in Africa (Ddamulira *et al*., 2015; Symphorien *et al*., 2017). The crop is mostly grown in the drier eastern and northern parts of Uganda (Ddungu, 2015). According to Symphorien *et al*. (2017), cowpea ranks fourth in production among legumes in Uganda, following beans, groundnuts, and soybeans.

1.1.4 Cowpea production constraints

In Sub-Saharan Africa (SSA) cowpea productivity is low due to a wide array of abiotic and biotic stresses (Horn and Shimelis, 2020). The constraints can be grouped into field and storage constraints:

Field constraint includes pests and diseases, poor soil fertility, unreliable rainfall, weeds, limited access to quality seeds, inputs, and high labour costs. Losses due to insect pests and diseases coupled with low yield potentials of the local cowpea varieties has led to reduced cultivation of cowpea in Uganda (Edema and Adipala, 1996).

Storage constraints includes weevil infestation, rodents, poor drying, high moisture, inadequate storage facilities, and poor packaging, which can lead to post-harvest losses and reduced grain quality. Local production systems are not well-documented, however, reports from research stations in Uganda indicates that storage pests are a major constraint of cowpea grains (Sola *et al*., 2014). The cowpea weevil, synonym to cowpea bruchid (notably *Callosobruchus maculatus*) causes severe damage to most pulses (Hajam, and Kumar, 2022) with the larval stage feeding on the grains (Grazziotin *et al*., 2020).

1.1.5 An overview of bruchid infestation and its management

Bruchid (*Callosobruchus maculatus*) is a major post-harvest pest responsible for significant losses in both quantity and quality of stored grains. It degrades the nutritional value and appearance of the produce (Kabole, 2019), and compromises the cooking quality of cowpea-based meals (Mulungu, 2007; Misangu, 2007). If not managed, bruchid infestations can result in total grain loss (Idoko & Ileke, 2020). Although chemical preservatives are commonly used to protect stored cowpea, they pose health and environmental risks and can contribute to pesticide resistance (Van Boxtel *et al*., 2000; Sano *et al*., 2018). While botanicals offer a safer and eco-friendly alternative (Sano *et al.*, 2018; Kpoviessi *et al.*, 2019), their effectiveness is often limited by inappropriate delivery methods, inconsistent dosages, and variable efficacy. A sustainable solution lies in developing bruchid-resistant cowpea varieties. This approach minimizes environmental impact while maintaining both yield and quality (Olakojo *et al*., 2007; Ismail, 2010; Boukar *et al.*, 2016).

1.1.5.1 Cowpea resistance to bruchid infestation

Genetic improvement for bruchid resistance is a cost-effective and sustainable strategy for reducing post-harvest losses in cowpea. However, progress may be constrained by linkage drag, variation among insect populations and a narrow genetic base (Kpoviessi *et al*., 2019). Host-plant resistance involves three mechanisms: antixenosis, which deters insect oviposition or feeding; antibiosis, which impairs insect development, reproduction or survival; and tolerance, which minimises damage and associated yield or seed-quality losses (Abang *et al.*, 2018; Kpoviessi *et al.,* 2019; Pratyusha, 2022).

Seed characteristics, including colour, size, hardness, phenolic compounds, tannins and α-amylase inhibitory activity, may influence bruchid oviposition, larval development and

survival, although their effects vary among genotypes (Lattanzio *et al.*, 2005; Saruchi *et al.*, 2014; Tripathi *et al*., 2020).

Molecular studies have identified markers and quantitative trait loci (QTLs) associated with bruchid resistance. Miesho *et al.* (2019) analysed 217 cowpea mini-core accessions using 41,948 single-nucleotide polymorphism (SNP) markers and identified 11 SNPs associated with egg laying, seed damage, bruchid emergence, development period and the Dobie susceptibility index. For example, SNP 2_20268 on Vu08 was associated with seed holes and bruchid emergence, while SNPs 2_36627, 2_54737 and 2_35230 on Vu07 were associated with emergence and susceptibility-related traits. Similarly, Kpoviessi *et al.* (2022) identified 14 quantitative trait nucleotides associated with six resistance traits in 107 accessions from six countries and reported 11 candidate genes within 10–30 kb of significant associations.

Using $F_{2:3}$ progenies derived from the susceptible parent MU9 and resistant parent WC42, Unzimai *et al.* (2024) identified ten QTLs across seven cowpea chromosomes under no-choice infestation with Callosobruchus maculatus. MDP6.1 on Vu06 explained 25.9% of the phenotypic variance in median development period, while NEI9.1 on Vu09 explained 28.6% of the variance in bruchid emergence. These findings suggest that different genomic regions contribute to distinct resistance components.

Overall, bruchid resistance in cowpea is genetically complex and influenced by host genotype, insect population, resistance traits and experimental conditions. Although identified markers and QTLs provide useful resources for breeding, their transferability across genetic backgrounds and infestation conditions requires validation. Investigating marker–trait associations and candidate genes in cowpea MAGIC populations could further elucidate the genetic basis of resistance and support the development of resistant varieties.

1.2 Problem statement

Cowpea is an important food and income crop for smallholder farmers in Uganda and sub-Saharan Africa. Uganda has made efforts to improve cowpea production through the release of several improved varieties, including SECOW 1T, SECOW 2W, CPKUNDE, CP White, SECOW 3B, SECOW 4W and SECOW 5T, as well as the more recently released NAROCOWPEA 1–6. These varieties contribute to improving cowpea production and farmer access to improved planting materials. However, cowpea production and storage remain

affected by post-harvest losses caused by the cowpea bruchid (*Callosobruchus maculatus*), which reduces grain quantity and quality (Zakka & Nwosu, 2019).

A major constraint is the lack of cowpea varieties with effective and durable resistance to *C. maculatus*. The improved varieties released in Uganda have not been reported to possess effective resistance to the bruchid (Weldekidan, 2019). Although some resistant genotypes have previously been identified, their resistance has shown limited durability, partly because of monogenic resistance and the existence of different bruchid biotypes (Shade *et al.*, 1999). Previous studies have identified SNPs, QTLs and candidate genes associated with bruchid resistance (Miesho *et al.*, 2019; Abdullahi *et al.*, 2022; Unzimai *et al.*, 2024), but the genetic basis and durability of resistance remain insufficiently understood. In particular, the Makerere Research Centre for Crop Improvement (MaRCCI) cowpea MAGIC population, which harbours substantial genetic diversity and recombination, remains largely unexplored for bruchid resistance. This presents an opportunity to identify novel sources of resistance and elucidate the genomic regions and candidate genes underlying this trait.

If the lack of effective and durable bruchid resistance is not addressed, continued infestation by *C. maculatus* will result in substantial post-harvest losses, deterioration of grain quality, and reduced availability of cowpea for food and income. Smallholder farmers may also continue to depend on chemical insecticides, which can be costly, less accessible and associated with health and environmental risks (Zakka & Nwosu, 2019). Therefore, identifying novel and potentially durable sources of bruchid resistance and understanding their genetic basis are essential for supporting the development of resistant cowpea varieties and more sustainable bruchid management in Uganda.

1.3 Objectives

The general objective of this study was to contribute to development of improved cowpea genotypes with resistance to bruchids (*Callosobruchus maculatus*) in Uganda

1.3.1 Specific objectives

1. To identify genotypes with resistance to bruchids (*Callosobruchus maculatus*) in a cowpea MAGIC population.
2. To identify genomic regions and candidate genes associated with resistance to bruchid (*Callosobruchus maculatus*) in a cowpea MAGIC population.

1.4 Hypothesis

1. There is significant genetic variability and resistant cowpea genotypes to bruchid infestation among the MAGIC population of cowpea genotypes.

2. There are genomic regions and candidate genes in the cowpea MAGIC populations associated with bruchid resistance traits.

1.5 Justification

Bruchid infestation remains a major constraint to cowpea (*Vigna unguiculata*) production, particularly during post-harvest storage, causing substantial economic losses and threatening food security in regions heavily reliant on this legume. Genetic resistance offers a sustainable, cost-effective, and environmentally friendly alternative to chemical control measures. Moreover, comprehensive understanding of the genetic basis of bruchid resistance in cowpea is needed.

This study seeks to address this critical knowledge gap by identifying genomic regions and candidate genes associated with bruchid resistance using a Multi-Parent Advanced Generation Inter-Cross (MAGIC) cowpea population. Through genomic region and candidate identification the research aims to uncover key loci tightly linked to resistance traits. The identification of these genomic regions will not only advance the fundamental understanding of the genetic mechanisms involved but also facilitate the development of molecular markers for use in marker-assisted selection (MAS).

The resistant genotypes identified in this study offer valuable genetic resources for use as controls in future research and as parental lines in breeding programs targeting bruchid resistance. This work supports the genetic improvement of cowpea, with practical implications for reducing storage losses, enhancing food security, and promoting sustainable agriculture in bruchid-prone regions.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview and historical documentation of bruchids on legumes

Bruchid beetles are among the most destructive pests of stored legumes worldwide, particularly in regions where pulses such as cowpea serve as staple foods. The family Bruchidae comprises an estimated 1,300 species, but only about 20 are considered major pests of stored grains, especially in developing countries (Moss, 1994). Historically, the documentation and scientific study of bruchids date back to the 18th century when Linnaeus (1758) first classified them under the genus Bruchus. Subsequent taxonomic revisions, including the work of Southgate (1979) and Moss (1994), led to clearer identification of economically important species. Four species *Callosobruchus maculatus, C. chinensis, Acanthoscelides obtectus,* and *Zabrotes subfasciatus* have been recognized globally as the most harmful storage pests (Moss, 1994). Other *Callosobruchus* species such as *C. analis, C. rhodesianus, and C. subinnotatus* have also been recorded as significant pests (Southgate, 1979). Among these, *C. maculatus* stands out due to its widespread distribution and severe damage to cowpea seeds during both field and storage stages. Bruchids have co-evolved with legume host plants for centuries, shaping ecological interactions and seed predation dynamics (Southgate *et al*., 1978). Their global distribution mirrors the cultivation of their host plants, with high adaptability to tropical and temperate climates (Soundararajan *et al*., 2012). Because legume grains are critical for food and income security in many developing countries, the longstanding presence of bruchids continues to pose major challenges to storage, seed quality preservation, and sustainable crop production.

2.2 Biology of *collosobruchus maculatus*

Callosobruchus maculatus, the cowpea bruchid, belongs to the family *Chrysomelidae* (subfamily *Bruchinae*) and is the most economically important pest of stored cowpea. Its entire life cycle occurs within the seed, making it damaging and difficult to control. Females lay eggs on the seed surface, and upon hatching larvae bore directly into the seed where they feed and develop through four larval instars before pupation (Srivastava & Subramanian, 2016). Under optimal environmental conditions of 27–32°C and 60–75% relative humidity, the life cycle is completed in 3–4 weeks, allowing rapid population growth and multiple generations in a single season. Several studies have investigated the developmental biology and fitness of *C. maculatus*. Devina *et al*. (2019) demonstrated that fecundity varies across generations, with female beetles depositing an average of 47–69 eggs depending on environmental conditions. The egg stage is particularly vulnerable to low oxygen, whereas

later larval instars (3rd and 4th) can tolerate hypoxic conditions for extended periods (Cheng *et al*., 2015). Adult females typically live for 11–12 days, while males live slightly longer (Devina *et al*., 2019). Environmental factors strongly influence bruchid development and reproduction. Temperature not only affects the beetle's life cycle duration but also influences plant physiological responses. Moderate temperatures promote the synthesis of defensive secondary metabolites such as phenolics, while extreme temperatures can suppress plant defenses (Castillo *et al*., 2013). Relative humidity between 60–80% also enhances phenolic production and bruchid survival, making both climatic factors central to pest population dynamics. Genetically, bruchid beetles exhibit a haplodiploid reproductive system, with males developing from unfertilized eggs and females from fertilized eggs (Musa *et al*., 2022). This system has implications for population genetics, sex ratios, and resistance evolution (Pitnick *et al*., 2009). Advances in molecular research have identified genes linked to development and pesticide resistance, informing potential future strategies for targeted pest control (Tuan *et al*., 2016; Nicolai & Moshagen, 2018).

2.3 Damage and economic importance of bruchids

Bruchid beetles attack cowpea both in the field and in storage. Field infestation begins when adult beetles lay eggs on developing pods; larvae penetrate the pods and begin feeding on seeds before harvest (Murdock *et al*., 2003). During storage, adults lay eggs on dry seeds, visible as small white patches, and the larvae burrow into the seeds where they cause extensive damage (Umeozor, 2005). Their feeding destroys the cotyledons, leaving characteristic exit holes and often reducing seeds to powder. The consequences of bruchid damage are severe. Infested seeds exhibit significantly reduced germination rates, nutritional deterioration, weight loss of up to 60%, reduced protein content by 45–66% (Soundararajan *et al*., 2012; Poorani, 2012), and contamination by fungal pathogens facilitated by metabolic heating (Mager *et al*., 2020). Within three to six months of storage, bruchids can destroy up to 90% of stored cowpea if no control measures are applied (Soundararajan *et al*., 2012). In Nigeria alone, annual losses range between 30–80%, translating into financial losses exceeding USD 300 million (Brisibe *et al.*, 2011). These losses not only affect food security but also limit farmers' ability to store grains for improved market prices, forcing premature sales at lower value. Although chemical insecticides, biological control agents, and cultural techniques have been deployed, these methods are often expensive, environmentally risky, or inconsistent. The development and use of resistant cowpea varieties represent the most sustainable long-term solution to minimizing post-harvest losses.

2.4 Breeding efforts for bruchid resistance in cowpea

Breeding for resistance to *C. maculatus* has attracted substantial attention because host-plant resistance provides an environmentally safe, cost-effective, and durable method of bruchid management. Cowpea resistance to bruchids is typically governed by quantitative trait loci (QTLs) associated with antixenosis, antibiosis, or tolerance (Zhu-Salzman & Zeng, 2015). Effective breeding programs therefore require a thorough understanding of the genetic architecture governing these resistance mechanisms. Historically, screening of cowpea germplasm has identified only a few resistant sources among thousands of accessions. Out of approximately 7,000 cowpea germplasm lines evaluated, only the accession TVu-2027 was initially confirmed to show strong resistance (Singh, 1985; Asante & Mensah, 2007). Related accessions such as TVu-1952 and TVu-1953 also demonstrated resistance but differed in several phenotypic traits (Singh, 1985). However, resistance in TVu-2027 has been overcome by virulent biotypes of *C. maculatus*, illustrating the evolutionary pressure exerted by pests on host resistance genes (Weldekidan, 2019; Dikane, 2022). Several additional genotypes including IT82D-889, IT86D-880, IT84S-2246-4, IT97K-499-35, and IT98K-205-8 have been identified as moderately resistant across diverse environments (Boxtel *et al*., 2000). Screening of 217 Ugandan mini-core accessions also identified genotypes 2419, WC42, TVu-2027, and IT84S-2246 as resistant sources (Weldekidan, 2019b). Despite these findings, complete immunity to bruchid attack is rare; even resistant lines often suffer minimal but non-zero damage (Gbaye & Holloway, 2011). Modern breeding approaches such as marker-assisted selection (MAS), genomic selection, and the use of multi-parent populations (e.g., MAGIC populations) have accelerated the identification and introgression of resistance loci (Omoigui *et al*., 2019). These strategies have enhanced the efficiency of breeding programs but require reliable molecular markers linked to resistance traits.

2.5 Molecular markers and genomic studies on bruchid resistance

The availability of the cowpea reference genome and high-density SNP markers has greatly advanced genetic studies of resistance traits. DNA-based markers are highly reproducible, cost-effective, and suitable for analyzing large populations with genetic variability (Omomowo & Babalola, 2021). Recent genomic studies have mapped several QTLs and candidate genes associated with resistance to *C. maculatus*. A genome-wide association study (GWAS) by Miesho *et al.* (2019) identified eleven SNP markers significantly associated with bruchid resistance traits such as egg number, holes, emergence rate, development period, and the Dobie susceptibility index. Six candidate genes *Vigun08g132300, Vigun08g158000,*

Vigun06g053700, Vigun02g131000, Vigun01g234900, and *Vigun01g201900* were identified as potential contributors to resistance. Another study by Unzimai *et al*. (2024) reported ten QTLs associated with bruchid resistance across chromosomes *Vu02, Vu03, Vu06, Vu04, Vu08, Vu11,* and *Vu09,* demonstrating the quantitative and polygenic nature of the trait. The use of multi-parent advanced generation inter-cross (MAGIC) populations represents a promising approach for identifying stable loci across diverse genetic backgrounds (Tataru *et al.*, 2017). These populations offer increased recombination, reduced linkage drag, and greater allele diversity than bi-parental populations, improving the power to detect novel resistance loci (Boukar *et al*., 2016). However, limited research exists on bruchid resistance within cowpea MAGIC populations, representing an important knowledge gap.

