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Identification of candidate genes associated with resistance to bruchid (*Callosobruchus maculatus*) in cowpea

Belay Miesho[1,2] | **Mehari Hailay**[1] | **Ulemu Msiska**[1] | **Awio Bruno**[1,3] | **Geoffrey M. Malinga**[4,5] | **Patrick Obia Ongom**[1,3] | **Richard Edema**[1,3] | **Paul Gibson**[1,3] | **Patrick Rubaihayo**[1] | **Samuel Kyamanywa**[1]

[1]Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda

[2]College of Agriculture, Aksum University-Shire campass, Shire, Ethiopia

[3]Makerere University Regional Center for Crop Improvement (MaRCCI), Makerere University, Kampala, Uganda

[4]Faculty of Science, Department of Biology, Gulu University, Gulu, Uganda

[5]Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

Correspondence
Belay Miesho, College of Agriculture, Aksum University-Shire campass, Shire, Ethiopia
Email: belaymiesho@yahoo.com

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Abstract

Cowpea is an important legume crop widely grown in sub-Saharan Africa for food and feed. However, it is largely challenged by bruchid, a serious storage pest resulting in losses in quantity and quality of grains. Therefore, this research was designed to contribute to the breeding of cowpea resistance to bruchid through the identification of candidate genes associated with resistance to bruchid. A total of 217 mini-core cowpea accessions were genotyped and phenotyped for their reactions to bruchid. To determine the genomic regions linked with bruchid resistance, 41,948 polymorphic SNP markers were used. Genome-wide association study identified 11 SNPs linked to the average number of eggs, holes, insect emergence and development period and Dobie susceptibility index. Gene search via Phytozome identified six candidate genes (*Vigun08g132300*, *Vigun08g158000*, *Vigun06g053700*, *Vigun02g131000*, *Vigun01g234900* and *Vigun01g201900*) associated with the resistance traits. These candidate genes could be incorporated into the farmers preferred but susceptible cowpea varieties to bruchid. The SNP markers associated with the resistance traits can be used in marker-assisted breeding for accurate and rapid screening of cowpea resistant genotypes to bruchid.

KEYWORDS
genome-wide association, genomic region, SNP, *Vigun*

1 | INTRODUCTION

Cowpea is an important leguminous crop that is well-adapted to the drier regions of the tropics. It provides more than half the plant protein in the diets of people in many developing countries, especially in sub-Saharan Africa (Aliyu &Wachap, 2014; Bhattarai et al., 2017; Boukar, Fatokun, Huynh, Roberts, & Timothy, 2016). However, the crop is particularly susceptible to infestation, both in the field and during storage, to several insects including bruchids (Boukar et al., 2012). The inheritance of bruchid resistance traits (average number of eggs, insect emergence and insect development period) is quantitative and complex and little efforts have been made to understand the genetic basis of such complex traits (Miesho et al., 2018). It is challenging to improve complex traits through conventional breeding approaches, but the use of molecular markers enhances the selection process although it requires a good understanding of how variations at the DNA level relate to phenotypic variations. Genome-wide association studies (GWAS) has emerged as an effective tool to identify multiple related candidate genes regulating important traits in both self- and cross-pollinated crops (Lorenz, Hamblin, & Jannink, 2010; Lucas et al., 2011). GWAS use collections of diverse lines that have genotyped and phenotyped traits of interest. The technique is used for identifying genomic loci that are linked with quantitative traits (Varshney, Terauchi, & McCouch, 2014). In comparison to quantitative trait loci (QTL) studies that are achieved using pedigrees (e.g. bi-parental crosses), GWAS have the advantage of detecting

smaller chromosomal regions affecting a trait and provide precise estimates of the size and direction of the effects of alleles in known loci (Abdel-Shafy, Bortfeldt, Tetens, & Brockmann, 2014).

