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GENOME-WIDE ASSOCIATION STUDY REVEALS SINGLE NUCLEOTIDE POLYMORPHISMS AND CANDIDATE GENES FOR RESISTANCE OF COWPEA TO APHIDS

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp.) is a major crop grown mainly in the arid and semi-arid regions for food and nutritional security. Its production, however, is generally hampered by its susceptibility to sap-sacking aphids (*Aphis craccivora* Koch). Resistance breeding for cowpea improvement against aphids, has been limited by inadequate understanding of genes responsible for resistance to this cosmopolitan pest. The objective of this study was to identify single nucleotide polymorphisms (SNPs) and candidate genes, associated with resistance of cowpea to aphids. The study evaluated 209 genotypes of the multi-parent advanced generation intercross (MAGIC) population, together with 5 MAGIC parents, cross three different locations for two seasons in Uganda. Significant genetic variation ($P<0.001$) for aphid resistance was detected in this germplasm. Results revealed three stable and significant SNPs, including *2_30668*, *2_43528* and *2_43747;* being associated with resistance to aphids. Eleven candidate genes were detected within the significant loci; including 7 genes on chromosome I (*Vu01*), 3 on chromosome VII (*Vu07*) and 1 on chromosome IX (*Vu09*). These putative genes have functions related to host plant resistance and plant defence responses, possibly against cowpea aphids. The significant SNP markers and genes reported may be deployed in marker-assisted breeding strategy, for faster development of aphid resistant cowpea varieties in Uganda.

Key Words: *Aphis craccivora*, genes, SNPs, Uganda, *Vigna unguiculata*

RÉSUMÉ

Le niébé (*Vigna unguiculata* L. Walp.) est une plante cultivée de grande importance, cultivée principalement dans les régions arides et semi-arides, pour assurer la sécurité alimentaire et nutritionnelle. Sa production est toutefois généralement entravée par sa sensibilité aux pucerons suceurs de sève (*Aphis craccivora* Koch). La sélection de la résistance pour l'amélioration du niébé contre les pucerons a été limitée par une compréhension insuffisante des gènes responsables de la résistance à ce ravageur cosmopolite. L'objectif de cette étude était d'identifier les polymorphismes d'un seul nucléotide (SNPs) et les gènes candidats associés à la résistance du niébé aux pucerons. L'étude a évalué 209 génotypes de la population MAGIC (multi-parent advanced generation intercross), ainsi que 5 parents MAGIC croisés dans trois endroits différents pendant deux saisons en Ouganda. Une variation génétique significative (P<0,001) pour la résistance aux pucerons a été détectée dans ce germoplasme. Les résultats ont révélé trois SNPs stables et significatifs, dont 2_30668, 2_43528 et 2_43747, associés à la résistance aux pucerons. Onze gènes candidats ont été détectés dans les loci significatifs, dont 7 gènes sur le chromosome I (*Vu01*), 3 sur le chromosome VII (*Vu07*) et 1 sur le chromosome IX (*Vu09*). Ces gènes putatifs ont des fonctions liées à la résistance de la plante hôte et aux réponses de défense de la plante, peut-être contre les pucerons du niébé. Les marqueurs SNP significatifs et les gènes rapportés peuvent être déployés dans une stratégie de sélection assistée par marqueurs, pour un développement plus rapide de variétés de niébé résistantes aux pucerons en Ouganda.

Mots Clés : *Aphis craccivora*, gènes, SNPs, Ouganda, *Vigna unguiculata*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), is a major crop grown mainly in tropical regions for food and nutritional security (Lonardi *et al*., 2019). The crop is well adapted to arid and semi-arid environments, owing to its high tolerance to drought conditions (Nunes *et al*., 2022). It is a major source of protein for human consumption (Dakora and Belane, 2019) and fodder for livestock (Boukar *et al*., 2016).

However, its production is heavily hindered by insect pests such as aphids (*Aphis craccivora* Koch) (Kityo *et al*., 2021). Aphids, apart from inflicting severe yield-reducing damages to the crop, also serve as vectors of several viruses of economic importance, including blackeye cowpea mosaic virus (BCMV) and cowpea aphid borne mosaic virus (CABMV) (Boa, 2014; MacWilliams *et al*., 2020). The pest causes considerable crop losses particularly at seedlings stage. Yield losses of 20 to 40% in cowpea have been reported in Asia and up to 35% in Africa have been estimated (Annan *et al*., 2000).

Recent efforts have been made to tap into genetic advances, through the cowpea breeding programmes at the International Institute of Tropical Agriculture (IITA) (Boukar *et al*., 2018). Although the results of phenotypic tests and molecular marker detection agreed in most cases, molecular markers detection is more reliable in identifying genotypes for resistance to cowpea aphid (Mofokeng and Gerrano, 2021). For instance, Bao-Lam *et al*. (2015) reported cowpea aphid resistance using field-based screenings; and consistently mapped significant loci on linkage Group 1 and Group 7. Through research efforts, genome mapping has also been used to identify markers associated with resistance of cowpea to aphids (Ongom *et al*., 2024).