2.6 Summary and Research Gaps

The literature demonstrates that bruchids, particularly *Callosobruchus maculatus*, are major pests of stored cowpea, causing significant yield and economic losses. Although several sources of resistance have been identified, complete and durable resistance remains scarce, and some resistant lines have been overcome by evolving bruchid biotypes. Molecular studies have identified QTLs and candidate genes associated with resistance, but these have been from studies using population-specific mapping and require validation in diverse genetic backgrounds.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials and methods for study 1

3.1.1 Study site

The study was conducted at the MaRCCI Biotechnology Laboratory, Makerere University Agricultural Research Institute, Kabanyalo (MUARIK) in Central Uganda. MUARIK is located 0°28′N and 32°37′E, at approximately 1,200 masl. The area receives mean annual rainfall of 1150 mm and has mean temperature of 21.5 ^{0}C. The experiment was conducted in two batches, from September to December 2023 and from December 2023 to February 2024.

3.1.2 Plant material

A total of 210 cowpea genotypes were used in this study. Of these, 183 lines were derived from the cowpea MAGIC population. In addition to the MAGIC lines, the study included three bruchid-resistant checks (2419, IT84S-2246, and WC-42), four released varieties (NAROCOWPEA1, NAROCOWPEA-2, NAROCOWPEA-3 and NAROCOWPEA-4), and 20 other cowpea genotypes. The resistant checks were previously reported as resistant to *Callosobruchus maculatus*. To generate fresh and uniform seeds for laboratory testing, all genotypes were planted at the MUARIK farm under standard field management practices. All plant materials harvested from the field were dried, cleaned, and stored under ambient conditions until use in the laboratory evaluations. All cowpea seeds used in the experiment were sterilized to prevent the presence of any stored-grain pests prior to infestation. Sterilization was achieved by placing the seeds in an oven at 57 °C for 1 hour, which effectively eliminated all larvae and any remaining bruchid eggs. The complete list of plant materials used and their mean performance in the experiment is presented in Appendix I (Table A1).

3.1.3 Bruchid rearing and infestation

Bruchids were collected from infested cowpea seeds stored in the gene bank at MaRCCI Kabanyolo. A total of 15 kg of a susceptible cowpea variety (IT71) was used to establish the stock culture. The seed was divided evenly into fifteen 1-liter, oxygen-permeable plastic jars. Adult *Callosobruchus maculatus* were introduced into each jar containing the susceptible seed. The jars were then placed in an incubator set at 27 ± 2°C and 70 ± 5%, relative humidity to facilitate bruchid multiplication. The cultures were maintained under these conditions for 21 days to allow a full generation cycle and establish a uniform stock colony.

The newly emerged adult bruchids from this stock culture were used for the artificial infestation of the experimental cowpea genotypes.

3.1.4 Experimental setup

For each of the 210 cowpea genotypes, 30 seeds were prepared for the three replications. Ten seeds constituted one experimental unit and were placed in a 90 mm × 10 mm sterile Petri dish. Six newly emerged *Callosobruchus maculatu*s adults (0–1 day old) were introduced into each dish at a ratio of 3 males to 3 females per dish. After infestation, the Petri dishes were left undisturbed for six days to allow mating and oviposition.

Due to the large number of genotypes and to minimize variation associated with handling, infestation timing, and laboratory position, treatments were arranged using an alpha-lattice design. The experiment comprised v = 210 cowpea genotypes evaluated in r = 3 replicates. Each treatment consisted of k = 10 seeds of a single genotype placed in one sterile Petri dish, with one petri- dish representing one experimental unit. Ten seeds per treatment were considered sufficient to provide an adequate number for assessing variation in infestation, adult emergence, seed damage, and other related resistance traits. This seed number was also comparable to that used by Miesho *et al*. (2019) and Unzimai *et al*. (2024) under similar experimental conditions in Petri dishes measuring 90 mm in diameter and 10 mm in depth.

Within each replicate, the 210 genotypes were allocated to s = 10 incomplete blocks, each containing k = 21 genotypes per block (10 blocks × 21 genotypes = 210 genotypes per replicate). The experiment was conducted in two batches (seasons). Thus, per batch, there were 3 replicates × 10 blocks × 21 genotypes = 630 experimental units (Petri dishes).

Three cowpea genotypes previously identified as resistant (IT84S-2246, 2419, and WC-42) were included as resistant checks for comparison. The experiment was intended to be repeated to obtain sufficient and reliable experimental data.

3.1.5 Data collection

Data collection focused on recording of key parameters related to bruchid infestation and cowpea seed response. For each treatment, initial seed weight (ISW), number of eggs laid (NE), number of emerged insects/bruchids (NEI), number of exit holes on seeds (ANH), residual seed weight (RSW), seed weight loss (WTL), percentage seed weight loss (PWL), median development period (MDP), growth index (GI), and Dobie susceptibility index (DSI)

were recorded. Observations and measurements were taken daily on each scheduled data-collection day. The description of data collection parameters and derived variables from description of data parameters in table 1 are listed or described in table 1 and table 2 below.

Table 1: Description of data collection parameters

Trait/parameter	Description	Period of collection
Initial seed weight (ISW in gm)	Weight (grams) of 10 randomly selected seeds per cowpea genotypes	At the onset of the experiment
Number of eggs laid (NE)	Egg counts were taken on each of the 10 seeds per progeny	6 days after the bruchid infestation
Number of insect/bruchid emergence (NEI)	Daily counts of insects that emerged, which are later removed	1st day of bruchid emergence till no more emergence for 11 consecutive days
Number of exit holes on seeds (ANH)	Count on the number of exit holes	At the end of the experiment
Residual seed weight (RSW in gm)	Weight (g) of cowpea seeds that remained after bruchid damage	At the end of the experiment

Adopted from Amusa *et al.*, 2013, 2014; Miesho *et al.*, 2018a; Kpoviessi *et al.*, 2021a and Unzimai *et al.*, 2024.

Table 2: Derived variables from description of data parameters in table 1.

Trait/parameter	Description	Formula
Weight loss/ cowpea progeny (WTL)	The difference between ISW and RSW	$WTL = ISW - RSW$
Percentage seed weight loss (PWL)	The difference between the ISW and RSW as expressed in %	$PWL = 100(ISW - RSW)/(ISW)$
Median development period (MDP)	Number of days from the middle of oviposition to 50% progeny emergence	
Growth index (GI)	Percentage of emerged adult bruchids over the MDP	$GI = \%NEI/MDP$

Dobie susceptibility index (DSI)	Percentage $\log_{10}$ of the F_1 progeny over the MDP	$DSI = (log_{10} F1 \times 100)/MDP$

Adopted from Wijenayake and Karunaratne, 1999; Dobie, 1997 and Unzimai *et al.*, 2024

Dobie Susceptibility Index (DSI): The formula used to calculate Dobie susceptibility index is $DSI = (log_{10} F1 \times 100)/MDP$ using the method developed by Dobie (1974). The resulting values were used to classify cowpea genotypes into resistance categories: 0–3: Resistant, 4–7: Moderately resistant, 8–10: Susceptible and ≥10: Highly susceptible.

3.1.6 Data analysis

Data was analyzed using a linear mixed model to evaluate genotype performance across two batches at a single laboratory location. Genotype, batch (season), and their interaction were treated as fixed effects because the study focused specifically on comparing performance across these two predefined experimental batches. Replications within batch, incomplete blocks within replication, and residual error were treated as random effects to account for the alpha-lattice experimental structure.

The combined analysis across the two batches was conducted using R statistical software 4.4.1 version ANOVA using lme4, asreml, Agricolae package at 5% significance level, based on the fitted mixed model. The linear model used for the combined analysis was:

$Y_{ijkl} = \mu + G_i + S_j + (GxS)_{ij} + R_{l(j)} + B_{k(lj),} + \epsilon_{ijkl}$ Where:

Y_{ijkl}: The observed value of the dependent variable for the ith genotype evaluated in the jth season (batch), in the lth replication within the jth season (batch), and in the kth incomplete block within that replication.

μ: Overall mean.

G_i: Fixed effect of the i th genotype.

S_j: Fixed effect of the j th season/batch.

$(GxS)_{ij}$: Fixed effect of the genotype × season interaction

$B_{k(lj)}$: Random effect of the k^{th} incomplete block nested within replication and season.

$R_{l(j)}$: Random effect of the lth replication nested within season.

ϵ_{ijkl}: Random residual error associated with each observation Y_{ijkl}.

This mixed-model framework appropriately accounts for both treatment effects and the hierarchical structure of the alpha-lattice design, thereby improving the precision of genotype

comparisons. Agronomic trait performance and correlation analyses among traits were also conducted using R Software version 4.4.1. Dobie's susceptibility index was used to classify genotypes into four categories resistant, moderately resistant, susceptible, and highly susceptible based on established threshold values.

The mixed linear model assumes independence of observations within genotype–batch/season combinations, normally distributed residuals, and homogeneity of variances across genotypes and batches. Random effects (replications, blocks, and residuals) are assumed to follow a normal distribution with mean zero and constant variance. Although the alpha-lattice design reduces local experimental variability through incomplete blocking model diagnostics were performed to assess potential residual spatial variation. The two seasons (batches) were treated as independent experimental runs conducted under controlled laboratory conditions.

Overall, this analytical approach provides a statistically robust framework for evaluating bruchid resistance among the 210 cowpea genotypes tested.

3.2 Materials and methods of study 2

The genetic basis of bruchid resistance in cowpea was explored using the Multi-parent Advanced Generation Inter-Cross (MAGIC) genotypes. Genotyping data encompassed the entire genome, enabling comprehensive analysis of genetic variants associated with resistance. Phenotypic data on bruchid resistance were meticulously collected to ensure accuracy.

3.2.1 Plant materials

Cowpea Multi-Parent Advanced Generation Inter-Cross (MAGIC) lines were used as the genetic resource for identifying genomic regions and candidate genes associated with bruchid (*Callosobruchus maculatus*) resistance. These lines, originally developed from eight highly diverse founder parents (IT84S-2049, IT84S-2246, IT89KD-288, IT82E-18, IT00K-1263, IT93K-503-1, CB27 and Suvita-2). The population represents a diverse population created through multiple rounds of inter-crossing among the founder parents, introduced from University of California Riverside. MAGIC populations are increasingly recognized as powerful genetic tools for dissecting complex traits due to their broad genetic variability, high recombination rates, and improved mapping resolution Xu *et al.* (2017).

3.2.2 Genotyping

The MAGIC population was genotyped with 51,128-SNP Illumina iSelect Bead Array, which identified 36,346 polymorphic SNPs, resulting in a polymorphism rate of 68.26% was utilized (Huynh *et al.*, 2017). The SNP marker data was acquired from the University of California, Riverside, through a collaboration with the Makerere University Regional Center for Crop Improvement (MaRCCI).

3.2.3 Genome-wide association and data analysis

Genome-wide association analysis (GWAS) was conducted using 137 cowpea MAGIC lines genotyped with a high-density SNP platform (Illumina iSelect 51K SNP BeadArray) from an initial population of 210 lines. The reduction in the number of lines included in the analysis was due to the application of genotypic data quality-control procedures. These procedures involved filtering based on genotype call rate, minor allele frequency (MAF > 0.05), and Hardy–Weinberg equilibrium. Lines with excessive missing genotype calls and insufficiently reliable genotypic data were excluded to ensure the quality and reliability of the association analysis. Consequently, 137 lines with acceptable genotypic data quality were retained for the final GWAS.

Marker–trait associations for all bruchid resistance traits were performed by TASSEL 5.2.23 using a mixed linear model to account for population structure and relatedness. Manhattan and QQ plots were generated using R software. Significant SNPs were mapped onto the cowpea reference genome to identify candidate genes. Genes located within approximately 50 kb of each significant marker and falling within the linkage disequilibrium (LD) region were examined. Functional annotations and known roles in plant defense were used to prioritize likely candidate genes associated with bruchid resistance.

3.2.4 Identification of genomic region and annotation of candidate genes

The genome-wide association studies (GWAS) and mixed linear models (MLM) techniques were used for analysis. The Statistical method MrLM was used to identify genomic regions of cowpea associated with bruchid resistance was used. And the Models used to identify the SNPs were mrMLM, FASTmrMLM, FASTmrMMA, ISIS EM-BLASSO, pLARmEB, and pKWmEB.

Candidate genes for bruchid resistance were identified using Phytozome13. The study used a range of 50 SNPs upstream and 50 downstream (Han *et al*., 2022). Functional annotations were sourced from the Legume Information System, and gene functions were analyzed to determine their relevance to bruchid resistance.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Results of study 1

Combined analysis of variance using R Software version 4.4.1 is presented in table 3, explaining on the effects of genotypes, batch, and their interaction on bruchid resistance related traits across the two experimental batches/rounds. This analysis enabled the partitioning of phenotypic variation into genetic and environmental components, thereby providing insight into the extent of genetic variability and the stability of trait expression across batches.

Table 3: Combined analysis of variance for the two batches

SOV	DF	NE	NEI	%NEI	MDP	GI	PWL	DSI	ANH
Batch	1	1158.1ns	1805***	46977***	2200***	37.9***	187660***	8.09ns	89714***
Genotype	209	1179 *	191.8***	612***	6.96 ***	1.08***	249**	3.50***	228***
Batch*Genotype	209	1146 *	168.0*	597***	6.19***	1.05***	245.05*	2.84 *	206.72*
Batch:Rep	4	76713	5716.3	6946	135.2	8.73	9124	150.11	25034
Batch:Rep:Block	54	4429	299.3	910	14.66	1.65	512	6.2	530
Residuals	782	946	133.1	415	4.32	0.72	184.16	2.34	153
Mean		63.02	21.41	39.4	24.36	1.62	36.52	4.86	28.59
LSD(5%)		34.85	13.07	23.1	2.36	0.96	15.38	1.73	14.01
CV(%)		48.8	53.89	51.73	8.53	52.15	37.16	31.46	43.24

*** Very highly significant ($p \leq 0.001$); ** Highly significant ($p \leq 0.01$); *Significant ($p \leq 0.05$); ns Non-significant ($p > 0.05$);

SOV, Source of variation; df, degree of freedom; NE, Number of eggs per 10 seeds; NEI, Number of emerged insects/bruchids; %NEI, percentage of emerged insects/bruchids; MDP, Median development period (days); GI, Insect growth index; Percent of seed weight loss; ANH, Average number of exit holes; DSI, Dobie susceptibility index, LSD, Least significant difference; and CV (%), Coefficient of variation

3.3.1 Variation in performance among the genotypes

The performance of all the 210 genotypes is in Appendix 2. A synthesis of the variation is presented in the sections below.

3.3.1.1 Variation in performance in insect derived parameters among the genotypes

Number of eggs laid (NE): The genotypic differences was significant (≤ 0.05) for NE, showing substantial genetic variation among the evaluated genotypes for this trait. NE values ranged from very low (<30 eggs in highly resistant genotypes such as WC 10 and MAGIC113) to very high (>100 eggs in susceptible genotypes such as MAGIC154 and MAGIC115). The mean NE across genotypes was 63.02 (Figure 1).

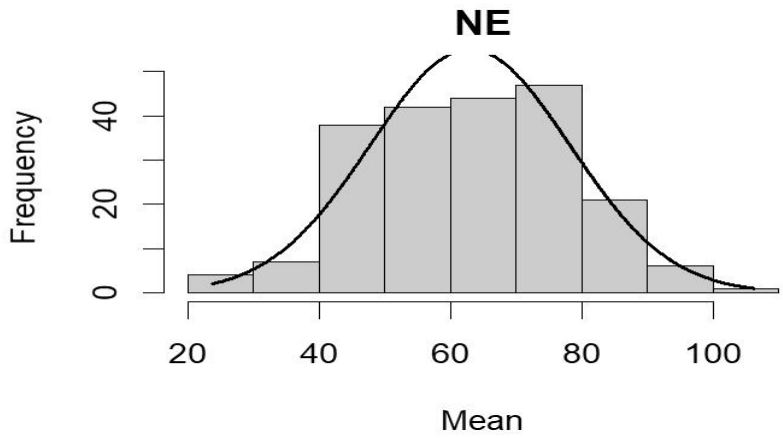


Figure 1: Frequency distribution of number of eggs laid (NE) across 210 cowpea genotypes evaluated for bruchid resistance

Number of bruchids/insect emerged (NEI): The NEI was evaluated across the cowpea genotypes indicating wide variation ($p \leq 0.001$) among them. The variation in NEI among genotypes show substantial differences in their ability to support bruchid development. NEI ranged from 4.67 (WC 10) to 38.50 (MAGIC091) with mean of 21.41 where the frequency of data distribution for number of bruchid emergence in the genotypes is indicated in the figure 2 below.