The development of genomic resources for cowpea has been more recent than for the majority of other crops and recent efforts have focused on molecular diversity and genetic linkage mapping (Boukar et al., 2016). Currently, molecular work on cowpea has focused on the study of quantitative trait loci (QTL) governing many agricultural and adaptive traits such as root architecture, seed size and resistance to *C. maculatus* (Burridge et al., 2016; Egbadzor, Yeboah, Danquah, Ofori, & Offei, 2013). Genetic mapping has been done using a range of methods, such as restriction fragment length polymorphism (RFLP) (Fatokun, Menancio-Hautea, Danesh, & Young, 1992), simple sequence repeat (SSR) (Fatokun, 2000) and single-nucleotide polymorphism (SNP) markers (Burridge et al., 2016; Egbadzor et al., 2013). Molecular markers together with phenotypic data can generate more reliable data than phenotype alone in crop improvement.

However, studies of quantitative trait loci (QTL) related to key bruchid resistance in cowpea are scarce (Tan et al., 2012). Fatokun (2000) reported that SSR marker Vm50 was closely associated with the delay in the emergence of *C. maculatus* explaining 20% of the variation. Fatokun (2002) also identified four QTL associated with bruchid resistance, and a major QTL accounted for 76% of the variation in the trait. However, the application of GWAS in cowpea improvement both at the global level in general and under the Ugandan condition, in particular is limited to the study of agronomic and nutritional traits and its use for the study of resistance, to *C. maculatus* are scant. The aim of this study was to identify genomic regions, candidate genes and their functions that control the bruchid resistance parameters such as average number of eggs (ANE), average number of emerged insects (ANI), average number of holes (ANH), median development period (MDP) and Dobie susceptibility index (DSI) from cowpea mini-core collections using SNP markers.

2 | MATERIALS AND METHODS

2.1 | Sources of cowpea genotypes

A total of 217 mini-core cowpea accessions, from ~60 countries across six continents representing a worldwide diversity of cultivated cowpea (Munoz-Amatriain et al., 2016), collected and genotyped by the University of California were procured through the Makerere University Regional Center for Crop Improvement (MaRCCI). To generate sufficient seeds for laboratory testing, each of the accessions was grown at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) (0°28′N and 32°37′E, approximately 1,200 masl), between September and December 2017.

2.2 | Infestation and data collection

Seeds of each of the 217 mini-core cowpea accessions were dried in an oven at 40°C for 24 hr to eliminate any bruchid (Callosobruchus maculatus [Fab.]) infestation coming from the field and to keep moisture level of the seeds uniform (Amusa et al., 2014). The experiment was conducted at the animal science laboratory, MUARIK from September 2017 to March 2018. Ten randomly selected seeds from each genotype were initially weighed and put into a Petri dish of size 90 × 15 mm. Each Petri dish was infested with two pairs of newly emerged male and female adult bruchids and covered to prevent the insects from escaping. The experiment was laid in a completely randomized design with two replicates per accession. The insects were left undisturbed in the Petri dishes for three consecutive days to allow for mating and oviposition before being removed (Amusa, Ogunkanmi, Bolarinwa, & Ojobo, 2013). Data on the average number of eggs laid by bruchid were recorded after removal of the insects. The number of emerged adult bruchids was counted daily until no more adults emerged for five consecutive days (Amusa et al., 2014), and the number of exit holes from each seed was counted. Data on the total number of adult bruchid that emerged and their median development period (i.e. the time from the middle of oviposition to the emergence of 50% of adult bruchids) of each accession were used to calculate its Dobie susceptibility index (DSI) using the formula of Dobie (1974).

2.3 | Genotyping

The 217 mini-core collections were genotyped at the University of California with the "Cowpea iSelect Consortium Array" available from Illumina (Illumina Inc., San Diego, CA; http://www.illumina.com/areas-of-interest/agrigenomics/consortia.html) containing 51,128 SNPs (Munoz-Amatriain et al., 2016). A total of 41,948 polymorphic and non-redundant SNP markers, with >5% minor allele frequency (MAF) and missing data lower than 20% filtered using TASSEL 5.2.1.5 (Bradbury et al., 2007) were used for subsequent analysis. Heterozygous markers were treated as missing data according to Boukar et al. (2012).

2.4 | Data analysis

The following parameters were used to assess resistance to bruchid: average number of eggs (ANE), average number of emerged insects (ANI), average number of holes (ANH), median development period (MDP) and Dobie susceptibility index (DSI). Each of the resistance parameters was subjected to one-way analysis of variance (ANOVA) to examine differences in the response of cowpea accessions for resistance to bruchid. Fisher's LSD test was used to separate the means of the genotypes. Pearson correlation was used to examine the relationships among resistance parameters including the DSI for the accessions. All analyses were conducted using GenStat Discovery, 16.1th Edition statistical package.