Plant reactions to insect attack may depend on plant genotypes, insect biotypes and environmental factors (Mofokeng and Gerrano, 2021). In fact, the existence of aphid biotypes has been noted as a major challenge to successful breeding of cowpea cultivars that are resistant to aphids (Attamah *et al*., 2024). For instance, it was reported that aphid

biotypes in Ghana were more aggressive than those in Nigeria when an aphid resistant cowpea line from Nigeria (IT99k-499-35) was found to be susceptible to aphids in Ghana (Kusi *et al*., 2010). These observations emerge from high genotype by environment (G x E) interactions, and existence of aphid biotypes. There is incomprehensive understanding of genomic regions and genes associated with resistance of cowpea to aphids, which would otherwise counter infestation and the associated damage caused by the aphid pest and its biotypes in Uganda. As such, the molecular basis of the gene-mediated aphid resistance remains elusive (Ongom *et al*., 2022).

In recent years, there has been rapid development of next generation sequencing, high-throughput genotype data, together with phenotypic data for utilisation to identify marker-trait associations *via* genome-wide association studies (GWAS) (Varshney *et al*., 2020). Compared to linkage mapping, GWAS has emerged as a powerful tool for detecting markers (single nucleotide polymorphisms (SNPs)), closely linked to quantitative traits, based on the principle of linkage disequilibrium (LD) between genetic markers and QTL that affect the trait (Geng *et al*., 2015).

The statistical model adopted is one of the setbacks to the power of detection of significant markers in GWAS (Yoosefzadeh-Najafabadi *et al*., 2022). Traditional popular statistical models (single-marker genome-wide scan models), such as mixed linear model (MLM), and general linear model (GLM) among others, have a number of limitations in detecting marginal effects quantitative trait nucleotides (QTNs), influenced by the polygenic background and stringent Bonferroni correction (Wang *et al*., 2016); as well as stringent thresholds of significance and mapping power (Wen *et al*., 2018).

To overcome these limitations, several multi-locus models have been developed and utilised for GWAS in several crops (Berhe *et al*., 2021; Vikas *et al*., 2022). Among them is the multi-locus random-SNP-effect mixed linear model (mrMLM) (Wang *et al*., 2016), a fast mrMLM (FASTmrMLM) (Zhang *et al*., 2018), a fast mrMLM efficient mixed-model association (FASTmrEMMA) (Wen *et al*., 2018), polygene background-control-based least-angle regression plus empirical Bayes (pLARmEB) (Zhang *et al*., 2017), Kruskal-Wallis test with empirical Bayes under polygenic background control (pKWmEB) (Ren *et al*., 2018); and integrative sure independence screening expectation maximisation Bayesian Least Absolute Shrinkage and Selection Operator Model (ISIS EM-BLASSO) (Tamba *et al*., 2017). In fact, the multi-locus models have become the state-of-the-art procedure for identifying genetic bases for complex traits, due to their power of detection and robustness (Zhang *et al*., 2019).

Additionally, crop improvement exploits germplasm diversity to generate phenotypic variation for traits under selection (Dwived *et al*., 2017). Therefore, genetic and phenotypic characterisation of germplasm collections is critical to warrant the development of resilient crop varieties that can sustain production under future crop production pest stress challenges.

Genetic improvement of cowpea relies on diversity in the phenotypes and genotypes of parents and populations, as well as heritable and repeatable quantitative trait loci (QTL) (Pariyar *et al*., 2021). Common bi-parental mapping populations possess allelic diversity of two parents; whereas multi-parent advanced generation inter-cross (MAGIC) mapping populations have higher allelic diversity, higher levels of recombination, and their genomes form a fine-scale mosaic of genome diversity of several parental lines (Cavanagh, 2008).

Combined with high density molecular markers, sequence information and analysis models, MAGIC populations serve as a new generation of mapping populations for QTL and gene discovery (Pariyar *et al*., 2021). The objective of this study was to identify single nucleotide polymorphisms and candidate genes

associated with resistance of cowpea to aphids in Uganda.

MATERIALS AND METHODS

Genetic material and experimental design. The study involved a mapping population of 214 (Table 1) genotypes, including 209 MAGIC and 5 parents from the MAGIC core set (Huynh *et al*., 2018), obtained from the cowpea gene bank maintained at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), in Central Uganda. The genotypes were screened for the six environments, through an alpha lattice experimental design, with 14 blocks, each containing 20 plots (14 blocks x 20 genotypes per block) and replicated twice.

Each genotype was planted in a two-row plot, of 8 plants within a row, at a seed rate of two per hill; and later thinned to one plant per hill. The blocks were separated by 1.5 m alleys with 1 m between plots. Plant spacing was 75 cm between and 25 cm within rows. No pesticides were used and the experiments were planted in isolated fields, to avoid confounding effects of pesticide drifts from other experimental fields.

Data collection. Aphid infestation and damage to cowpea in experimental plots were scored at 60 days after planting (DAP), when aphids caused distinct phenotypic variation among genotypes, as recommended by Huynh *et al*. (2015). Infestation and damage on each plant was assessed separately, on individual plants, using a scale of 1 to 5 (Omoigui *et al*., 2017; Souleymane *et al*., 2013), on 12 plants per plot.