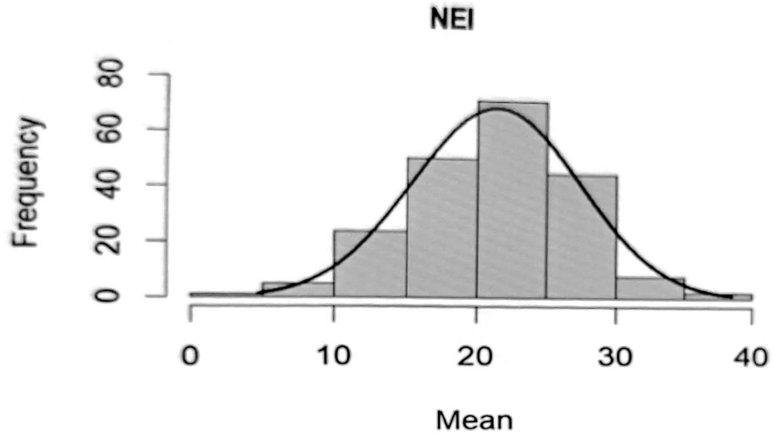


Figure 2: Frequency distribution of number of bruchids/insect emerged (NEI) across 210 cowpea genotypes evaluated for bruchid resistance

Percentage of adult bruchid/insects' emergence (%NEI): The analysis of variance revealed that there was very high significant variation ($p \leq 0.001$) in %NEI among the genotypes. Genotypes WC 10, MAGIC113, MAGIC060, and MAGIC284 showed lowest in %NEI (11.14–16.40%), reflecting strong resistance to bruchid infestation while, highly

susceptible genotypes like MAGIC317 had much higher %NEI (up to 73.9%). The distribution of the data for %NEI is shown in figure 3.

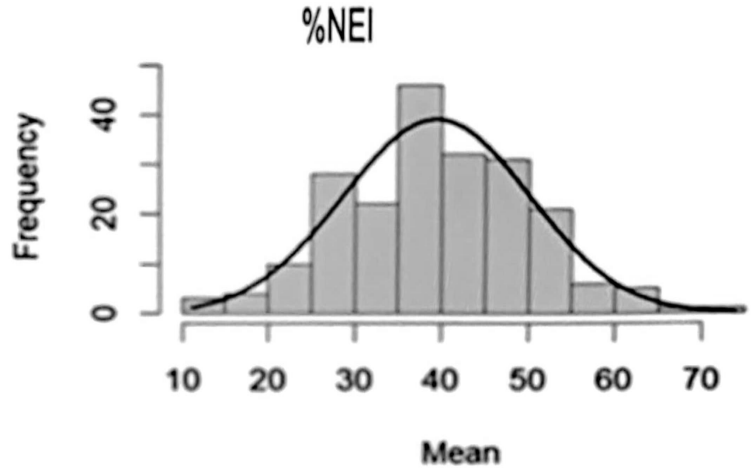


Figure 3: Frequency distribution of percentage of adult emergence (%NEI) across 210 cowpea genotypes evaluated for bruchid resistance

Growth index (GI): Growth index values ranged from very low (<1.0) to very high (>3.0), reflecting large differences in overall insect fitness across genotypes. Genotypes WC 10, MAGIC113, MAGIC060, and MAGIC284 observed to score between 0.43–0.66 in GI showing strong resistance to bruchid infestation while, low resistant genotypes MAGIC183, MAGIC292, MAGIC317 score 2.58, 2.90 and 3.30, respectively, with a mean GI of 1.62 (figure 4).

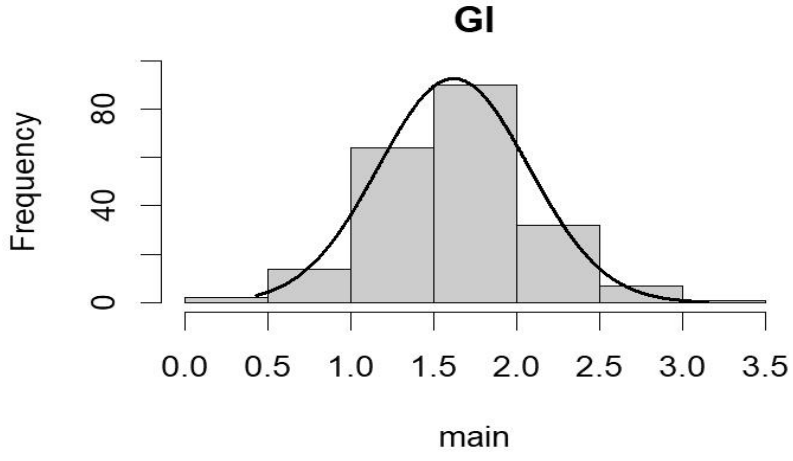


Figure 4: Frequency distribution of growth index (GI) across 210 cowpea genotypes evaluated for bruchid resistance

Median development period (MDP): MDP values showed very high significant variation ($p \leq 0.001$) among genotypes statistically and is shown (figure 5). MDP ranged from 22.00 days (MAGIC027) to 27.67 days (MAGIC199). Resistant genotypes tended to exhibit slightly prolonged development periods, showing delayed insect growth, while susceptible genotypes supported faster development. The extended life cycle and lower bruchid population growth in resistant genotypes contribute to reduced seed damage, while faster growth development and higher emergence rates in susceptible genotypes result in higher damage during storage.

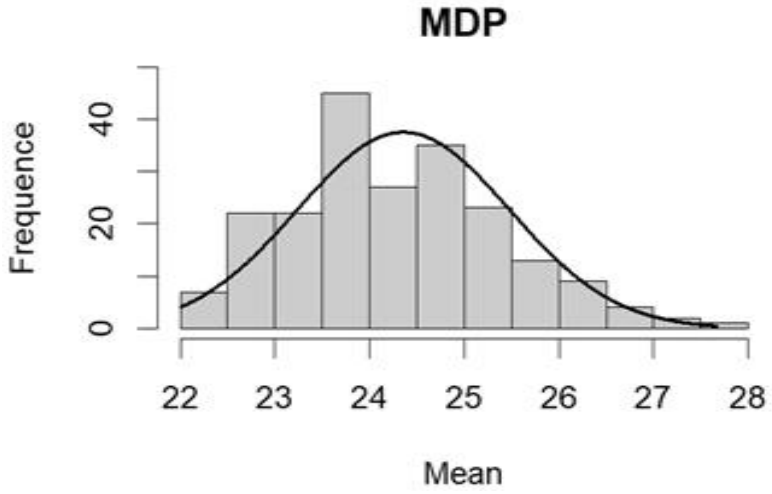


Figure 5: Frequency distribution of median development period across 210 cowpea genotypes evaluated for bruchid resistance

3.3.1.2 Variation in performance in seed derived parameters among the genotypes

Percentage seed weight loss (PWL): Very high significant differences ($p < 0.001$) were observed among genotypes for PWL. PWL ranged from 8.33% in NE 70 to 55.00% in MAGIC273. Out of the 210 genotypes evaluated, 136 recorded PWL values below 38.2%, while the remaining genotypes ranged between 38.2% and 55.0%. In this study, the checks performance was poorly ranked 124th (2419), 179th (WC-42), and 183rd (IT 84s-2246) for PWL. The frequency distribution of PWL values is shown in Figure 6.

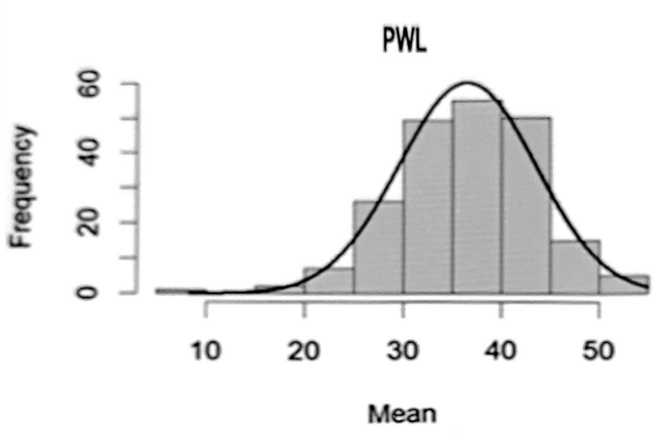


Figure 6: Frequency distribution of percentage seed weight loss (PWL) across 210 cowpea genotypes evaluated for bruchid resistance

Average number of holes per seed (ANH): Similarly, analysis of variance for ANH across the different genotypes revealed very high significant variation ($p \leq 0.001$) (figure 7). The mean value for ANH was 28.59. Genotype NE 70 scored the lowest ANH, with mean value of 4 while the highest ANH was scored by MAGIC154 with average value of 46.83.

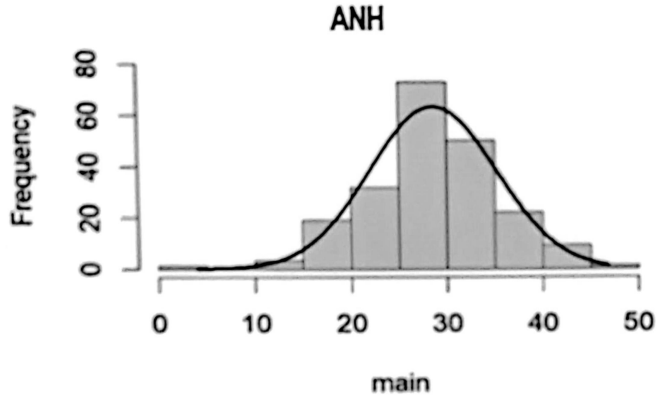


Figure 7: Frequency distribution of seed damage (average number of holes per seed (ANH)) across 210 cowpea genotypes evaluated for bruchid resistance

Dobie susceptibility index (DSI): The Frequency distribution of Dobie susceptibility index (DSI) across 210 cowpea genotypes for bruchid resistance is indicated in figure 8. Genotype WC-10 exhibited the highest level of resistance to bruchids among the evaluated genotypes, recording the lowest DSI value (1.44), whereas genotype MAGIC091 showed the highest susceptibility among the cowpea genotypes. Compared with the checks (2419, IT 84S-2246, and WC-42), genotype WC-10 exhibited markedly lower susceptibility, reducing DSI by 66.3%, 67.0%, and 67.7%, respectively. Based on DSI classification, genotypes WC-10,

MAGIC251, MAGIC072, MAGIC284, MAGIC060, and MAGIC113 were identified as the most resistant entries among the 210 genotypes evaluated. Although MAGIC284 and MAGIC072 demonstrated strong overall resistance, they showed moderate susceptibility in the individual batches, with DSI values exceeding 3.0. Among the 210 genotypes evaluated, the checks 2419, IT 84S-2246, and WC-42 ranked 53rd, 54th, and 58th, respectively, based on DSI values.

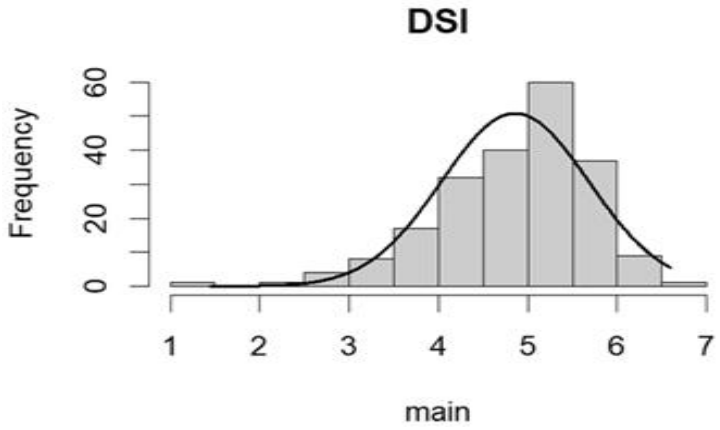


Figure 8: Frequency distribution of Dobie susceptibility index (DSI) across 210 cowpea genotypes evaluated for bruchid resistance

3.3.1.3 Ranking performance of genotypes in different batches

Means of top 20 and bottom 20 genotypes based on DSI for the first batch

Most of traits showed wide variability among the genotypes. As expected, the top 20 genotypes recorded lower average percentage of seed weight loss (PWL) values compared to the bottom 20 genotypes. PWL ranged from 4.33 to 37.67, with a mean of 19.98. Substantial variation was also observed for other parameters. The number of eggs laid ranged (NE) from 18.33 to 97.33 with the mean of 53.40. The number of insect/bruchids emerged (NEI) found to be varied between 1.00 and 46.33, with a mean of 17.15. The proportion of median development (MDP) values were relatively less variable, ranging from 21.33 to 26.00, with a mean of 23.10. Growth index (GI), which reflects the developmental performance of bruchids, varied from 0.26 to 3.30, with an average of 1.54. And number of exit holes (ANH) was observed to be ranged widely, from 0.33 to 40.67, with a mean of 17.74. The details of all the traits are summarized in table 4 below.

Analysis of bruchid resistance across two batches revealed clear differentiation among cowpea genotypes based on the Dobie susceptibility index (DSI). In the first batch,

MAGIC072 and WC 10 emerged as the most resistant genotypes, exhibiting DSI values near zero and minimal seed weight loss, egg numbers, and insect growth. MAGIC039 and MAGIC325 were also identified as resistant, with slightly higher DSI (2.28 and 2.91 respectively) as compared to the resistant genotypes in the first batch. In contrast, MAGIC333, MAGIC179, MAGIC091 and MAGIC091 were highly susceptible, showing extensive seed damage, high number of eggs laid, and accelerated insect development in the first batch.

Table 4: Mean of top 20 and bottom 20 genotypes based on DSI for the first batch

S/N	Genotypes	PWl	NE	NEI	%NEI	MDP	GI	DSI	ANH
1	MAGIC072	4.33	18.33	1.00	6.85	24.33	0.27	0.00	0.33
2	WC 10	4.67	25.67	1.33	6.03	24	0.26	0.39	2.00
3	NE 70	9.67	26.33	2.00	26.83	22.67	1.16	0.87	4.33
4	MAGIC051	9.67	17.67	8.00	38.52	23.00	1.65	1.72	8.00
5	MAGIC251	5.67	34.00	4.33	12.02	23.67	0.52	1.84	5.33
6	NAROCOWPEA 3	9.00	21.00	6.67	34.97	22.67	1.59	2.08	5.33
7	MAGIC048	9.33	28.33	5.33	28.33	21.00	1.38	2.17	8.33
8	MAGIC060	9.33	45.00	9.00	16.5	26.00	0.67	2.48	7.33
9	MAGIC300	9.67	31.33	11.67	38.74	22.00	1.77	2.57	13.67
10	MAGIC099	16.33	64.00	9.00	12.65	21.33	0.59	2.57	12.67
11	MAGIC070	16.67	42.67	11.00	29.45	22.00	1.29	2.66	10.00
12	MAGIC256	14.67	45.33	10.67	17.43	22.33	0.77	2.73	12.00
13	MAGIC113	10.33	58.33	5.00	10.86	22.00	0.49	2.77	7.67
14	MAGIC039	11.33	37.33	8.00	19.34	22.00	0.88	2.85	7.33
15	MAGIC325	9.67	35.00	7.33	19.17	21.67	0.89	2.91	7.67
16	MAGIC196	17.67	49.33	15.33	38.00	21.67	1.79	3.01	15.00
17	MAGIC231	7.67	39.67	8.67	21.52	22.33	0.94	3.02	7.67
18	MAGIC166	9.33	31.00	9.67	35.92	23.00	1.52	3.03	5.33
19	MAGIC125	14.33	40.67	9.33	20.71	22.67	0.93	3.08	9.67
20	MAGIC174	9.67	38.67	10.00	22.09	23.33	0.91	3.1	6.67
1	MAGIC132	49.67	109.33	32.33	31.56	24.33	1.3	6.21	35.00
2	MAGIC250	18.00	82.33	24.67	30.07	22.00	1.37	6.22	24.33
3	MAGIC324	27.33	71.67	26.67	38.92	22.67	1.72	6.24	25.33
4	SECOW 5T	24.33	73.67	24.33	34.31	22.00	1.57	6.24	25.67
5	MAGIC029	39.00	67.67	25.33	37.55	21.67	1.73	6.25	23.33
6	MAGIC141	21.00	93.67	26.33	31.25	22.67	1.39	6.26	29.00
7	MAGIC154	34.00	99.33	34.67	34.07	24.00	1.45	6.3	33.33
8	MAGIC001	36.33	84.00	30.00	38.9	23.33	1.67	6.34	31.33
9	MAGIC311	44.00	71.00	28.67	43.35	22.67	1.92	6.39	29.33