2.5 | Association mapping

GWAS study was performed on the average number of eggs (ANE), average number of emerged insects (ANI), average number of holes (ANH), median development period (MDP) and Dobie susceptibility index (DSI). GWAS was performed using the mixed linear model

(MLM) in the program TASSEL 5.2.1.5, incorporating Q matric and kinship data (K) (Zhang, Song, Cregan, & Jiang, 2016) as follows:

$$Y = X\beta + Wm + Qv + Zu + e,$$

where Y is a vector of phenotypic observations; β is a vector of unknown fixed effects except for the SNP marker under testing, m is a vector of fixed marker effects (i.e. SNP), v is a vector of subpopulation effects, u is a vector of unknown random effects and e is a vector of residual effects. Q is an incidence matrix of principal component scores of marker-allele frequencies. X, W and Z are incidence matrices of ones and zeros relating y to β, m and u, respectively. The covariance of u is equal to KVA, where K is the kinship matrix that was estimated with a random set of SNPs using the TASSEL program and VA is the additive variance estimated with the restricted maximum likelihood (REML). The kinship matrix estimation and the principal component analysis were performed in the TASSEL package. The optimum number of principal components/covariates included in the model for each trait was three. SNPs with a $-\log^{10}$ (p) score greater than 3.5 was treated as a significant threshold for marker–trait association analysis (Burridge et al., 2016; Contreras-Soto et al., 2017). The single trait–single environment association mapping procedure (Egbadzor et al., 2013; VSN International, 2012) was followed to identify SNP markers that are linked with the bruchid resistance traits. Quantile-quantile (QQ) plots were used to assess the presence of spurious associations.

2.6 | Gene prediction

To identify possible genes underlying the association signals detected by GWAS, the cowpea reference genome annotation accessible through Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er) was exploited. The physical positions of significant SNPs were searched on the cowpea genome browser to discover the relevant genes in the SNP vicinity. Annotated functions of the surrounding genes were investigated from the cowpea reference genome, accessible through Phytozome, for their involvement in bruchid resistance.

3 | RESULTS

3.1 | The response of the mini-core accessions to bruchid attack

Uniform levels of bruchid damage were recorded in all the replicates, resulting in distinct susceptibility and resistance responses to bruchid attack among cowpea genotypes (Table 1). Frequency distributions of the phenotypic data were continuous. Furthermore, the distribution of the accessions to the different resistance traits and resistant classes were fitting to normality (Figure 1), indicating the existence of additive gene effect.

The resistant accessions exhibited significantly less bruchid damage than the susceptible accessions (Table 1). These differences were confirmed by ANOVA, in which F values for phenotypic differences were highly significant ($p < 0.001$) and coefficient of variation (CV) ranged from 3.9% to 8.3% (Table 2).

TABLE 1 Means of genotypic performance under bruchid infestation (some representative genotypes)

Genotypes	ANE	ANI	ANH	MDP	DSI
1393-1-2-3(-)	0.4	0.1	0.1	29	0
IT84S-2246	0.9	0.1	0.1	41.5	0
IT95K-1491	0.5	0	0	42	0
IT97K-207-15	0.35	0.1	0.1	37.5	0
IT97K-499-39-1-1	0.5	0.1	0.1	42	0
IT97K-556-6	0.3	0	0	42	0
TVu-13305	0.25	0.15	0.15	38.75	0.381
IT97K-569-9	1.35	0.15	0.15	38	0.386
TVu-7778	0.7	0.15	0.15	39.5	0.386
TVu-1037	1.65	0.15	0.2	35.5	0.396
UCR_707	1	0.15	0.15	31	0.47
TVu-1280	1.35	0.2	0.2	34.5	0.873
IT89KD-288	2.05	0.3	0.3	38.5	1.239
TVu-15775	1.15	0.35	0.35	40.25	1.342
TVu-2398	0.9	0.25	0.25	28.75	1.343
FN-1-13-04	7.1	4.85	4.85	20.25	8.326
Xingove	19.45	4.7	4.7	19.75	8.469
58-57	6.35	4.6	4.3	19.25	8.638
TVu-8779	18.15	7.8	7.75	21.5	8.803
Ife_Brown	15.3	8.15	8.15	21.5	8.893
CB3	11	7.75	7.75	21.25	8.899
Vya	10.6	7.85	7.85	21.25	8.917
INIA-5E	18.4	6.15	6.15	20	8.969
TVu-13965	8.6	7.45	7.45	20.75	9.024
IT93K-452-1	18.4	9.3	9.3	21.75	9.05
N'diambour	10.25	5.85	5.8	19.5	9.066
TVu-13463	11.75	8.2	8.2	21	9.136
Mougne	10.15	7.05	7.1	19.5	9.533
Apagbaala	19.1	9.9	9.8	18.75	10.643
TVu-13939	17.85	9.55	9.35	18.5	10.707