For infestation severity, a scale of 1 = no (0) aphids, and 5 = >500 aphids denoting large continuous colonies; for damage severity, 1 = no symptom of attack, and 5 = severely stunted plant with severely curled and yellow leaves, stem and leaves covered with sooty mould or dead plant, was used. The scores were inverse transformed to obtain aphid resistance scores for analysis, from both infestation and damage scores.

Data analysis

Phenotypic trait analysis. Aphid resistance data for 214 genotypes (Table 1) were analysed based on both aphid infestation and damage resistance for variance. Descriptive data analyses and analysis of variance (ANOVA) were conducted in the R statistical package (R Core Team, 2024). To account for environmental variation in overall phenotypic differences, the best linear unbiased predictor (BLUPs) for each genotype were calculated, using R package lme4 (Bates *et al*., 2015), with the effect of environment, replicate within E, G, GE and error as random effects.

Single nucleotide polymorphism genotyping. Single nucleotide polymorphism (SNP) genotyping, was previously conducted as described by Muñoz-Amatriaín *et al*. (2017), for the founder parents and the MAGIC core set. Total genomic DNA from single plants was extracted from dried leaves, using Plant DNeasy (Qiagen, Germany); and genotyped using the Cowpea iSelect Consortium Array that contained 51,128 SNPs. SNPs were called using GenomeStudio software V.2011.1 (Illumina, Inc., San Diego, CA, United States); and the physical positions of the SNPs were determined using the IT97K-499-35 reference genome v1.0 (Lonardi *et al*., 2019).

Genome-wide association analysis. Six multi-locus algorithms were applied for GWAS, as implemented by Oteng-Frimpong *et al*. (2023) in R (R Core team, 2024). The threshold with a critical logarithm of odd value (LOD) score ≥ 3, was maintained to detect the association signals of by default (He *et al*., 2022); and to allow for robust QTNs at the last stage (Zhong *et al*., 2021). SNPs detected by at least 2 of the 6 methods were considered