10	WC 42	47.33	115.00	37.33	33.27	24.67	1.34	6.4	39.33
11	MAGIC320	23.00	71.00	30.67	49.83	22.00	2.27	6.4	30.67
12	MAGIC076	32.00	83.33	27.33	32.78	22.00	1.49	6.4	22.67
13	MAGIC245	33.67	89.33	35.67	42.51	23.67	1.83	6.47	29.00
14	MAGIC036	34.67	88.00	33.33	45.55	23.00	1.97	6.55	22.67
15	MAGIC021	45.00	92.00	34.67	39.95	22.00	1.79	6.6	28.33
16	MAGIC148	42.67	96.67	28.67	36.83	22.00	1.71	6.62	31.67
17	MAGIC333	29.00	75.00	32.33	42.45	22.33	1.9	6.67	32.67
18	MAGIC179	31.67	81.67	39	50.23	23.00	2.24	6.9	39.00
19	MAGIC091	47.67	103.33	40	39.63	22.00	1.79	7.2	39.67
20	MAGIC190	37.67	97.33	46.33	48.11	22.00	2.19	7.55	37.00
	Min	4.33	18.33	1.00	6.03	21.00	0.27	0.00	39.67
	Max	49.67	109.33	46.33	49.83	26.00	2.27	7.55	0.33
	Mean	22.68	61.87	19.79	30.93	22.69	1.37	4.40	19.14
	SD	14.23	28.72	13.18	11.90	1.02	0.54	2.24	12.37

Note: NE, Number of eggs per 10 seeds; NEI, Number of emerged insects; %NEI, percentage of emerged insects; MDP, Median Development Period (days); GI, Insect growth Index; Percent of Seed Weight Loss; ANH, Average number of exit holes; DSI, Dobie Susceptibility Index; Min, Minimum; Max, maximum and SD, Standard deviation

Means of top 20 and bottom 20 genotypes based on DSI for the second batch

Means of top 20 and bottom 20 genotypes based on DSI for second season is indicated in table 5. Wide ranges observed for NE (26-113.3)**,** NEI (3-42.7)**,** %NEI (3.6%-85.6%), DSI (1.8-6.7)**,** and ANH (19.3-65.7). This indicates that there was substantial phenotypic variability among genotypes, suggesting the presence of both highly resistant and susceptible lines. Seed physical traits such as ISW, RSW, and WTL had also a variation of 0.90-1.70, 0.4-1.10 and 0.3-1.10 respectively. The moderate mean values of GI (1.62) and DSI (4.58) further reflect differential bruchid developmental performance across genotypes. Overall, substantial variability was observed across all traits, indicating a diverse genetic pool within the evaluated genotypes.

MAGIC072 exhibited high resistance in Batch 1 but showed a moderate Dobie susceptibility index (DSI) in Batch 2. Conversely, MAGIC284 displayed resistance in Batch 2 while exhibiting a moderate DSI in Batch 1, suggesting batch-dependent variation in resistance expression. Among the susceptible phenotypes, MAGIC141 and MAGIC154 consistently remained susceptible in both batches. Stable bruchid resistance on genotypes WC10,

MAGIC251, MAGIC060, and MAGIC113 were observed and the genotypes are suitable candidates for breeding programs. However, variable performance which requires farther study was also shown by MAGIC072 and MAGIC284.

Table 5: Means of top 20 and bottom 20 genotypes based on DSI for second season

S/N	GENOTYPES	PWL	NE	NEI	%NEI	MDP	GI	DSI	ANH
1	MAGIC105	47.70	108.70	3.00	3.60	25.30	0.10	1.80	49.30
2	MAGIC223	42.50	28.00	4.70	18.30	24.30	0.70	2.10	39.00
3	WC 10	42.90	43.30	8.00	16.20	26.30	0.60	2.50	35.00
4	MAGIC284	51.30	88.00	6.70	9.20	27.00	0.30	2.70	31.70
5	MAGIC226	56.50	64.30	5.00	7.60	24.70	0.30	2.70	42.00
6	MAGIC113	51.80	50.30	5.70	11.80	26.30	0.50	2.70	30.30
7	MAGIC251	35.30	28.30	7.70	40.30	28.00	1.50	2.80	19.30
8	MAGIC060	36.80	66.70	6.00	11.50	23.70	0.50	2.90	38.70
9	MAGIC305	39.90	46.30	10.70	29.30	26.30	1.10	2.90	24.70
10	MAGIC006	56.20	37.70	7.30	22.30	26.70	0.80	3.00	36.00
11	MAGIC029	45.70	86.70	8.00	17.50	25.70	0.60	3.10	31.70
12	MAGIC204	38.90	38.00	14.00	46.10	26.70	1.70	3.20	42.70
13	NAROCOWPEA 2	48.20	55.70	8.00	19.10	25.70	0.70	3.20	37.00
14	MAGIC074	43.70	26.00	9.00	36.50	23.00	1.60	3.20	25.70
15	MAGIC119	57.60	82.00	8.30	11.00	27.30	0.40	3.30	35.70
16	MAGIC134	23.40	71.70	10.00	35.50	29.00	1.20	3.40	38.30
17	MAGIC281	39.70	30.70	12.00	33.60	25.00	1.50	3.40	34.00
18	LG 093	59.60	74.00	10.70	15.70	26.30	0.60	3.40	31.30
19	SUNSHINE	44.80	48.30	11.30	21.90	23.70	0.90	3.40	31.30
20	MAGIC049	57.30	78.00	17.30	18.60	24.70	0.80	3.50	31.30
1	NE 15	46.90	58.00	37.00	70.70	26.00	2.70	6.00	44.70
2	MAGIC154	54.40	113.30	31.00	27.60	25.00	1.10	6.00	60.30
3	MAGIC222	50.20	73.70	38.00	65.30	26.00	2.70	6.00	35.00
4	MAGIC003	53.60	42.30	35.00	85.50	24.70	3.50	6.00	32.70
5	MAGIC194	51.70	50.30	42.70	85.60	27.30	3.20	6.00	46.30
6	MAGIC043	65.70	52.70	30.70	58.60	24.30	2.40	6.00	37.30
7	MAGIC225	60.20	82.30	22.70	30.10	22.00	1.40	6.10	45.70
8	MAGIC266	52.70	47.30	29.00	73.80	24.30	3.20	6.10	38.00
9	MAGIC202	53.30	78.30	40.30	58.80	26.30	2.30	6.10	43.30
10	MAGIC141	43.10	55.30	25.00	44.20	22.30	2.00	6.20	27.70
11	MAGIC298	58.30	95.70	36.00	40.30	25.30	1.60	6.20	31.00
12	MAGIC243	54.20	53.00	24.30	46.40	22.30	2.10	6.20	42.70
13	MAGIC136	40.10	54.30	36.00	77.40	25.00	3.20	6.20	24.70
14	MAGIC075	52.80	48.70	25.00	67.50	22.30	3.10	6.20	44.70

15	IT82E-18	55.50	95.70	32.70	37.80	23.30	1.60	6.20	46.00
16	MAGIC233	60.20	58.00	36.00	64.70	25.30	2.50	6.30	46.70
17	MAGIC267	52.40	66.70	31.30	50.60	24.00	2.10	6.30	35.00
18	MAGIC328	64.30	42.70	32.00	78.10	22.00	3.60	6.60	35.30
19	MAGIC044	58.00	102.30	31.00	45.70	22.00	2.00	6.70	65.70
20	MAGIC027	41.20	105.00	29.30	43.70	21.70	2.00	6.70	22.70
	Min	23.40	26.00	3.00	3.60	21.70	0.10	1.80	19.30
	Max	65.70	113.30	42.70	78.10	29.00	3.60	6.70	65.70
	Mean	49.72	63.21	20.46	39.45	24.93	1.62	4.58	37.26
	SD	8.85	23.66	12.75	23.71	1.82	1.01	1.68	9.37

Note: NE, Number of eggs per 10 seeds; NEI, Number of emerged insects; %NEI, percentage of emerged insects; MDP, Median development period (days); GI, Insect growth index; Percent of seed weight loss; ANH, Average number of exit holes; DSI, Dobie susceptibility index; Min, Minimum; Max, maximum and SD, Standard deviation.

3.3.3 Correlation of parameters for the combined batches

This presents the result of correlation analysis of traits related to bruchid resistance in the artificially infected cowpea genotypes based on the genotype means (figure 9). Several traits exhibited strong positive correlations with the NEI ($P < 0.001$), including the ANH ($r = 0.57$), NE ($r = 0.53$), SWL ($r = 0.46$), and DSI ($r = 0.83$). DSI showed significant positive correlations ($P < 0.001$) with ANH ($r = 0.51$), NE ($r = 0.47$), PWL ($r = 0.52$), GI ($r = 0.54$), and %NEI ($r = 0.52$). However, negative significance correlation ($P < 0.001$) was observed for DSI with MDP ($r = -0.13$), NE with GI ($r = -0.33$) and NE with %NEI ($r = -0.032$) . The strongest positive correlation was observed between GI and %NEI ($r = 0.98$), followed by DSI and NEI ($r = 0.83$), WTL and PWL ($r = 0.82$), and WTL and ANH ($r=0.77$). These findings suggest that higher number of insect emergence (NEI) is associated with increased susceptibility.

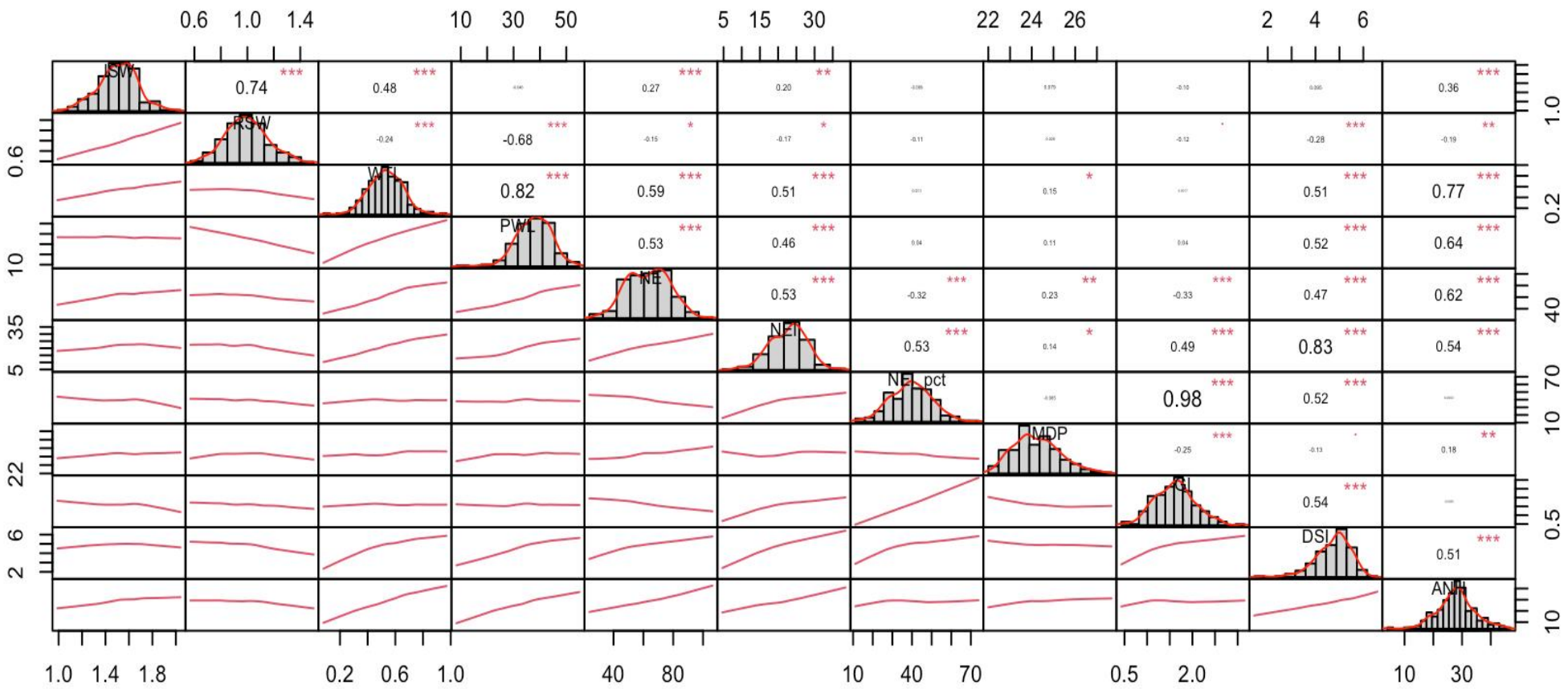


Figure 9: Pearson's correlation coefficient of tested traits for phenotypic bruchid resistance study based on the genotype means

Note: Correlation coefficients (r) for experimental parameters, under *Callosobruchus maculantus* infestation was *** significant at P<0.001; **significant at p<0.01; *significant at p<0.05; and ns not significant at P>0.05; NE, Number of eggs laid per 10 seeds; NEI, Number of emerged insects; %NEI, percentage of emerged insects; MDP, Median development period (days); GI, Insect/bruchid growth index; Percent of seed weight loss; ANH, Average number of exit holes; DSI, Dobie susceptibility index

4.2 Results of study 2

Genotyping of a diverse set of cowpea genotypes from MAGIC population was performed using high-density genome-wide markers to ensure comprehensive genome coverage and high mapping resolution. Phenotypic evaluations for bruchid resistance–related traits were conducted under controlled laboratory conditions to ensure data accuracy and reliability. Table 6 summarizes the detected significant single nucleotide polymorphisms (SNPs) associated with bruchid resistance traits across combined experimental batches. Figure 10 also demonstrates the identified significant SNPS in manhatan and QQ plots in traits of **(A) s**eed weight loss percentage, (B) number of emerged bruchids, (C) growth index, (D) Dobie susceptible index and (F) average number of holes.

4.2.1 Percentage of seed weight loss (PWL)

Three significant SNPs associated with PWL, a key indicator of bruchid resistance using the pLARmEB model, were identified. These SNPs were located on chromosomes *Vu03*, *Vu04*, and *Vu09,* respectively. The first marker 2_10459 (A/G) at 0.42 Mb on Chromosome *Vu03* explained 4.9% of the phenotypic variation, marker 2_47089 (A/G) at 10.83 Mb on *Vu04* also explained 3.63 % of the phenotypic variation while the third marker 2_34448 (A/T) at 29.20 Mb on *Vu09* explained 3.54 % of the phenotypic variation respectively.

4.2.2 Number of emerged adult bruchids (NEI)

A significant SNP 2_14396 (A/C) associated with NEI was detected on chromosome *Vu10* at position 40.61 Mb using both pLARmEB and ISIS EM-BLASSO models. This SNP showed a QTN effect of 1.61, with LOD scores ranging from 3.02 to 3.04 and −log10(P) values between 3.72 and 3.74. The marker explained approximately 4.7% of the phenotypic variance, with a minor allele frequency of 0.18.