ANE, average number of eggs; ANH, average number of holes; ANI, average number of emerged insects; DSI, Dobie susceptibility index; MDP, median development period.

3.2 | Correlation

Highly significant ($p < 0.001$) correlations were observed among the traits (Table 3).

The average number of eggs, insect emergence and average number of holes were positively correlated to DSI while median development period was correlated negatively with other traits. Positive and significant ($p < 0.001$) correlations were observed among the average number of eggs, average number of holes and insect emergence, suggesting that these resistance traits are likely under the same genetic control.

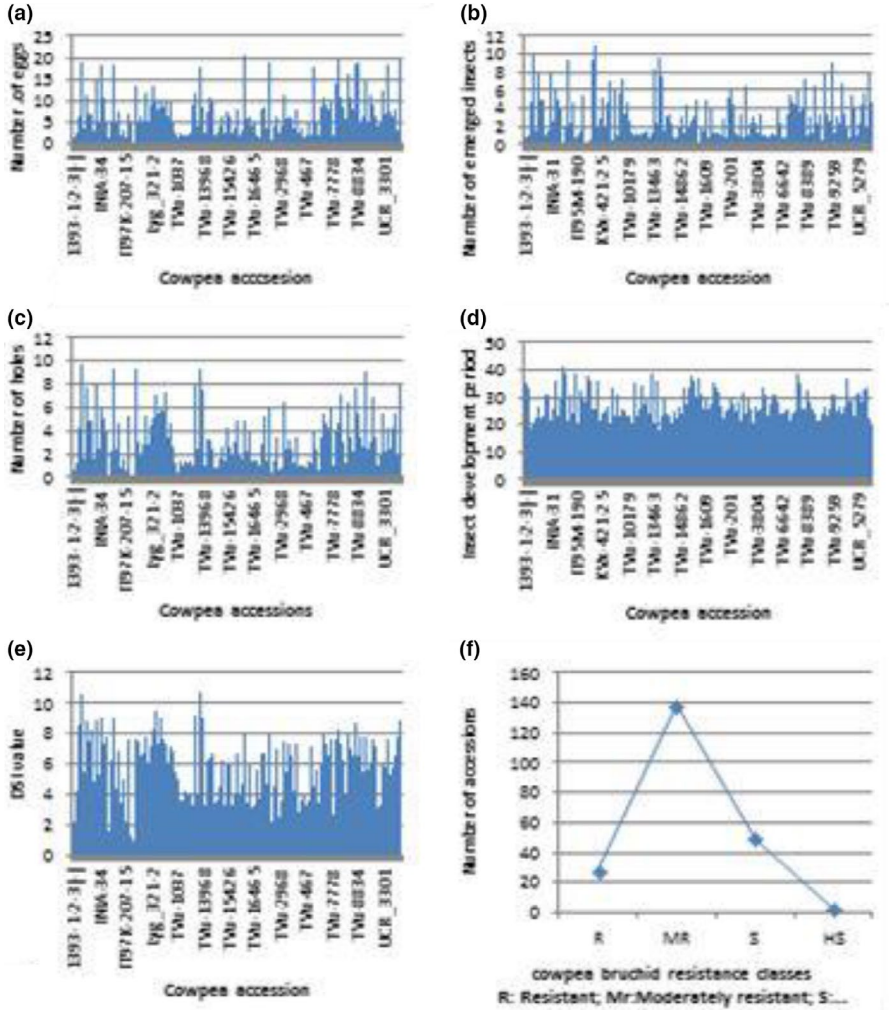

FIGURE 1 Distribution of cowpea accessions in the different bruchid resistance traits; average number of eggs (a), average number of emerged insects (b), average number of holes (c), Median development period (d), Dobie susceptibility index (e), and resistance classes (f)