TABLE 1. List of genotypes and their sources, used in GWAS in the study

Genotype	Source	Genotype	Source	Genotype	Source	Genotype	Source	Genotype	Source	Genotype	Source	Genotype	Source	Genotype	Source
IT82E-18	IITA	MAGIC-081	UCR	MAGIC-172	UCR	MAGIC-246	UCR	MAGIC-323	UCR	MAGIC074	UCR	MAGIC146	UCR	MAGIC232	UCR
IT845-2049	IITA	MAGIC-082	UCR	MAGIC-174	UCR	MAGIC-248	UCR	MAGIC-324	UCR	MAGIC076	UCR	MAGIC148	UCR	MAGIC240	UCR
IT93K-503-1	IITA	MAGIC-083	UCR	MAGIC-175	UCR	MAGIC-257	UCR	MAGIC-327	UCR	MAGIC077	UCR	MAGIC149	UCR	MAGIC242	UCR
ITOOK-1263	IITA	MAGIC-087	UCR	MAGIC-178	UCR	MAGIC-260	UCR	MAGIC-329	UCR	MAGIC080	UCR	MAGIC153	UCR	MAGIC243	UCR
SUVITA-2	INERA	MAGIC-089	UCR	MAGIC-181	UCR	MAGIC-262	UCR	MAGIC-333	UCR	MAGIC085	UCR	MAGIC154	UCR	MAGIC247	UCR
MAGIC-004	UCR	MAGIC-094	UCR	MAGIC-183	UCR	MAGIC-266	UCR	MAGIC006	UCR	MAGIC086	UCR	MAGIC157	UCR	MAGIC249	UCR
MAGIC-005	UCR	MAGIC-097	UCR	MAGIC-185	UCR	MAGIC-268	UCR	MAGIC008	UCR	MAGIC090	UCR	MAGIC162	UCR	MAGIC265	UCR
MAGIC-017	UCR	MAGIC-104	UCR	MAGIC-188	UCR	MAGIC-269	UCR	MAGIC009	UCR	MAGIC092	UCR	MAGIC163	UCR	MAGIC267	UCR
MAGIC-019	UCR	MAGIC-106	UCR	MAGIC-192	UCR	MAGIC-271	UCR	MAGIC010	UCR	MAGIC095	UCR	MAGIC166	UCR	MAGIC270	UCR
MAGIC-024	UCR	MAGIC-107	UCR	MAGIC-194	UCR	MAGIC-272	UCR	MAGIC011	UCR	MAGIC098	UCR	MAGIC170	UCR	MAGIC276	UCR
MAGIC-027	UCR	MAGIC-115	UCR	MAGIC-195	UCR	MAGIC-273	UCR	MAGIC015	UCR	MAGIC099	UCR	MAGIC176	UCR	MAGIC280	UCR
MAGIC-028	UCR	MAGIC-116	UCR	MAGIC-199	UCR	MAGIC-274	UCR	MAGIC020	UCR	MAGIC101	UCR	MAGIC177	UCR	MAGIC284	UCR
MAGIC-035	UCR	MAGIC-117	UCR	MAGIC-200	UCR	MAGIC-275	UCR	MAGIC021	UCR	MAGIC105	UCR	MAGIC179	UCR	MAGIC285	UCR
MAGIC-036	UCR	MAGIC-118	UCR	MAGIC-202	UCR	MAGIC-277	UCR	MAGIC023	UCR	MAGIC109	UCR	MAGIC182	UCR	MAGIC288	UCR
MAGIC-038	UCR	MAGIC-122	UCR	MAGIC-204	UCR	MAGIC-279	UCR	MAGIC029	UCR	MAGIC110	UCR	MAGIC184	UCR	MAGIC297	UCR
MAGIC-039	UCR	MAGIC-125	UCR	MAGIC-205	UCR	MAGIC-281	UCR	MAGIC030	UCR	MAGIC111	UCR	MAGIC189	UCR	MAGIC298	UCR
MAGIC-040	UCR	MAGIC-126	UCR	MAGIC-208	UCR	MAGIC-282	UCR	MAGIC032	UCR	MAGIC112	UCR	MAGIC190	UCR	MAGIC300	UCR
MAGIC-043	UCR	MAGIC-129	UCR	MAGIC-209	UCR	MAGIC-286	UCR	MAGIC033	UCR	MAGIC113	UCR	MAGIC191	UCR	MAGIC301	UCR
MAGIC-046	UCR	MAGIC-132	UCR	MAGIC-211	UCR	MAGIC-287	UCR	MAGIC044	UCR	MAGIC114	UCR	MAGIC198	UCR	MAGIC302	UCR
MAGIC-048	UCR	MAGIC-134	UCR	MAGIC-214	UCR	MAGIC-292	UCR	MAGIC045	UCR	MAGIC119	UCR	MAGIC207	UCR	MAGIC304	UCR
MAGIC-050	UCR	MAGIC-136	UCR	MAGIC-217	UCR	MAGIC-293	UCR	MAGIC049	UCR	MAGIC120	UCR	MAGIC216	UCR	MAGIC314	UCR
MAGIC-052	UCR	MAGIC-139	UCR	MAGIC-224	UCR	MAGIC-295	UCR	MAGIC051	UCR	MAGIC130	UCR	MAGIC219	UCR	MAGIC320	UCR
MAGIC-061	UCR	MAGIC-141	UCR	MAGIC-233	UCR	MAGIC-296	UCR	MAGIC053	UCR	MAGIC131	UCR	MAGIC220	UCR	MAGIC325	UCR
MAGIC-064	UCR	MAGIC-152	UCR	MAGIC-234	UCR	MAGIC-303	UCR	MAGIC054	UCR	MAGIC135	UCR	MAGIC225	UCR	MAGIC336	UCR
MAGIC-068	UCR	MAGIC-155	UCR	MAGIC-236	UCR	MAGIC-311	UCR	MAGIC060	UCR	MAGIC138	UCR	MAGIC227	UCR	MAGIC59	UCR
MAGIC-073	UCR	MAGIC-160	UCR	MAGIC-237	UCR	MAGIC-315	UCR	MAGIC067	UCR	MAGIC140	UCR	MAGIC229	UCR		
MAGIC-078	UCR	MAGIC-171	UCR	MAGIC-238	UCR	MAGIC-317	UCR	MAGIC072	UCR	MAGIC144	UCR	MAGIC231	UCR		

INERA: Institut de l'Environment et des Recherches Agricole, Burkina Faso; UCR: University of California – Riverside, United States; IITA: International Institute of Tropical Agriculture, Nigeria

stable, and thus used for candidate gene exploration.

Candidate gene identification. For candidate gene identification, a search was conducted on the cowpea phytozome (https://phytozome-next.jgi.doe.gov/jbrowse/index.html?data=genomes%2FVunguiculata_v1_2) accessed on 29th May, 2024, using the significant signals from the position ranges captured in the significant SNPs. Genes within the 50 kb range upstream and downstream the significant SNPs were reported as candidate genes for aphid resistance (Chen *et al*., 2023).

RESULTS

Phenotypic variability

Resistance of cowpea to aphids. There were significant (P<0.001) variances among the genotypes on the resistance traits, based on both infestation resistance and damage resistance (Table 2). There were also significant (P<0.05) variations of resistance across locations, genotype x season interaction, genotype x location interaction (for infestation resistance) and three way interactions.

Marker coverage. The SNPs tested were spread throughout the cowpea genome on all the eleven chromosomes and were representative of the whole genome (Fig. 1). A heat map showing chromosomal regions, is presented with high number of SNPs within 1 Mb window size. The vertical axis displays the chromosomes; while the horizontal axis shows chromosome length. Legend (0–193) insert indicates the SNP density; on top of each chromosome there is an insert reflecting the total number of SNPs per chromosome.