Table 6: Significant single nucleotide polymorphism (SNPs) associated with bruchid resistance in cowpea MAGIC population

Experiment	Trait	SNP ID	Allele	CHR.	Marker position (Mb)	Model	QTN effect	LOD score	'-log10(P)'	r2(%)	MAF
Combined batches	PWL	2_10459	A/G	*Vu03*	0.42	5	1.61	3.67	4.40	4.85	0.47
	PWL	2_47089	G/T	*Vu04*	10.83	5	-1.42	3.49	4.21	3.63	0.38
	PWL	2_34448	A/T	*Vu09*	39.21	5	-1.68	3.00	3.70	3.54	0.21
	NEI	2_14396	A/C	*Vu10*	40.61	4,5	1.61	3.02-3.04	3.72-3.74	4.7	0.18
	GI	2_23649	A/G	*Vu03*	48.04	2,4	(0.15) -(0.15)	3.01-3.37	3.70-4.08	6.01-6.42	0.14
	DSI	2_00381	A/G	*Vu03*	47.28	5	2.00E-04	3.11	3.82	4.67-6.0	0.15
	DSI	2_14396	A/C	*Vu10*	40.61	1,4,5	(0.30) -(0.36)	4.28-5.44	5.05-6.25	9.31-13.30	0.18-0.19
	ANH	2_40705	A/C	*Vu05*	42.50	4	-2.61	3.95	4.70	6.61	0.12
	ANH	2_43843	T/G	*Vu10*	26.55	5	2.03	3.84	4.58	6.3	0.22
	ANH	2_49489	A/G	*Vu10*	28.07	4	2.37	3.76	4.50	8.61	0.21

Note: SNP= Single Nucleotide Polymorphism, CHR= Chromosome, MAF= Minor allele frequency, LOD= logarithm of odds

Models: (1),mrMLM; (2) FASTmrMLM; (3) FASTmrMMA; (4) ISIS EM-BLASSO; (5) pLARmEB; (6), pKWmEB

Manhatan and QQ plots of significant SNPs in the studied traits

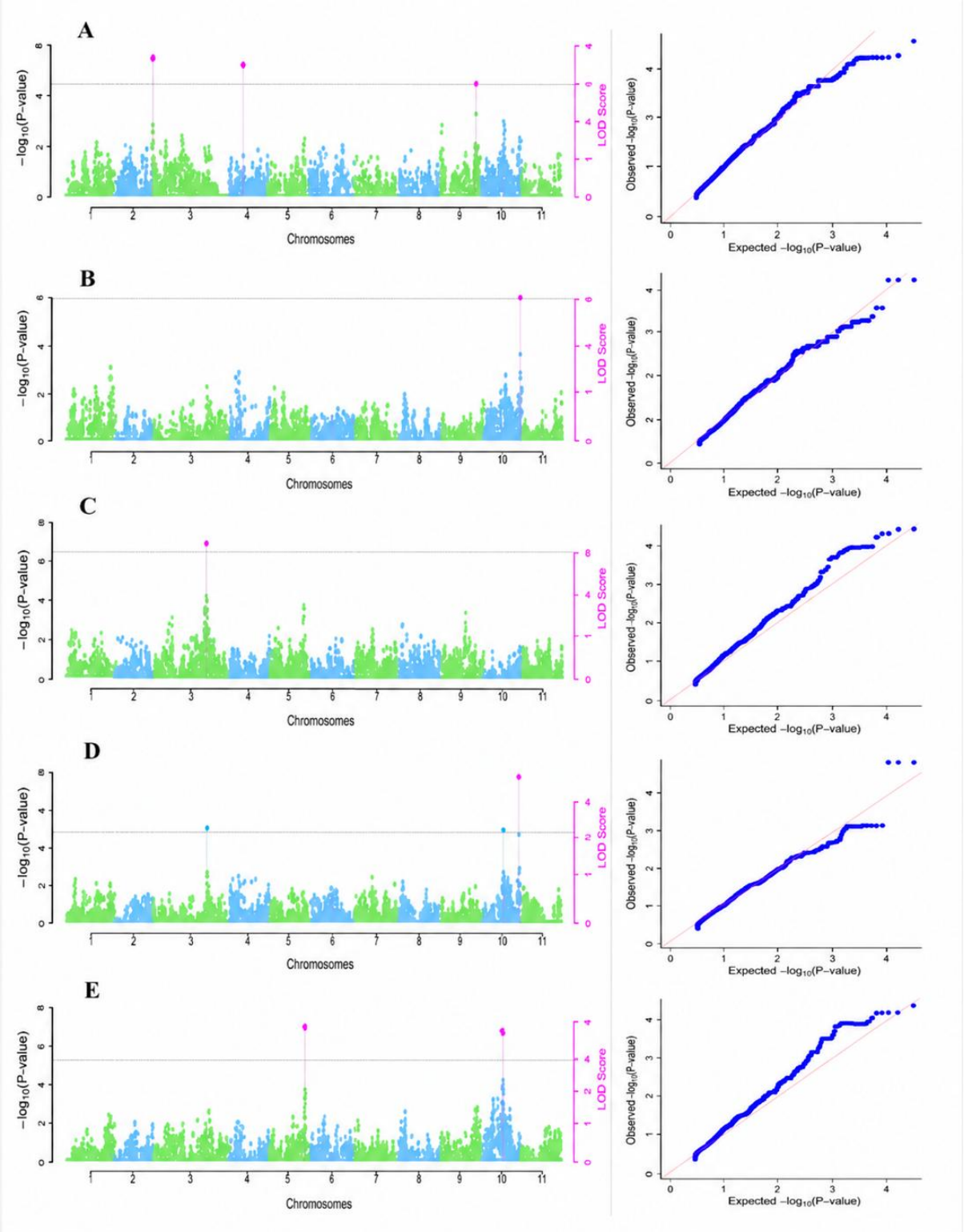


Figure 10: Manhattan plots for genomic wide association of signals for significant traits of combined batches [(A) Seed weight loss percentage, (B) Number of emerged bruchids, (C) growth index, (D) Dobie susceptible index and (E) Average number of holes]. [The y-axis represents the p-value of the marker-trait association on a -log10 scale and the x-axis relates to the bruchids and the Horizental line is 5% Bonferoni threshold line].

4.2.3 Growth index (GI)

Significant SNP associated with Growth index (GI), was identified on chromosome *Vu03* using FASTmrMLM and ISIS EM-BLASSO models. The marker 2_23649 (A/G) at 48.04 Mb on Chromosome *Vu03* explained 6.01% - 6.42% of the phenotypic variation.

4.2.4 Dobie susceptibility index

Two significant SNPs associated with the DSI were detected. SNP 2_00381(A/G), located at 47.28 Mb on chromosome *Vu03* was identified using the pLARmEB model and showed a small QTN effect (2.00×10^{-4}), explaining 4.67% of the phenotypic variance. The second SNP, 2_14396 (A/C), located at 40.61Mb on chromosome *Vu10* was consistently detected using three models (mrMLM, ISIS EM-BLASSO, and pLARmEB). This SNP showed stronger effects, with QTN values ranging from 0.30 to 0.36, LOD scores between 4.28 and 5.44, explained up to 13.30% of the phenotypic variance. These results indicate that DSI is influenced by multiple loci with varying effect sizes.

4.2.5 Average number of holes

Three significant SNPs associated with the average number of holes (ANH) were identified on chromosomes *Vu05* and *Vu10*. Two SNPs on chromosome *Vu10* (2_43843 and 2_49489) showed positive QTN effects, explaining between 6.3% and 8.6% of the phenotypic variation. One SNP 2_40705 (A/C) on chromosome *Vu05* showed a negative QTN effect (−2.61).

4.2.6 Population structure of MAGIC populations

To investigate the population structure, Principal Component Analysis (PCA) was conducted. The first two principal components (PC1 and PC2) accounted for 7.65% of the total genetic variation. A scatter plot of PC1 versus PC2 revealed five distinct clusters, each representing genetically similar sub-populations within the MAGIC population (Figure 11) likely reflecting distinct breeding lines or origins. These groupings reflect the diverse genetic backgrounds of the MAGIC founder lines. To account for population structure in subsequent genome-wide association studies (GWAS) and minimize the risk of false positives, PC1 and PC2 were included as covariates.

"To investigate the genetic structure of the 210 MAGIC genotypes, Principal Component Analysis (PCA) was conducted. The first two principal components (PC1 and PC2)

accounted for 7.65% of the total genetic variation. The PCA scatter plot revealed five distinct genetic clusters, indicating the presence of genetic differentiation among the MAGIC genotypes (Figure 11). To further understand the relationship between genetic diversity and disease resistance, the distribution of resistant, moderately resistant and susceptible genotypes was examined across the five clusters. This analysis provided insights into whether disease resistance was associated with particular genetic groupings or distributed across the population. PC1 and PC2 were subsequently included as covariates in the GWAS models to account for population structure and minimize the risk of false-positive associations."

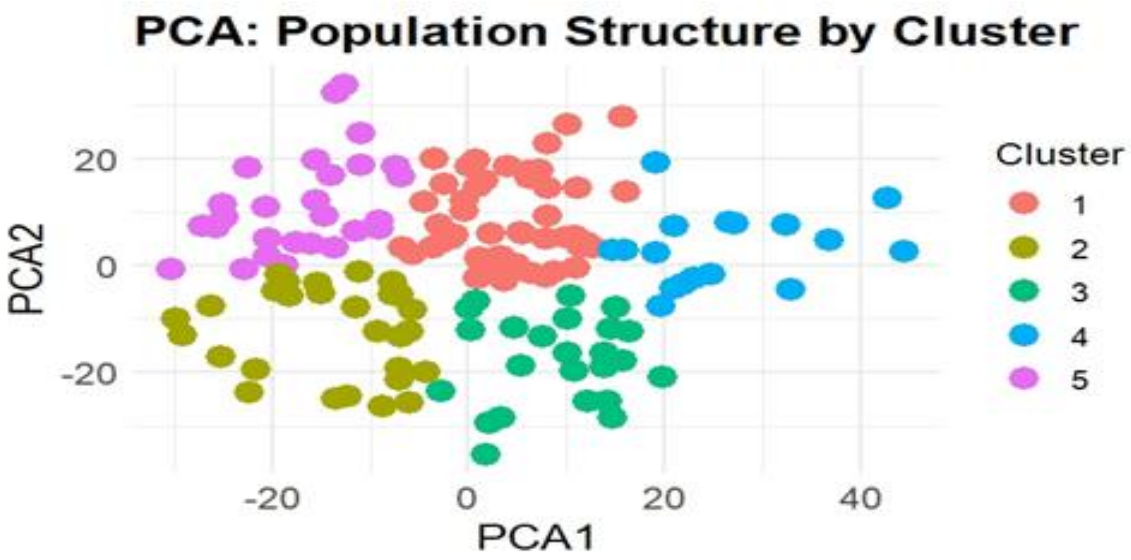


Figure 11: PCA-plot based clustering displaying the relationship between and among the sources of genotypes

4.2.6 Candidate genes

Candidate genes located within or near significant SNP regions were identified and functionally annotated. List of identified candidate genes with molecular and biological roles linked to bruchid resistance are indicated in table 7. A total of eleven candidate genes were identified across chromosomes *Vu03, Vu04, Vu09, and Vu10*. These genes are associated with known plant defense mechanisms, including protease inhibitors, detoxification enzymes (cytochrome P450s), transcriptional regulators (MYB and Mediator complex proteins), and metabolic enzymes involved in stress and defense responses. Identification of these genes provides strong biological support for the detected marker trait associations and suggests their potential role in conferring resistance against *Callosobruchus maculatus.*

Table 7: List of candidate genes with molecular and biological roles linked to bruchid resistance

TRAIT	CHR	POSITION	GENE ID	FUNCTION
ANH	Vu10	28065479	Vigun10g095200	This family is composed of trypsin-alpha amylase inhibitors, seed storage proteins and lipid transfer proteins from plants. Defence (e.g., protease inhibitors defending against herbivores or pathogens).
PWL	Vu03	420041	Vigun03g006100	Glycosylation regulates hormones, so identifying the glycosyltransferases (GTs) that balance plant hormones and understanding their roles is crucial. Some GTs also contribute to plant resistance against abiotic stress.
PWL	Vu09	39205242	Vigun09g218100	They are now recognised to bind DNA, RNA, protein and/or lipid substrates.
GI	Vu03	43325794	Vigun03g264300	P450 detoxification helps An. sinensis survive pyrethroid insecticides.
GI	Vu03	43325794	Vigun03g264000	The proteomic analyses confirmed the results of previous studies of A. angustifolia embryogenic cell lines regarding the importance of proteins related to defence responses.
NEI	Vu10	40612173	Vigun10g191500	Mediator is a multi-subunit protein complex that regulates gene expression in eukaryotes by integrating physiological and developmental signals and transmitting them to the general RNA polymerase II machinery.
NEI	Vu10	40612173	Vigun10g192300	SAM binding site according to the Inter Pro database
NEI	Vu10	40612173	Vigun10g191800	These enzymes could help in the degradation of toxic compounds, act directly as insecticidal proteins, or assist in modifying other defence-related compounds.
DSI	Vu10	40612173	Vigun10g192400	Studying genes and TR changes can reveal insights into protein properties like binding affinity and stability, as well as phenotypic traits such as stress resistance.
DSI	Vu03	47278916	Vigun03g290200	Binds to and activates the ODO1 and EOBI pro, moters via MYB binding sites (MBS) 5'-AAACCTAAT-3' and 5'-CTAACT-3' (PubMed: 20543029, PubMed: 21585571, PubMed: 22499185, PubMed: 23275577).
DSI	Vu03	40612173	Vigun03g290400	Binds to and activates the ODO1 and EOBI promoters via MYB binding sites (MBS) 5'-AAACCTAAT-3' and 5'-CTAACT-3' (PubMed: 20543029, PubMed: 21585571, PubMed: 22499185, PubMed: 23275577).

4.3 Discussion

4.3.1 Discussion of study 1

There is strong genetic variability among the genotypes for all of the resistance traits evaluated. This is highly desirable for breeding because it indicates substantial genetic control, meaning that selection for resistance is likely to be effective. Batch significantly affected most traits; however, the batch effect was not significant (ns) for number of eggs (NE) and Dobie susceptibility index (DSI). These results suggest that environmental conditions between batches strongly influenced infestation intensity, whereas NE and DSI remained relatively stable across batches. Comparative analysis across two batches revealed that genotype WC 10 consistently displayed the highest resistance to bruchids, maintaining low DSI, minimal seed damage, and low insect development in both batches. MAGIC251, MAGIC060 and MAGIC0113 also showed relatively stable resistance. However, MAGIC141 and MAGIC154 were consistently highly susceptible across both batches. Some genotypes, including MAGIC072 and MAGIC284, displayed variable responses across batches, performing well in one batch but less consistently in the other. This batch-dependent response may reflect environmental variation, differences in experimental conditions, pathogen pressure and genotype-specific sensitivity to these factors. Such genotype × batch interactions suggest that the expression of resistance may be influenced by experimental and environmental conditions. The results therefore identify both stable sources of resistance with consistent performance across batches and genotypes whose responses are more sensitive to batch variation, reinforcing the importance of multi-batch and multi-environment evaluation for reliable identification of resistance sources. One important distinction: “batch-dependent effects” does not necessarily mean the genotype itself is unstable. If batch represents different experimental conditions, the interaction could reflect a genuine genotype × environment interaction rather than simply an experimental error.

4.3.1.1 Insect derived parameters

Number of eggs laid (NE): There was significant variation among the genotypes for NE, however, no significant variation was shown across the batches. According to Badii *et al.* (2013), variation in egg counts may reflect differences in seed surface characteristics, such as texture, glossiness, or the presence of deterrent secondary metabolites. In the current study, low number of eggs 23.83, 29, 31.17 and 34.5 were recorded on the resistant genotypes of MAGIC072, MAGIC051, MAGIC251 and WC 10, respectively. This indicate that those genotypes had reduced oviposition preference by female beetles as compared to the other genotypes. Similar significant reduction in oviposition of bruchids on resistant cowpea genotypes was also reported by Amusa *et al.* (2014) and Woldemicheal (2019). This supports findings by Sharma & Thakur (2014) and Amusa *et al.* (2014) that oviposition deterrence is an essential resistance mechanism (*antixenosis*). In this study, genotypes with fewer eggs consequently had lower seed weight loss and reduced insect emergence, supporting the idea that reduced oviposition and low insect emergence are a strong predictor of resistance. Overall, genotypes with fewer eggs exhibited lower seed weight loss and reduced adult emergence, confirming that reduced oviposition is a strong indicator of bruchid resistance and is an important trait for selection in breeding programs.

Number of bruchid/ insect emergence (NEI): The study demonstrated the existence of very high significant differences ($p \leq 0.001$) among cowpea genotypes for resistance to bruchids in NEI. The NEI was substantially lower in resistant genotypes, suggesting effective antibiosis that inhibits larval survival or development. Similar observations have been reported by Jacobson (1990), Buleti (2020), and Dikane (2022), who attributed it to traits such as secondary metabolites, seed coat hardness, or nutritional factors that may be unfavorable for larval growth. Resistant genotypes, notably WC 10, MAGIC113, MAGIC251, MAGIC251 and MAGIC284 recorded very low NEI values, reflecting strong suppression of larval survival and unsuccessful adult emergence. These genotypes are resistant genotypes and likely express antibiosis-based resistance mechanisms. The results also show that susceptible genotypes such as MAGIC091, MAGIC202 and MAGIC286 supported high adult emergence of bruchids indicating weak defensive mechanisms and greater suitability for larval feeding and development. NEI proved to be a reliable indicator of host suitability and resistance, as it directly reflects the cumulative effects of oviposition success, larval survival, and

development. Genotypes with consistently low NEI represent valuable sources of resistance for bruchid management and breeding programs. The correlation result confirms that high association (83%) between NEI and DSI parameters supports the result observed in NEI trait.