Genome-wide association and gene annotations. Manhattan plots for resistance based on infestation resistance (A) and damage (B) resistance, together with their corresponding QQ plots (a and b), were extracted from the analysis, using the six methods (Fig. 2). The multi-locus model revealed three significant and stable SNPs on chromosomes 1, 7 and 9 (Table 3). Three significant and stable SNPs were identified including *2_30668*, *2_43528* and *2_43747* on

TABLE 2. Analysis of variance for resistance of cowpea to aphids in the MAGIC population

Source of variation	Degrees of freedom	Mean squares	
		Infestation resistance	Damage resistance
Rep	1	4.496***	1.13***
Rep*Block	26	0.023*	0.16***
Genotype	213	0.145***	0.10***
Season	1	2.180***	208.87***
Location	2	13.845***	21.64***
Genotype*season	213	0.018*	0.11***
Genotype*Location	415	0.038***	0.08ns
Season*Location	2	6.509***	17.83***
Genotype*Location*Season	363	0.030***	0.07ns
Residual	927	0.015	0.07
Total	2,164		

*, ** and *** = significant at P< 0.05, P< 0.01 and P< 0.001, respectively; ns = non-significant

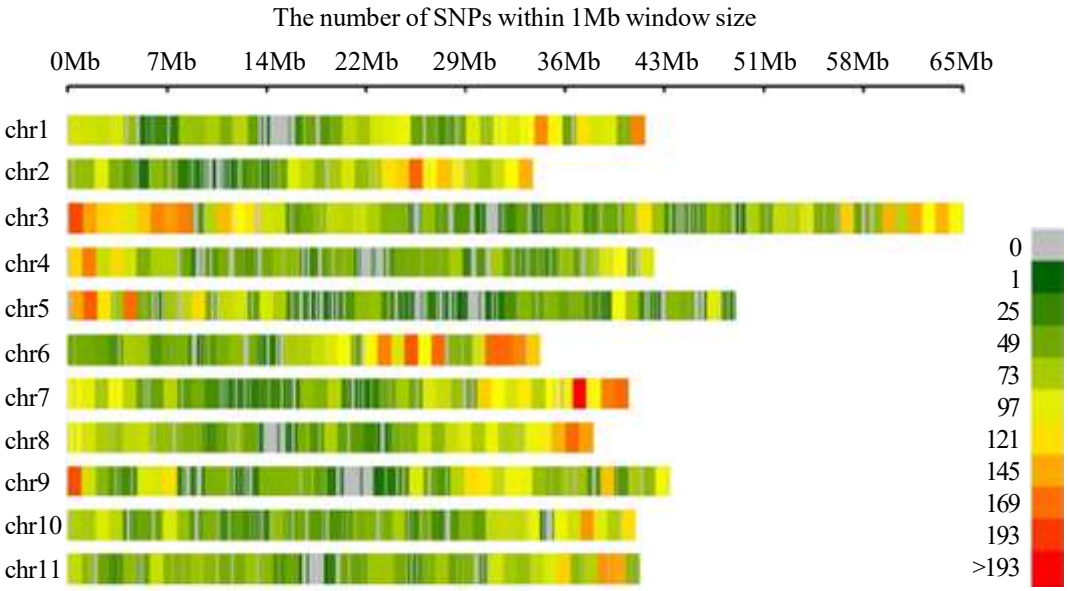

Figure 1. SNP distribution across the 11 chromosomes of cowpea.

3 chromosomes including; Chr 1, Chr 7 and Chr 9 as indicated in Table 3. Eleven candidate genes were detected including; 7 genes (*Vigun01g235100*, *Vigun01g235200*, *Vigun01g235300*, *Vigun01g235350, Vigun01g235400*, *Vigun01g235500 and Vigun01g235600*) on chromosome 1, 3 genes (*Vigun07g046450, Vigun07g046500 and Vigun07g046550*) on chromosome 7 and 1 gene (*Vigun09g087200*) on chromosome 9 (Table 3).

DISCUSSION

Phenotypic variability. The significant ($P<0.001$) variations among the genotypes on the resistance trait, based on both infestation resistance and damage resistance (Table 2), is an indication of the existence of considerable genetic variability for resistance to aphids among the MAGIC genotypes evaluated; which is useful in selection of superior genotypes, which can then be used for the development of genetic stocks for hybridisation programs or the release of a crop variety in crop improvement (Salgotra *et al*., 2023). The phenotypic differences among genotypes on the resistance trait in the present study were distinguishable and significant, with significant variations in resistance across genotypes, seasons and locations based on both infestation and damage (Table 2). This observation suggests that the MAGIC population of cowpea is genetically diverse, providing an opportunity for genetic improvement of cowpea on various traits, including resistance to aphids.

The significant differences of the genotypes on the resistance traits could be due to biochemical factors affecting behavior and metabolic functions in the aphid pest. The MAGIC population used in this study was, therefore, suitable for exploration of genetics controlling resistance to aphid in cowpea, given the broad diversity it encompasses (Huynh *et al*., 2018). The significant ($P<0.05$) variations of resistance across environments (locations) and, genotype x environment interactions, indicate the relative importance of G x E interactions on the studied trait. This high GxE interactions may also imply that that the cowpea MAGIC founder parents carry many alleles that are differentially adapted to different environments (Huynh *et al*., 2018).

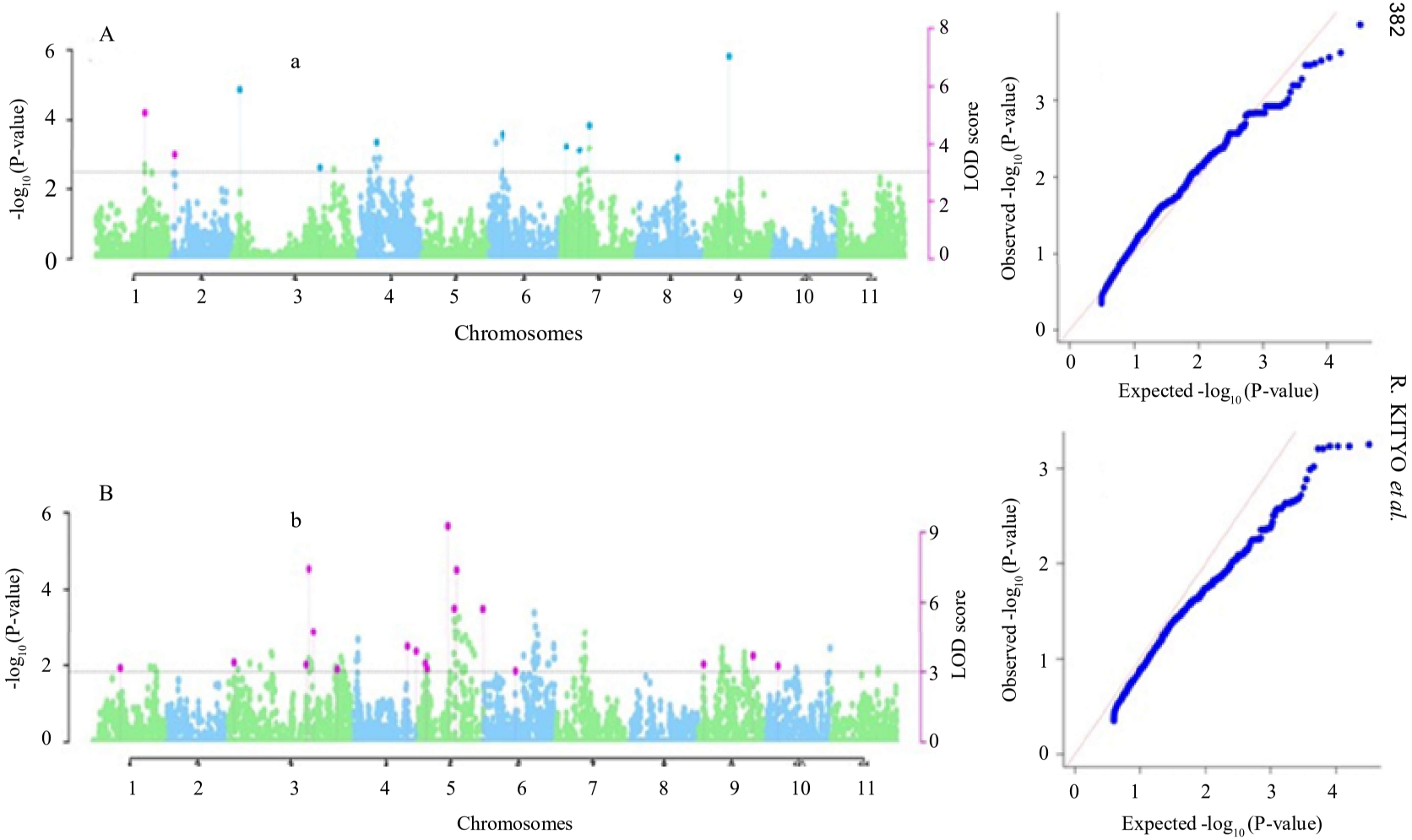

Figure 2. Manhattan plots and QQ plots for aphid resistance based on infestation and damage.

TABLE 3. Significant and stable SNP based on a multi-locus GWAS

Resistance trait	Method	RS#	Chr	Marker position (bp)	QTN effect	LOD score	'-log10(P)'	r2 (%)	MAF	Allele
Infestation	MrMLM	2_30668	1	40683737	-0.1243	4.979	5.7743	11.7382	0.0448	C/G
Infestation	FASTmrMLM	2_30668	1	40683737	-0.1028	4.5573	5.3348	8.225	0.0444	C/G
Infestation	PLARmEB	2_30668	1	40683737	-0.0882	4.6441	5.4254	0.8452	0.0444	C/G
Infestation	ISIS EM-BLASSO	2_30668	1	40683737	-0.1009	4.6847	5.4677	7.928	0.0444	C/G
Damage	MrMLM	2_30668	1	40683737	-0.1243	4.979	5.7743	11.7382	0.0448	C/G
Damage	FASTmrMLM	2_30668	1	40683737	-0.1028	4.5573	5.3348	8.225	0.0444	C/G
Damage	PLARmEB	2_30668	1	40683737	-0.0882	4.6441	5.4254	0.8452	0.0444	C/G
Damage	ISIS EM-BLASSO	2_30668	1	40683737	-0.1009	4.6847	5.4677	7.928	0.0444	C/G
Infestation	PLARmEB	2_43528	7	4764365	-0.0362	3.3388	4.0548	0.2912	0.1098	G/C
Damage	PLARmEB	2_43528	7	4764365	-0.0362	3.3388	4.0548	0.2912	0.1098	G/C
Infestation	FASTmrEMMA	2_43747	9	11698945	-2.00E-04	3.5141	4.2402	2.27E-06	0.0911	A/T
Damage	PLARmEB	2_43747	9	11698945	-0.0329	3.113	3.8154	0.2041	0.0911	A/T