Percentage of adult insect emergence (%NEI): The very high significant genotypic variation observed in %NEI highlights the critical role of host genetics in regulating insect survival and emergence. As has been reported by Weldekidan (2019) the resistant genotypes were characterized by extended adult emergence period while adult emergence in susceptible lines was rapid. Lower %NEI values indicate enhanced resistance through reduced larval survival or impaired development within seeds, revealing the presence of exploitable resistance sources among genotypes. Despite the high biological variability associated with insect-related traits, %NEI remains a robust and informative parameter for resistance assessment, especially when integrated with complementary traits such as growth index and development period. As has been shown in the correlation analysis, high strongest positive correlation was observed between GI and %NEI ($r = 0.98$) thus, %NEI can be reliably used alongside complementary traits to identify and select cowpea genotypes with strong bruchid resistance for breeding programs.

Growth index (GI): Significant genotypic effect was observed for GI among the tested genotypes which indicates that this trait exhibited genetic variation and hence can be good for selecting resistant genotypes, making it a promising parameter for determining levels of bruchid resistance. Since GI integrates both survival and developmental speed, reduced GI values are often associated with enhanced host resistance. For instance, the resistant genotypes WC 10, MAGIC113, MAGIC060 and MAGIC284 scored low GI values 0.43, 0.47, 0.58 and 0.66, respectively; while susceptible genotypes MAGIC317 and MAGIC219 score high GI values (3.15 and 2.61) and were also observed to have high %NEI 73.87 and 60.75, respectively with shorter MDP, characteristic of susceptible responses. A recent study by Innocent (2023), reported that progenies with lower GI are likely to have higher anti-nutritional factors than those with a higher GI. Earlier, Gatehouse *et al.* (1979) also found a higher level of *trypsin* inhibitors in *Tvu* 2027 cowpea (bruchid resistant an IITA line) compared to the susceptible varieties of cowpea. Therefore, GI is a reliable integrative parameter for selecting bruchid-resistant genotypes in cowpea breeding programs.

Median development period (MDP): The very high significant genotypic differences among the tested genotypes indicate that MDP is a stable and reliable trait for assessing bruchid resistance. Longer development periods are often associated with unfavorable host conditions for insect growth, implying that resistant genotypes delays insect development. The significant batch and interaction effects highlight the sensitivity of MDP to environmental conditions; however, the consistency of genotypic differences suggests that MDP can serve as a robust selection criterion. The MDP observed in the first batch (21–31 days) is consistent with previous research showing that *C. maculatus* typically completes its development within 3–4 weeks under favorable conditions (Sharmeen, 2007; Srivastava & Subramanian, 2016). Resistant genotypes generally exhibited longer MDP values, consistent with the principle that delayed development reduces bruchid population growth and is a hallmark of antibiosis resistance. Therefore, MDP can be effectively used as a selection criterion in breeding programs aimed at developing cowpea bruchid resistant varieties.

4.3.1.2 Seed derived parameters

Percent of seed weight loss (PWL): Seed weight loss ranged widely (5.37–55.12%), mirroring the extensive damage potential of bruchids as described in similar reports (Umeozor, 2005; Soundararajan *et al.*, 2012). Resistant genotypes (WC 10 and MAGIC060) showed minimal weight loss due to few numbers of bruchid emergence, lower growth index, lower percentage of adult emergence and reduced seed damage. Since weight loss directly affects grain quality, market value, and germination, genotypes with low PWL provide major advantages for storage and food security. This confirms that PWL can be considered as a direct and economically meaningful indicator of post-harvest resistance.

Average number of holes per seed (ANH): The average number of exit holes per seed (ANH) is a direct indicator of bruchid damage in stored grains. Susceptible genotypes recorded higher ANH values, consistent with earlier findings reported by Weldekidan (2019). Among the evaluated genotypes, MAGIC251 recorded the second lowest ANH after MAGIC200, indicating strong resistance and a high potential for maintaining seed quality during exposure of the genotypes to bruchids. This genotype also exhibited a low number of emerged bruchids and a reduced percentage of weight loss. The high variability observed for ANH demonstrates its strong discriminatory ability, making it a reliable and valuable selection criterion in breeding programs targeting post-harvest resistance.

Dobie susceptibility index (DSI): A very highly significant difference ($p \leq 0.001$) among genotypes was observed for DSI, indicating substantial genetic variation in resistance. In the present study, Dobie susceptibility index (DSI) was used to quantify and categorize genotypes according to their level of bruchid resistance. Similar approaches have been reported by Dobie (1974), Tengey et al. (2023), Weldekidan (2019), and Unzimai (2024).

Lower DSI values were recorded for genotypes such as WC10, MAGIC251, MAGIC060, and MAGIC113, indicating reduced susceptibility to bruchid infestation. These findings are consistent with earlier reports showing that resistant genotypes typically exhibit longer developmental periods, fewer adult emergence, and lower growth indices (Elsawy *et al*., 2024). In the current study, these genotypes also exhibited lower seed damage and reduced weight loss percentages. Since DSI integrates multiple developmental traits, the results suggest that several physical defense mechanisms, physiological, or biochemical may be operating simultaneously. This observation is consistent with the complex inheritance patterns of bruchid resistance described in the literature (Zhu-Salzman & Zeng, 2015).

A significant genotype × batch interaction was also observed for all the traits (Table 3), indicating that genotype performance varied across batches. Practically, this implies that a genotype performing well in one batch may not necessarily perform similarly in another. Nevertheless, the identified resistant genotypes consistently exhibited resistance across both batches. As an integrative index, DSI effectively captures multiple resistance mechanisms and enables the identification of genotypes with stable and durable bruchid resistance across batches.

The relatively high variability observed in the present study is comparable to that reported in previous cowpea–*Callosobruchus maculatus* bioassays. For example, Tengey *et al*. (2022) reported coefficients of variation ranging from 33.1 to 48.1% for seed damage-related measurements in cowpea genotypes challenged with *C. maculatus*. Similarly, Kusi *et al.* (2025) reported coefficients of variation of 47.7% for oviposition and 64.1% for hatchability, while the coefficient of variation for percentage seed-weight loss reached 33.6% at 26 days after infestation. These findings demonstrate that relatively high experimental variability can occur in cowpea–bruchid assays, particularly for traits involving insect oviposition, emergence and seed damage.

The variability among replicates and blocks may have resulted from a combination of biological and experimental factors. *C. maculatus* populations are not completely uniform in their biological responses. Previous studies have demonstrated significant population-level differences in development time, adult emergence, adult weight and female fecundity even when insects were maintained under similar environmental conditions (Kingsolver *et al*., 2011). Even though, the experimental procedures were standardized as far as possible, some degree of biological and experimental heterogeneity may have contributed to the relative variability among replicates and blocks.

Correlations also provide valuable insights into the dynamics of bruchid infestations and their impact on cowpea seeds, highlighting how different factors interact to influence seed damage and losses. The correlation analysis revealed strong and significant relationships among several traits, with coefficients reaching as high as ~0.98, indicating a high degree of interdependence. GI and DSI were positively associated with multiple traits, suggesting they may be influenced by common underlying factors or genetic regions. Moderate correlations ($r \approx 0.5–0.8$) further support partial linkages among traits, while the presence of a few negative correlations indicates potential trade-offs, where improvement in one trait could lead to a reduction in another. Overall, the pattern of correlations highlights coordinated trait interactions and supports the likelihood of shared genetic control, consistent with the presence of QTL hotspot regions.

4.3.2 Discussion of study 2

The genome-wide association study (GWAS) of bruchid resistance in cowpea identified several significant associations that provide insights into the genetic basis of resistance-related traits. The present study employed pLARmEB, FASTmrMLM, ISIS EM-BLASSO and mrMLM to identify significant single-nucleotide polymorphisms (SNPs) associated with percentage weight loss (PWL), number of insects/bruchids emerged (NEI), growth index (GI), average number of exit holes (AHN), and Dobie susceptibility index (DSI). Similarly, Kpoviessi *et al.* (2022) identified significant QTNs for percentage weight loss, bruchid emergence, DSI and mean number of holes, demonstrating the utility of multi-locus GWAS for dissecting the genetic architecture of bruchid resistance in cowpea. The detection of significant loci for multiple resistance-related traits is therefore consistent with previous evidence that cowpea resistance to bruchids is controlled by multiple genomic regions.

Significant GWAS associations for PWL were identified on chromosomes *Vu03*, *Vu04* and *Vu09* in the cowpea MAGIC population. The SNP 2_10459 on *Vu03* exhibited a positive QTN effect (+1.61), indicating an association with increased seed weight loss and, consequently, greater susceptibility. In contrast, SNPs 2_47089 on *Vu04* and 2_34448 on *Vu09* showed negative QTN effects (−1.42 and −1.68, respectively), suggesting associations with reduced weight loss and increased resistance. These results indicate that PWL is influenced by multiple loci with varying effect sizes. The findings matches with the multi-locus GWAS of Kpoviessi *et al*. (2022), which identified significant QTNs associated with PWL and further supported the contribution of multiple genomic regions to variation in seed weight loss under bruchid infestation. Because PWL reflects the cumulative feeding and developmental damage caused by bruchids, loci associated with reduced weight loss may be useful for breeding; however, their effects should be validated across environments and genetic backgrounds before routine application in marker-assisted selection (MAS).

A significant association with NEI was detected on chromosome *Vu10*, identifying this region as a potential genomic region contributing to bruchid resistance. The QTN effect of 1.61 indicates an association with variation in bruchid emergence. However, the low coefficient of determination (r^2 = 4.7%) and minor allele frequency (MAF = 0.18) indicate that this locus explains only a small proportion of the phenotypic variation, suggesting that additional genetic factors contribute to NEI. Miesho *et al*. (2019), for example, identified SNPs associated with insect emergence and other bruchid-resistance parameters in a GWAS of 217 cowpea mini-core accessions. Similarly, Unzimai *et al*. (2024) identified QTL associated with bruchid emergence in cowpea, providing further evidence that variation in insect emergence has a quantitative genetic basis. The *Vu10* region identified in the present study may therefore harbour genes involved in seed defence, seed biochemical composition, or other mechanisms affecting insect survival and development.

Significant QTNs associated with GI were identified on chromosome *Vu03*. The growth index is an important indicator of bruchid performance during storage because it reflects the ability of the insect population to develop and multiply on a particular cowpea genotype. Associations with reduced GI may therefore indicate genomic regions that contribute to restricting bruchid development and enhancing resistance during storage. Previous studies have similarly shown that resistant cowpea genotypes can adversely affect bruchid development and emergence, while QTL mapping has identified genomic regions associated

with bruchid developmental characteristics (Unzimai *et al*., 2024). The markers identified in the present study explained 6.01% and 6.42% of the phenotypic variance, respectively, indicating potentially useful association. Nevertheless, these values represent moderate contributions and should not be interpreted as evidence that the identified markers independently control GI. Their utility for MAS will require validation in independent populations and environments. Despite this limitation, the markers provide promising candidates for further evaluation in breeding programmes aimed at improving bruchid resistance and storage stability.

Two significant associations for DSI were identified in the cowpea MAGIC population on chromosomes *Vu03* and *Vu10*. The DSI integrates aspects of bruchid development and emergence and is widely used to differentiate resistant and susceptible cowpea genotypes. The identification of DSI-associated loci on *Vu03* and *Vu10* provides further evidence that overall bruchid susceptibility is controlled by multiple genomic regions. Unzimai *et al.* (2024) similarly reported the significant QTL DSI 3.2MW on *Vu03* near marker 2_18835 (5.0 Mb), flanked by markers 2_14315 (4.7 Mb) and 2_28278 (5.4 Mb), and explaining 10.6% of the phenotypic variance for DSI. However, marker 2_00381 identified in the present study at 47.28 Mb is located in a substantially different region of *Vu03* from the QTL reported by Unzimai *et al*. (2024). This difference suggests that *Vu03* may harbour more than one genomic region contributing to bruchid resistance rather than a single resistance locus.

A particularly notable association for DSI was detected on *Vu10* at 40.61 Mb, explaining 9.31–13.3% of the phenotypic variation. This relatively large proportion of explained variance, makes the locus a promising candidate for further validation and potential application in MAS. However, the magnitude of phenotypic variance explained should not, by itself, be interpreted as evidence for a major gene, since replication, fine-mapping and functional validation are required to establish the underlying genetic mechanism. The SNP exhibited an A/C allele substitution and was consistently detected across three GWAS models, providing additional support for its reliability as a candidate association. Previous GWAS studies have likewise identified SNP markers and genomic regions associated with DSI and other bruchid-resistance traits in cowpea (Miesho *et al*., 2019; Kpoviessi *et al*., 2022). In addition, inheritance studies have reported relatively high narrow-sense heritability for DSI, indicating potential for genetic improvement of this trait (Kpoviessi *et al*., 2021). Collectively, these findings support the interpretation that DSI is a complex quantitative trait influenced by

multiple genetic factors and that the *Vu10* locus identified here represents a promising candidate for further validation and incorporation into breeding strategies.

Three significant SNPs associated with AHN were also identified in the MAGIC population on chromosomes *Vu05* and *Vu1*0. Two SNPs, 2_43843 and 2_49489, exhibited positive effects, whereas SNP 2_40705 showed a negative effect, indicating an association with reduced seed damage. Collectively, these loci explained 6.3–8.6% of the phenotypic variance, suggesting that the number of seed holes is influenced by several genomic regions, with individual loci making relatively modest contributions to phenotypic variation. This finding is consistent with Miesho *et al.* (2019), who identified SNPs associated with the number of holes and other bruchid-resistance traits in cowpea. Kpoviessi *et al*. (2022) likewise detected QTNs associated with mean number of holes using multi-locus GWAS, further supporting the genetic basis of variation in seed damage caused by bruchid infestation.

Candidate-gene analysis identified loci on *Vu10* associated with defence, stress and metabolic pathways, including Vigun10g191800, which may contribute to mechanisms involved in plant defence, detoxification or seed biochemical responses to insect infestation. Additional candidate genes identified on Vu03 included Vigun03g290200 and Vigun03g290400, which contain SANT and MYB-binding domains, respectively, suggesting possible roles in transcriptional regulation of defence-related processes. Vigun03g006100 may also be involved in glycosylation and hormone-related processes, which are important for plant development and stress responses. Furthermore, the identification of cytochrome P450-related genes and mediator-complex components suggests that multiple regulatory and metabolic pathways may contribute to cowpea responses to bruchid infestation. These candidate-gene annotations should, however, be regarded as hypotheses requiring experimental validation rather than definitive evidence of gene function. The proximity of a significant SNP to an annotated gene does not necessarily establish that the gene is causal. Expression analysis, fine-mapping, gene editing and functional assays will therefore be important for confirming the roles of these candidate genes.

Overall, the present study identifies several genomic regions and candidate SNP markers associated with important components of bruchid resistance in cowpea. The detection of significant loci for PWL, NEI, GI, DSI and AHN across multiple chromosomes reinforces the quantitative and genetically complex nature of resistance. The identified markers, particularly

those consistently detected across multiple GWAS models, provide promising targets for further validation and potential integration into MAS and genomic selection. However, validation across independent populations, environments and genetic backgrounds is essential before their routine deployment in breeding programmes. Ultimately, these findings provide a valuable foundation for developing cowpea cultivars with improved resistance to storage bruchids, with potential benefits for grain quality, post-harvest stability, food security and sustainable pest management, particularly in cowpea-producing regions such as Uganda.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 GENERAL DISCUSSION

Integrating phenotypic evaluation with genomic analysis provides a comprehensive approach to understanding the genetic basis of bruchid resistance in cowpea (*Vigna unguiculata*). Phenotypic screening identifies resistant genotypes, while genome-wide association studies (GWAS) help identify genomic regions associated with resistance-related traits. Combining these approaches can support the development of cowpea varieties with improved resistance to Callosobruchus maculatus, a major storage pest that reduces seed quality and quantity.

The phenotypic evaluation identified four promising resistant genotypes: WC10, MAGIC251, MAGIC060 and MAGIC113. These genotypes consistently recorded low values for number of eggs (NE), number of emerged insects (NEI), average number of holes (ANH), growth index (GI), percentage weight loss (PWL) and Dobie susceptibility index (DSI) across the two experimental batches. Their performance suggests reduced suitability for bruchid infestation and development. Similar findings on variation in resistance-related traits among grain legume genotypes have been reported by Keneni *et a*l. (2012). The consistency observed across experimental batches strengthens the potential of these genotypes as resistance sources. However, further evaluation against different bruchid populations and under varying storage conditions is necessary to establish the stability of their resistance. These genotypes could therefore serve as potential donor parents in breeding programmes aimed at improving cowpea storage resistance.