TABLE 4. Significant SNPs, candidate genes and functional annotations

Significant SNP	Chromosome	Candidate Gene (Identifier)	Functional annotations
2_30668	1	*Vigun01g235100*	K17469 - sulfate transporter 2, low-affinity (SULTR2)
		Vigun01g235200	K17469 - sulfate transporter 2, low-affinity (SULTR2)
		Vigun01g235300	PTHR33059:SF4 - F28K19.24-RELATED
		Vigun01g235350	No associated InterPro accession: Unintegrated signatures
		Vigun01g235400	5.5.1.4 - Inositol-3-phosphate synthase / Myo-inositol-1-phosphate synthase
		Vigun01g235500	PTHR31614//PTHR31614:SF5 - FAMILY NOT NAMED // ALLERGEN-LIKE PROTEIN BRSN20-RELATED
		Vigun01g235600	PTHR33305//PTHR33305:SF10 - FAMILY NOT NAMED // ETHYLENE INSENSITIVE 3-LIKE 4 PROTEIN-RELATED
2_43528	7	*Vigun07g046450*	PTHR12374:SF26 - F25A4.19 PROTEIN
		Vigun07g046500	PTHR10209//PTHR10209:SF201 - OXIDOREDUCTASE, 2OG-FE II OXYGENASE FAMILY PROTEIN // KAR-UP OXIDOREDUCTASE 1
		Vigun07g046550	PF13966 - zinc-binding in reverse transcriptase (zf-RVT), Reverse transcriptase zinc-binding domain
2_43747	9	*Vigun09g087200*	KOG1282//KOG1283 - Serine carboxypeptidases (lysosomal cathepsin A) // Serine carboxypeptidases

The genotype x environment interactions result in non-stable performances between the genotypes across environments (Abebe *et al*., 2024).

These results suggest the existence of different patterns of genetic associations across environments, as well as the importance of stability in cowpea breeding for the resistance trait processes to aphids; being controlled by certain genes in the resistant cowpea genotypes (Boukar *et al*., 2020). From previous studies, genotype x environment interactions are known to be important for many agronomic traits of importance in many crops (Akande, 2009; Gerrano *et al*., 2019; Asher *et al*., 2022). Previous field-based studies exhibited clear differences among cowpea lines in their resistance to aphid infestations and damage (Omoigui *et al*., 2017), allowing for the identification of some resistance sources. These observations, coupled with the fact that resistance genes from different sources are non-allelic and independent (Ongom *et al*., 2022), suggest the need to identify genes involved in aphid resistance to support improvement of cowpea on this trait. These observations also emphasize the importance of genetic diversity in detection of DNA markers and candidate genes associated with resistance of cowpea to aphids.

Marker coverage. Scanning of the entire cowpea genome in the present study was made possible by a highly dense SNP marker system (Fig. 1), in addition to high genetic diversity among the genotypes, allowed for the revelation of three significant SNPs on three different chromosomes, potentially harbouring genes underlying resistance to field cowpea aphids (Table 3).

The positive and negative sign of the quantitative trait nucleotide (QTN) effect values, were used as the criteria for selecting superior alleles. If the QTN effect value is positive, the genotype of code 1, which was obtained by GWAS, is the superior allele. On the other hand, if the QTN effect value is negative, the other genotype is the superior allele (Qi *et al*., 2020). In the present study, all the QTN effects were negative, implying that the alternative genotypes are the superior alleles. These observations indicate the possibility of identifying superior alleles for the aphid resistance trait in cowpea.

Genome-wide association. The significant marker-trait associations for resistance to aphids detected on chromosome *Vu01*, flagged by SNP marker *2_30668* at position 80.59 cM, *Vu07* flagged by SNP variant *2_43528* at position 18.48 cM and *Vu09* flanked by SNP variant *2_43747* at position 39.71 cM (Table 3); imply that resistance genes of cowpea to aphids can be traced using these SNPs and chromosomal positions on the cowpea genome.

In a similar study, the locus *Vu01* was previously reported to potentially harbour genes associated with resistance of cowpea to aphids (Ongom *et al*., 2022). Boa-Lam *et al*. (2015) and Huynh *et al* (2015) consistently mapped loci on chromosomes I and VII, being associated with resistance of cowpea to aphids, possibly conferring a phloem-based defence mechanisms against cowpea aphid feeding; indicating the presence of resistance genes in these regions. Ouedraogo *et al*. (2021) also found the locus *Vu07* to be associated with resistance of cowpea to aphids, using single sequence repeat (SSR) markers in a greenhouse experiment.