The identification of additional resistance sources is important because reliance on a limited number of resistant accessions may constrain long-term breeding progress. Incorporating WC10, MAGIC251, MAGIC060 and MAGIC113 into breeding programmes could broaden the genetic diversity available for bruchid resistance improvement. Previous studies have also demonstrated the importance of identifying and characterising cowpea germplasm for resistance to *C. maculatus* (Miesho *et al*., 2019; Kpoviessi *et al*., 2022). Nevertheless, the selected genotypes should also be evaluated for agronomic performance, seed quality and other desirable characteristics before their use in variety development.

The genomic analysis identified ten single-nucleotide polymorphism (SNP) markers significantly associated with bruchid resistance-related traits on chromosomes *Vu03, Vu04*, *Vu05, Vu09 and Vu10*. These associations are involved in percentage seed weight loss, growth index, number of emerged insects, Dobie susceptibility index and average number of holes. The findings suggest that resistance is influenced by multiple genomic regions. This agrees with Miesho *et al*. (2019), who identified SNP markers associated with cowpea bruchid resistance, and Kpoviessi *et al*. (2022), who reported resistance-associated genomic regions using GWAS approaches. Unzimai *et al*. (2024) also investigated quantitative trait loci associated with bruchid resistance in cowpea, further demonstrating the value of genomic approaches in identifying useful resistance regions.

Chromosomes *Vu03* and *Vu10* were particularly noteworthy because they contained markers associated with multiple resistance-related traits. The co-localisation of associations involving NEI, DSI, GI and ANH suggests that some genomic regions may influence related components of resistance. However, these patterns could result from pleiotropy, linkage between neighbouring genes or multiple causal variants. Further validation is therefore required to establish the genetic relationships underlying these associations. Markers that demonstrate consistent effects in independent populations could eventually support marker-assisted selection, reducing reliance on repeated phenotypic screening alone.

Candidate-gene analysis identified genes annotated as being involved in plant defence, detoxification, stress responses, transcriptional regulation and metabolism. These functions are potentially relevant to plant–insect interactions. For example, protease inhibitors may interfere with insect digestion, while cytochrome P450 enzymes participate in the metabolism of diverse compounds. Transcriptional regulators may also influence the expression of genes involved in plant defence. Similar approaches have been used to identify candidate genes associated with cowpea bruchid resistance (Miesho *et al*., 2019; Kpoviessi *et al*., 2022). However, the functions of the candidate genes identified in this study remain putative and require further confirmation through gene-expression analysis, functional characterisation and biochemical studies.

Overall, the phenotypic and genomic findings provide complementary information for improving cowpea resistance to bruchids. The four selected genotypes represent promising resistance sources, while the associated SNP markers and candidate genes provide

opportunities for further genetic investigation. Future research should validate the markers in independent breeding populations, determine whether the selected genotypes carry favourable alleles at the associated loci, and investigate the functional roles of prioritised candidate genes. These efforts could contribute to the development of cowpea varieties with more stable bruchid resistance, reduced post-harvest losses and improved seed quality.

5.2 CONCLUSIONS

The study established that four cowpea genotypes, namely WC10, MAGIC251, MAGIC060, and MAGIC113, exhibited strong and consistent resistance to bruchid infestation based on multi-trait evaluation across the two experimental batches; In contrast, MAGIC044, MAGIC179, and MAGIC091 were the most susceptible to bruchid infestation among the tested cowpea genotypes. The resistant genotypes demonstrated desirable levels of resistance across the evaluated parameters, suggesting their potential as useful sources of resistance for cowpea improvement programmes.

The study further identified ten SNPs that showed significant associations with key bruchid resistance-related traits, including percentage seed weight loss, number of eggs laid, growth index, number of exit holes, and Dobie susceptibility index. These significant marker–trait associations indicate substantial genetic control of the evaluated resistance parameters and provide useful genomic information for further genetic studies and breeding applications.

Markers located on chromosomes *Vu03* and *Vu10* were consistently associated with multiple bruchid resistance-related traits, highlighting these genomic regions as important regions underlying bruchid resistance in cowpea. In particular, SNPs 2_14396, 2_23649, and 2_49489 were identified as promising candidate markers for further validation and potential application in marker-assisted breeding programmes aimed at developing cowpea varieties with improved resistance to bruchid infestation.

In addition, eleven candidate genes associated with resistance-related mechanisms were identified within the significant genomic regions. The genomic regions linked to average number of holes (ANH), percentage weight loss (PWL), growth index (GI), number of emerged insect/bruchids (NEI), and Dobie susceptibility index (DSI) have been identified. Their identification provides a basis for further functional validation to elucidate their specific roles in bruchid resistance and their potential application in cowpea genetic

improvement. They provide promising targets for marker-assisted breeding for durable resistance.

5.3 RECOMMENDATIONS

1. WC10, MAGIC251, MAGIC060, and MAGIC113 have been identified as cowpea genotypes with resistance to *Callosobruchus maculatus*. Future studies should focus on validating these genotypes under diverse environmental conditions and storage systems. Detailed investigations into biochemical and physiological resistance mechanisms such as seed coat characteristics and defense related compounds are necessary. Gene expression profiling during pest infestation will further elucidate resistance pathways. Long-term studies should also assess the stability and durability of resistance across generations.
2. From a genomic perspective, priority should be given to the validation of identified SNPs and functional characterization of candidate genes. Comparative genomic analyses with other legume species may reveal conserved resistance mechanisms. The development of reliable molecular markers will support marker-assisted selection and genomic selection, accelerating breeding progress.
3. Investigate resistance mechanisms, including seed coat characteristics, defence compounds, and gene expression during bruchid infestation.
4. Collectively, these efforts will contribute to the development of cowpea varieties with durable resistance to bruchids, reducing post-harvest losses, enhancing food security, and promoting sustainable pest management in cowpea-producing regions.

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APPENDICES

Appendix 1: The plant materials used in the study and their mean performance for both batches

S/N	Genotypes	ISW	RSW	WTL	PWL	NE	NEI	%NEI	MDP	GI	DSI	ANH
1	MAGIC001	1.35	0.74	0.61	44.17	57.5	28.00	61.06	23.83	2.54	6.01	32.67
2	MAGIC003	1.43	1.00	0.43	31.83	59.83	26.17	53.44	23	2.24	5.15	19.17
3	MAGIC005	1.9	1.24	0.66	36.67	62.5	15.00	25.7	24.67	1.08	4.24	29.17
4	MAGIC006	1.36	0.93	0.43	35.67	39.67	10.67	30.34	24.83	1.25	3.95	26.67
5	MAGIC010	1.52	0.93	0.6	40.00	72.5	24.00	37.81	25.67	1.43	5.31	29.67
6	MAGIC011	1.31	0.91	0.41	32.17	29.83	10.67	51.86	22.67	2.23	4.13	17.67
7	MAGIC013	1.38	0.93	0.45	35.83	47.83	18.33	52.48	22.83	2.26	5.25	24.33
8	MAGIC015	1.72	1.17	0.55	33.83	48.33	16.00	42.27	23.5	1.8	4.27	29.00
9	MAGIC016	1.53	0.98	0.55	36.83	77.33	26.00	47.62	24.67	1.89	5.71	35.83
10	MAGIC017	1.62	1.06	0.57	36.83	69.5	22.67	33.18	23.5	1.39	4.93	31.33
11	MAGIC019	1.78	1.1	0.68	38.00	53.33	23.00	48.1	25.67	1.88	4.32	33.83
12	MAGIC020	1.55	0.94	0.62	40.67	69.00	24.00	38.72	24.5	1.57	4.82	29.17
13	MAGIC021	1.34	0.75	0.6	45.67	72.83	31.83	48.24	22.83	2.1	6.24	29.67
14	MAGIC024	1.35	0.79	0.56	41.67	58.33	20.83	46.55	24.00	1.84	5.09	28.5
15	MAGIC025	1.48	0.94	0.54	37.83	71.17	14.00	22.19	25.83	0.91	3.65	24.00
16	MAGIC027	1.59	1.09	0.5	31.17	74.33	20.67	37.22	22.00	1.67	5.54	18.00
17	MAGIC028	1.37	0.89	0.48	35.67	64.33	14.00	25.15	24.17	1.06	4.74	30.67
18	MAGIC029	1.33	0.78	0.55	42.33	77.17	16.67	27.53	23.67	1.16	4.65	27.5
19	MAGIC030	1.57	0.9	0.67	43.67	77.83	28.33	46.9	24.5	1.82	5.72	29.33
20	MAGIC032	1.48	1.00	0.48	29.83	55.5	23.5	52.51	24.83	2.11	5.53	26.67
21	MAGIC034	1.67	1.09	0.58	36.67	61.17	25.00	53.91	24.33	2.24	5.34	31.5
22	MAGIC036	1.5	0.88	0.62	42.67	73.00	23.5	40.3	26.5	1.56	5.12	29.00
23	MAGIC039	1.98	1.33	0.65	32.67	55.5	17.5	28.36	23.83	1.17	4.21	26.67
24	MAGIC041	1.56	1.02	0.54	37.5	50.33	17.5	39.52	24.00	1.63	3.85	26.83
25	MAGIC043	1.68	0.82	0.87	51.17	73.33	31.17	45.08	23.83	1.88	6.08	32.67
26	MAGIC044	1.59	0.88	0.72	43.83	90.00	28.17	40.38	22.67	1.77	6.3	44.83
27	MAGIC046	1.14	0.65	0.49	42.67	63.67	19.00	35.19	23.00	1.53	5.24	28.67
28	MAGIC048	1.57	1.23	0.35	24.5	39.5	12.17	32.1	22.5	1.44	3.43	22.83
29	MAGIC049	1.62	1.17	0.46	33.33	75.00	18.67	22.45	23.83	0.96	3.72	26.33
30	MAGIC051	1.64	1.24	0.4	24.33	29.00	15.83	51.26	24.00	2.09	3.63	23.17
31	MAGIC057	2.04	1.5	0.53	27.67	73.5	15.83	19.91	24.17	0.83	4.64	28.5
32	MAGIC060	1.55	1.25	0.3	23.83	55.83	7.5	14.02	24.83	0.58	2.69	23.00
33	MAGIC061	1.52	0.9	0.62	38.83	77.83	20.33	27.28	23.67	1.16	4.9	31.00
34	MAGIC062	1.66	1.05	0.61	40.67	65.17	23.00	37.99	24.5	1.52	5.19	28.17
35	MAGIC064	1.32	0.99	0.33	32.33	48.33	18.67	39.66	23.17	1.68	4.98	26.5
36	MAGIC065	1.48	0.91	0.57	34.67	53.33	17.5	38.35	23.83	1.66	5.04	41.17

37	MAGIC066	1.68	1.17	0.52	34.5	65.00	18.17	34.77	24.33	1.42	5.01	27.5
38	MAGIC068	1.48	0.96	0.53	37.5	71.33	22.67	38.87	23.67	1.68	5.26	29.33
39	MAGIC070	1.41	1.00	0.41	29.17	49.00	12.5	34.71	23.5	1.42	3.36	23.5
40	MAGIC072	1.56	1.36	0.2	15.17	23.83	11.83	43.9	24.83	1.74	2.67	18.17
41	MAGIC074	1.63	1.08	0.55	33.83	42.83	16.17	44.99	22.67	2.00	4.28	24.00
42	MAGIC075	1.46	0.84	0.62	40.67	83.67	24.5	46.09	23.67	2.06	5.89	36.83
43	MAGIC076	1.45	0.94	0.51	35.17	64.00	24.00	43.00	22.67	1.9	5.7	26.00
44	MAGIC078	1.27	0.77	0.5	40.17	75.17	21.83	25.84	24.83	1.03	4.76	28.33
45	MAGIC081	1.34	0.96	0.38	30.33	49.00	14.67	35.26	22.83	1.57	4.24	16.33
46	MAGIC085	1.61	1.00	0.6	38.00	85.00	26.17	37.04	27.17	1.38	4.72	34.5
47	MAGIC086	1.67	0.81	0.86	49.00	60.67	23.83	44.44	23.67	1.86	5.76	32.17
48	MAGIC087	1.54	0.82	0.72	46.5	87.33	23.67	29.67	25.17	1.2	5.15	39.83
49	MAGIC088	1.9	1.23	0.67	37.00	54.00	20.67	38.6	26.17	1.43	4.38	30.00
50	MAGIC089	1.47	1.00	0.47	32.83	56.00	22.33	46.29	25.17	1.81	5.27	28.17
51	MAGIC090	1.62	0.95	0.67	43.17	79.67	24.00	31.17	23.00	1.33	4.99	33.17
52	MAGIC091	1.51	0.78	0.74	46.83	80.67	38.5	55.16	24.00	2.27	6.59	42.17
53	MAGIC092	1.21	0.85	0.36	28.33	60.00	15.17	27.53	24.67	1.14	4.52	25.5
54	MAGIC095	1.59	0.93	0.67	43.00	73.17	15.67	28.93	23.67	1.27	4.41	27.5
55	MAGIC096	1.77	1.1	0.67	38.33	76.17	18.17	28.76	24.17	1.2	5.03	34.67
56	MAGIC097	1.45	0.95	0.5	39.83	55.83	15.83	38.19	23.00	1.68	5.11	29.17
57	MAGIC098	1.59	1.19	0.4	23.67	48.00	12.00	30.7	23.33	1.34	4.52	25.33
58	MAGIC099	1.54	1.02	0.52	34.17	68.83	15.5	29.25	23.67	1.2	3.85	21.33
59	MAGIC100	1.46	0.83	0.63	42.33	49.67	21.5	58.85	23.00	2.58	5.54	35.00
60	MAGIC104	1.51	0.94	0.57	37.67	74.17	27.33	39.75	24.83	1.52	4.79	31.00
61	MAGIC105	1.67	1.01	0.67	39.5	86.33	12.33	18.67	23.83	0.83	3.08	34.5
62	MAGIC106	1.51	0.91	0.6	40.33	68.83	27.67	42.69	24.5	1.71	5.54	26.67
63	MAGIC107	1.2	0.86	0.35	28.17	56.17	20.67	42.93	26.5	1.54	4.54	19.00
64	MAGIC113	1.41	1.01	0.4	31.17	54.33	5.33	11.33	24.17	0.47	2.75	19.00
65	MAGIC114	1.54	1.09	0.45	29.00	56.00	22.17	48.82	24.17	1.99	4.91	24.17
66	MAGIC115	1.44	0.74	0.7	48.5	90.83	28.83	42.00	24.5	1.83	6.02	36.67
67	MAGIC116	1.44	0.82	0.62	44.00	92.33	29.5	33.38	24.33	1.4	5.73	38.33
68	MAGIC117	1.47	0.96	0.51	35.33	59.5	20.33	40.57	23.33	1.67	5.02	25.17
69	MAGIC119	1.48	0.86	0.62	42.00	73.67	14.00	21.43	24.67	0.93	3.77	28.33
70	MAGIC120	1.87	0.9	0.97	52.00	83.83	24.5	28.91	24.83	1.17	5.00	44.17
71	MAGIC125	1.41	0.94	0.47	34.17	43.17	15.5	38.21	23.00	1.61	4.24	19.17
72	MAGIC126	1.58	0.97	0.61	40.17	70.33	20.33	28.77	23.83	1.22	4.45	37.00
73	MAGIC129	1.46	1.06	0.39	28.17	51.83	15.5	36.29	24.83	1.48	4.45	24.83
74	MAGIC130	1.89	1.31	0.58	32.17	76.5	16.83	21.81	24.00	0.87	4.15	31.5
75	MAGIC132	1.37	0.7	0.67	46.83	81.33	23.00	30.37	25.00	1.23	5.22	35.83
76	MAGIC134	1.69	1.25	0.44	27.67	64.83	15.00	42.76	26.33	1.69	4.28	30.5
77	MAGIC136	1.75	1.31	0.43	27.83	49.33	26.33	53.52	23.83	2.22	5.01	21.00