Thus, the present study reaffirms the possibility of the loci *Vu01* and *Vu07* being associated with resistance of cowpea to aphids and loci *Vu09* being novel, as far as this trait in cowpea is concerned. Other studies have implicated expression of these loci under an array of conditions, including salinity (Reinders *et al*., 2005), mechanical wounding and insect feeding (Sharma *et al*., 2014), pathogens and stress signalling (Smith *et al*., 2007) and resistance to insects in different plants (Prince *et al*., 2014; Shoala *et al*., 2018). These

observations emphasize the power of multi-locus GWAS in deciphering the genetic control of resistance of cowpea to aphids.

Gene annotations. The identified candidate genes in the present study have plant defence-related functions (Table 4). For instance, genes *Vigun01g235100* and *Vigun01g235200* (SNP variant *2_30668*) have roles related to sulphate transport (K17469 - sulphate transporter 2, low-affinity (SULTR2) (Ding *et al*., 2016). Sulphate transporters (SULTRs) are an essential plant transporter class, responsible for the absorption and distribution of sulphur, which is an essential plant growth element (Puresmaeli *et al*., 2023).

Studies have revealed that members of this (SULTRs) gene family are also involved in responding to environmental stress (Kumar *et al*., 2015; Vatansever *et al*., 2016). Indeed, the multifaceted plant defence responses, initiated by sulphur-containing defence compounds (SDCs), should provide novel tools for plant breeding to endow crops with efficient defence responses to invaders (Künstler *et al*., 2020). The genes reported here being involved in transporting these compounds, could be playing central roles in resistance of cowpea to aphids.

The candidate gene, *Vigun01g235400*, also flagged by SNP variant *2_30668* on chromosome 1 (Table 4) is a Myo-inositol-1-phosphate synthase (MIPS), based on functional gene annotations. Inositol phosphate synthase (IPS) is a rate-limiting enzyme in myoinositol biosynthesis, which regulates stress responses in plants and animals (Ni *et al*., 2019). Inositol is the precursor for many inositol-containing compounds such as signalling molecules and plays important roles in many essential processes, including growth regulation, hormonal regulation, membrane trafficking, and signal transduction (Kaur *et al*. 2013; Tan *et al*. 2013). In fact, MIPS genes play a critical role in response to stresses including protecting plants from environmental stress factors (Kusuda *et al*., 2015).

The candidate gene, *Vigun07g046500,* flagged by SNP variant *2_43528* on chromosome 7, belongs to the "Oxidoreductase, 2OG-FE II Oxygenase Family Proteins" (Liang *et al*., 2023). Oxidoreductase enzymes are involved in plant defence mechanism, typically assisting in reactive oxygen species (ROS) generation, which serve as signalling molecules and activate signal transduction pathways during stress (Das and Sen, 2024). Induction in peroxidase activity has been implicated as an immediate response of plants to biotic stresses, including insect attack. For instance, Singh *et al*. (2013) observed induced peroxidase activity in sap and total soluble protein (TSP) of cowpea leaves after infestation with chewing and sap-sucking insects.

The candidate gene, *Vigun09g087200,* detected on chromosome 9 (flagged by SNP variant *2_43747*), is a Serine carboxypeptidase. Such enzymes are involved in the biosynthesis of a range of structurally diverse and ecologically relevant natural compounds that provide chemical defence against pathogens and herbivores (Mugford and Milkowski, 2012).

The evidence presented here regarding the identified SNPs and candidate genes, indicates their potentially significant roles in plant defence systems in host plant resistance; and could be responsible for resistance of cowpea to aphids. These will be provide the basis for marker assisted selection in the breeding of aphid resistant varieties of cowpea in Uganda and elsewhere.

CONCLUSION

Three significant SNP variants located on chromosomes I, VII and IX, are identified being associated with resistance of cowpea to aphids (*Aphis craccivora*). Eleven candidate genes are detected whose functional annotations point to plant defence systems and could be involved in resistance of cowpea to aphid. The study uncovered significant SNPs

(*2_30668*, *2_43528* and *2_43747*) and candidate genes (*Vigun01g235100*, *Vigun01g235200*, *Vigun01g235300,* *Vigun01g235350,* *Vigun01g235400*, *Vigun01g235600*, *Vigun07g046450*, *Vigun07g046500*, *Vigun07g046550*, *Vigun09g087200*, and *Vigun09g087200*) for aphid resistance thereby contributing towards a better understanding of the genetic control of this insect pest in cowpea. The SNP markers reported here should be tested further for consistent associations in different genetic backgrounds. This will enhance confidence in the utilisation of these SNPs in marker-assisted breeding for aphid resistance. Once validated, these SNP markers may be deployed in marker-assisted selection (MAS) for faster development of aphid-resistant cultivars of cowpea in Uganda.

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