78	MAGIC139	1.58	1.05	0.53	34.17	76.67	24.83	38.12	25.83	1.44	4.78	29.5
79	MAGIC140	1.47	0.84	0.63	43.5	83.33	24.5	31.54	25.67	1.29	5.23	39.5
80	MAGIC141	1.63	1.13	0.5	32.17	74.5	25.67	37.74	22.5	1.69	6.22	28.33
81	MAGIC144	1.62	0.99	0.63	38.33	91.5	22.5	25.93	26.17	0.99	5.11	34.83
82	MAGIC147	1.44	1.06	0.37	25.67	67.00	28.33	47.79	25.17	1.86	5.36	33.00
83	MAGIC148	1.48	0.71	0.77	50.5	83.17	26.00	39.35	23.67	1.73	5.81	35.5
84	MAGIC149	1.62	1.26	0.36	26.5	75.83	20.00	26.4	23.33	1.13	4.75	27.67
85	MAGIC153	1.48	0.99	0.49	37.67	69.00	21.83	31.7	25.33	1.23	4.93	30.17
86	MAGIC154	1.6	0.88	0.72	44.17	106.3	32.83	30.85	24.5	1.28	6.15	46.83
87	MAGIC157	1.62	0.91	0.71	43.83	95.00	23.83	26.44	25.5	1.04	5.17	34.00
88	MAGIC158	1.39	0.88	0.51	38.33	73.33	28.17	41.32	25.17	1.69	5.73	27.17
89	MAGIC162	1.44	0.9	0.53	37.33	66.17	13.5	26.33	25.00	1.13	3.89	28.17
90	MAGIC163	1.56	1.03	0.53	32.17	73.83	29.00	39.29	24.00	1.59	5.18	36.33
91	MAGIC166	1.55	1.1	0.45	29.17	52.83	18.5	37.22	26.17	1.43	3.93	19.67
92	MAGIC167	1.56	0.89	0.67	42.83	63.00	24.5	53.39	24.17	2.22	5.53	30.33
93	MAGIC169	1.37	0.87	0.5	36.67	66.67	22.33	40.62	23.17	1.79	5.79	25.67
94	MAGIC173	1.48	0.86	0.61	42.83	64.83	24.83	39.56	23.83	1.59	5.01	35.67
95	MAGIC174	1.64	1.18	0.47	30.00	46.00	14.17	27.77	24.5	1.1	3.95	25.33
96	MAGIC176	1.39	0.76	0.63	45.5	58.00	23.5	37.08	24.67	1.47	4.68	27.33
97	MAGIC178	1.58	1.05	0.53	34.00	57.83	28.5	54.58	26.00	2.11	4.45	30.17
98	MAGIC179	1.67	0.98	0.68	42.83	72.17	32.83	51.11	23.67	2.16	6.34	43.83
99	MAGIC180	1.46	1.00	0.46	32.5	48.17	21.5	54.66	27.00	2.05	4.74	22.33
100	MAGIC183	1.54	0.86	0.68	43.00	78.00	29.17	51.94	25.5	2.2	5.62	38.5
101	MAGIC184	1.33	0.93	0.4	31.17	57.5	23.5	46.12	25.5	1.8	5.26	20.33
102	MAGIC185	1.25	0.79	0.46	36.5	41.83	18.67	49.3	23.33	2.14	4.76	26.83
103	MAGIC190	1.84	1.04	0.8	43.67	80.83	29.67	35.22	24.00	1.52	5.78	29.33
104	MAGIC191	1.74	1.29	0.46	28.5	62.00	20.5	41.15	25.00	1.66	4.28	24.5
105	MAGIC192	1.57	1.05	0.52	31.17	51.83	24.17	57.49	24.67	2.3	5.56	26.00
106	MAGIC194	1.6	1.06	0.54	33.67	55.67	28.5	53.91	25.33	2.06	5.23	30.83
107	MAGIC196	1.69	1.3	0.39	25.17	42.67	21.17	54.21	22.17	2.44	4.05	25.67
108	MAGIC197	1.39	0.94	0.45	35.83	67.17	32.17	62.44	25.17	2.49	5.72	30.67
109	MAGIC199	1.47	1.02	0.45	31.00	53.5	20.83	51.29	27.67	1.81	3.95	17.5
110	MAGIC200	1.7	1.32	0.38	20.67	47.5	22.5	46.15	22.83	2.02	4.27	12.00
111	MAGIC201	1.68	1.18	0.5	31.33	37.00	16.33	45.78	25.00	1.79	4.56	24.17
112	MAGIC202	1.83	1.11	0.72	40.17	80.17	37.17	46.87	24.67	1.89	5.92	36.67
113	MAGIC204	1.5	1.09	0.4	26.83	53.33	17.5	34.87	24.67	1.35	3.62	31.00
114	MAGIC205	1.85	1.1	0.75	41.5	88.00	29.83	40.48	25.33	1.58	5.48	43.5
115	MAGIC207	1.22	0.66	0.56	46.17	70.17	23.83	51.39	23.00	2.25	5.99	30.5
116	MAGIC209	1.5	1.06	0.44	30.00	50.5	18.5	35.4	23.67	1.49	4.55	28.83
117	MAGIC211	1.85	1.07	0.78	42.33	85.33	25.67	35.93	24.17	1.52	5.33	40.33
118	MAGIC212	1.63	0.94	0.7	46.17	74.00	17.17	25.88	25.5	1.04	4.75	36.67

119	MAGIC216	1.71	1.1	0.62	36.17	65.5	25.00	38.73	26.5	1.48	5.08	41.17
120	MAGIC219	1.65	1.13	0.51	34.33	43.00	21.83	60.75	23.5	2.61	5.28	25.83
121	MAGIC220	1.26	0.8	0.46	34.67	45.00	16.00	47.99	23.17	2.08	5.1	27.67
122	MAGIC222	1.41	1.03	0.38	31.17	66.5	26.83	44.37	25.00	1.8	5.01	26.17
123	MAGIC223	1.6	1.16	0.44	30.33	42.5	12.67	24.28	23.83	1.03	3.25	29.17
124	MAGIC225	1.27	0.62	0.65	49.83	84.67	22.67	26.47	23.67	1.14	4.95	33.00
125	MAGIC226	1.35	0.83	0.51	41.5	64.33	14.5	20.59	24.5	0.85	3.89	31.67
126	MAGIC231	1.45	1.03	0.42	29.00	52.5	11.00	24.79	23.83	1.02	3.49	27.33
127	MAGIC232	1.53	0.85	0.68	44.33	89.00	27.17	29.8	26.00	1.14	5.25	38.17
128	MAGIC233	1.58	1.02	0.56	39.17	65.00	29.00	46.54	24.83	1.86	5.55	35.00
129	MAGIC234	1.56	1.3	0.26	17.00	45.5	17.83	50.96	23.67	2.17	4.44	14.83
130	MAGIC236	1.46	0.89	0.57	39.33	67.00	27.00	45.01	23.83	1.87	5.15	29.33
131	MAGIC238	1.14	0.8	0.35	31.17	48.83	17.17	39.9	24.83	1.62	4.28	23.67
132	MAGIC240	1.65	0.88	0.77	46.17	64.67	24.83	49.66	27.33	1.78	5.03	31.00
133	MAGIC241	1.21	0.76	0.44	34.83	46.00	17.5	51.49	24.00	2.24	5.03	23.67
134	MAGIC243	1.44	0.91	0.53	37.33	47.5	21.00	46.54	23.00	2.02	5.75	30.5
135	MAGIC245	1.63	0.96	0.67	41.83	93.33	31.67	42.01	26.33	1.64	5.66	35.17
136	MAGIC249	1.16	0.78	0.37	32.17	55.67	22.5	46.53	24.33	1.83	4.68	22.67
137	MAGIC250	1.65	1.16	0.49	31.5	82.83	28.00	43.14	24.33	1.66	5.86	27.33
138	MAGIC251	1.37	1.14	0.22	22.17	31.17	6.00	26.17	25.83	1.01	2.32	12.33
139	MAGIC256	1.67	1.13	0.54	31.00	40.17	19.00	50.4	24.33	1.98	4.16	31.17
140	MAGIC258	1.38	0.76	0.63	45.83	72.5	25.33	41.09	25.33	1.71	5.58	33.5
141	MAGIC260	1.48	0.82	0.67	46.17	50.17	18.5	39.18	24.67	1.65	5.01	30.83
142	MAGIC263	1.37	1.06	0.32	27.5	48.83	19.67	38.37	24.5	1.52	3.75	21.5
143	MAGIC266	1.3	0.77	0.53	39.5	47.17	25.83	60.49	23.83	2.6	5.84	28.83
144	MAGIC267	1.73	1.09	0.64	37.33	75.17	28.67	43.54	23.00	1.9	5.36	30.67
145	MAGIC270	1.22	0.78	0.44	36.33	43.5	26.67	58.91	22.5	2.61	5.24	26.83
146	MAGIC272	1.36	0.87	0.49	35.67	53.67	20.83	44.57	23.17	1.89	5.36	16.67
147	MAGIC273	1.46	0.65	0.81	55.00	76.83	30.33	42.99	27.00	1.59	5.39	38.33
148	MAGIC275	1.69	1.11	0.58	37.67	78.67	25.00	37.44	24.5	1.55	5.57	30.83
149	MAGIC276	1.4	0.95	0.45	31.67	52.5	21.33	47.91	23.67	1.98	5.38	23.5
150	MAGIC277	1.54	0.93	0.61	40.83	60.00	26.33	53.65	22.83	2.38	5.9	35.5
151	MAGIC278	1.57	0.96	0.61	38.67	54.00	26.17	57.49	25.17	2.29	5.7	32.83
152	MAGIC279	1.07	0.72	0.35	34.83	54.5	22.17	43.96	23.83	1.82	4.56	17.83
153	MAGIC281	1.64	1.12	0.52	29.83	37.17	13.5	31.54	23.33	1.38	3.67	25.33
154	MAGIC282	1.09	0.57	0.53	46.67	50.67	22.83	49.82	23.00	2.18	5.79	28.00
155	MAGIC283	1.58	1.15	0.43	32.83	65.17	18.83	39.07	24.00	1.58	5.12	22.67
156	MAGIC284	1.23	0.84	0.39	32.33	60.33	7.33	16.42	25.5	0.66	2.9	19.83
157	MAGIC286	1.67	1.02	0.65	40.33	82.33	33.5	41.64	24.17	1.68	5.86	39.83
158	MAGIC287	1.31	0.82	0.49	38.67	45.33	19.00	44.38	24.67	1.78	4.22	21.33
159	MAGIC292	1.38	0.79	0.59	42.33	43.83	26.33	65.01	25.83	2.57	4.9	28.00

160	MAGIC293	1.7	1.18	0.53	33.83	75.83	29.00	44.09	25.67	1.76	5.48	31.83
161	MAGIC296	1.44	0.92	0.53	37.00	77.67	19.67	33.72	23.17	1.45	5.00	24.83
162	MAGIC297	1.63	1.04	0.59	39.17	65.33	27.00	46.75	24.00	1.9	5.72	29.33
163	MAGIC298	1.47	0.98	0.49	40.00	76.17	28.5	38.47	25.33	1.51	5.08	26.67
164	MAGIC300	1.57	1.01	0.56	35.00	63.17	24.83	39.32	25.00	1.64	4.12	32.00
165	MAGIC301	1.11	0.81	0.3	41.17	70.5	17.17	32.27	23.5	1.33	4.33	27.00
166	MAGIC302	1.44	0.91	0.54	37.67	67.33	24.5	35.7	26.00	1.39	5.04	31.67
167	MAGIC303	1.52	0.98	0.53	39.17	64.83	23.17	45.13	25.00	1.72	4.87	28.33
168	MAGIC305	1.24	0.92	0.32	28.5	46.17	13.67	32.72	25.67	1.28	3.89	19.33
169	MAGIC311	1.65	0.99	0.66	36.33	54.67	22.00	46.52	25.33	1.81	5.32	32.83
170	MAGIC313	1.35	0.91	0.45	34.5	45.17	19.00	45.8	24.83	1.84	4.95	23.67
171	MAGIC314	1.58	1.12	0.46	27.83	65.33	16.00	29.48	25.17	1.17	4.09	35.17
172	MAGIC315	1.62	1.18	0.45	31.33	68.67	17.67	24.48	25.5	0.99	4.37	25.17
173	MAGIC317	1.52	1.03	0.49	32.5	41.17	28.17	73.87	23.67	3.15	5.93	29.67
174	MAGIC320	1.58	0.99	0.59	37.67	72.83	22.00	36.29	23.33	1.61	5.45	35.00
175	MAGIC324	1.58	1.03	0.55	36.5	68.33	22.83	37.05	24.67	1.53	5.38	30.83
176	MAGIC325	1.81	1.25	0.56	32.5	70.17	16.00	22.26	24.00	0.92	4.04	29.83
177	MAGIC326	1.41	1.03	0.38	28.17	85.17	17.33	29.87	24.83	1.19	4.55	22.00
178	MAGIC327	1.71	1.04	0.67	38.17	75.00	25.67	32.99	25.33	1.29	5.22	35.83
179	MAGIC328	1.67	1.01	0.66	43.00	42.00	23.67	57.98	22.5	2.61	5.8	25.17
180	MAGIC331	1.66	1.08	0.58	36.67	72.67	22.17	38.17	22.67	1.71	5.73	26.83
181	MAGIC333	1.58	0.91	0.67	42.33	68.83	29.83	43	24.00	1.87	6.17	40.00
182	MAGIC335	1.4	0.86	0.54	42.00	55.5	16.33	26.48	23.17	1.15	4.35	24.17
183	MAGIC336	1.45	0.85	0.59	36.33	91.5	22.83	24.3	24.67	0.99	4.57	30.83
184	IT84S-2246	1.62	0.92	0.7	40.33	69.5	24.17	43.26	25.00	1.71	5.5	44.00
185	IT93K-503-1	1.57	0.97	0.6	35.83	61.83	19.00	38.51	24.17	1.61	4.81	31.83
186	IT89KD-288	1.38	0.78	0.6	44.00	63.83	25.33	43.25	25.00	1.7	4.72	26.5
187	IT82E-18	1.33	0.81	0.52	40.33	81.83	26.5	38.15	23.00	1.68	5.99	33.5
188	NAROCP 2	1.36	0.8	0.56	40.5	61.67	15.33	32.67	24.00	1.38	4.62	29.33
189	NAROCP 3	1.11	0.82	0.29	27.83	29.67	15.83	45.68	24.33	1.89	3.5	18.67
190	NE15	1.41	0.81	0.6	42.00	78.5	21.83	28.66	26.33	1.06	4.72	31.83
191	NE 50	1.66	1.09	0.57	34.83	65.67	25.5	38.00	24.17	1.49	4.84	31.17
192	NE 70	1.19	0.88	0.32	31.17	38.67	12.83	39.00	23.33	1.67	3.31	16.00
193	WC 42.	1.18	0.7	0.49	42.33	87.33	26.83	35.08	26.83	1.28	5.32	28.83
194	WC 29	1.38	0.96	0.42	29.17	51.83	9.83	19.94	23.17	0.89	3.86	21.00
195	WC 36	1.25	0.58	0.67	52.00	73.00	17.17	28.66	23.33	1.24	5.11	32.67
196	SECOW 5T	1.08	0.7	0.38	37.67	54.00	26.33	60.05	23.83	2.5	5.92	28.83
197	SUNSHINE	1.41	0.88	0.53	35.5	52.83	15.67	27.91	23.00	1.21	4.52	24.33
198	LG 083	1.3	0.89	0.41	34.5	49.5	22.17	47.13	24.83	1.85	5.01	19.5
199	LG 093	1.04	0.61	0.43	40.5	51.67	11.5	30.45	24.67	1.25	3.39	21.00
200	LGC085	1.41	0.78	0.63	45.67	67.83	21.83	40.39	25.33	1.63	5.05	26.67

201	WC 10	1.26	0.98	0.29	23.67	34.5	4.67	11.14	25.17	0.43	1.44	18.5
202	SEC 6WST	1.19	0.85	0.34	30.17	48.17	22.67	51.92	24.00	2.14	5.29	23.67
203	NAROCOWPEA-1	1.32	0.74	0.58	42.33	74.67	22.67	33.29	25.5	1.3	5.27	31.67
204	NAROCOWPEA-2	1.22	0.72	0.5	42.00	78.17	28.17	35.04	25.83	1.35	5.37	29.17
205	NAROCOWPEA-3	1.15	0.7	0.45	37.83	47.67	20	46.57	23.17	1.95	5.16	28.67
206	NAROCOWPEA-4	1.22	0.9	0.33	26.67	46.83	11.17	40.77	23.83	1.73	4.15	19.83
207	NE15	1.45	1.38	0.08	8.33	66.00	24.5	35.6	22.17	1.59	5.33	25.00
208	IT 84s-2246 Check	1.11	0.61	0.5	43.67	46.33	14.17	35.67	23.33	1.55	4.46	21.17
209	2419 Check	0.99	0.61	0.37	38.00	41.00	21.00	48.83	24.00	2.01	4.27	24.17
210	WC-42 Check	1.39	0.79	0.61	43.00	77.33	24.33	34.41	26.67	1.25	4.37	29.83