Source of variation	*df*	Variables				
		ANE	ANI	ANH	MDP	DSI
Genotype	216	44.91***	10.66***	9.97***	51.10***	9.45***
Residual	217	0.11	0.05	0.05	1.07	0.05
Mean		5.75	2.63	2.58	26.25	5.06
Minimum		0.25	0.10	0.10	18.50	0
Maximum		20.65	11.00	9.80	41.50	10.71
CV%		5.80	8.20	8.30	3.90	4.5
SEM		0.23	0.15	0.15	0.73	0.16

TABLE 2 Analysis of variance for the resistance of cowpea accessions to *Callosobruchus maculatus* infestation

ANE, average number of eggs; ANH, average number of holes; ANI, average number of emerged insects; DSI, Dobie susceptibility index; MDP, median development period.
***$p \leq 0.001$.

3.3 | SNP-based association analyses

The results for evaluation of model fit for mixed linear models with Q (structure) matrices (Figure 2), showed the best fit model to consider Q for all the traits (ANE, ANI, ANH, MDP and DSI).

Eleven significant regions (LOD > 3.5) associated with the cowpea bruchids resistance traits were identified on six chromosomes (Table 4). The SNPs *2_36050* on chromosome 8, *2_37236* on chromosome 6, *2_38306* on chromosome 7 and *2_38338* on chromosome 9 had allelic effects of −6.5, −6.4, −6.2 and −3.1 on the average number of eggs respectively. SNPs *2_36627* on chromosome 7 had −2.1 allelic effects on the average number of emerged insects. SNPs *2_32564* and *2_37091* on chromosome 1; and *2_15764* on chromosome 2 had 2.1 and 5.1; and 1.8 allelic effects on insect development

TABLE 3 Correlation coefficients (*r*) for cowpea genotype under *Callosobruchus maculatus* artificial infestation

Trait	ANE	ANI	ANH	MDP	DSI
ANE	1				
ANI	0.79	1			
ANH	0.83	0.96	1		
MDP	−0.46	−0.47	−0.48	1	
DSI	0.78	0.84	0.87	−0.77	1

ANE, average number of eggs; ANH, average number of holes; ANI, average number of emerged insects; DSI, Dobie susceptibility index; MDP, median development period.
All correlations are significant ($p < 0.001$).

period respectively. Likewise, SNPs *2_54737* and *2_35230* on chromosome 7 had −1.4 and −1.5 allelic effects on DSI respectively. SNP *2_20268* on chromosome 8 had allelic effects on the average number of eggs (−1.5), emerged insects (−0.7) and the average number of holes (−0.7).

Several regions with minor allelic effects on the phenotypic expression of the resistance traits were detected (Table 4). 45.5% of the proportion of phenotypic variance for the average number of eggs was explained by five genomic regions (*2_20268*, *2_36050*, *2_37236*, *2_38306* and *2_38338*). About 21.1% of the variation among accessions in their DSI values was accounted by SNPs *2_54737* and *2_35230*. Similarly, SNP *2_20268* explained 10.7, 9.1 and 8.5% of the phenotypic variations on the average number of holes, average number of eggs and average number of emerged insects, respectively, indicating strong linkage among the traits. Likewise, 21.9% of the phenotypic variation among accessions on their median development period was accounted by SNPs *2_15764*, *2_32564* and *2_37091*.

GWAS identified five candidate genes and one upstream gene involved in the inheritance of seed resistance to bruchids (Table 5). *Vigun08g132300* on chromosome 8 linked to SNP *2_20268* was involved in reducing the average number of holes, insect emergence and average number of eggs. *Vigun08g158000* on chromosome 8 linked to *2_36050* was involved in reducing the average number of eggs. *Vigun06g053700* on chromosome 6 was also involved in reducing oviposition. Candidate gene *Vigun02g131000* and a downstream gene *Vigun01g201900*, 1482bp away from SNP *2_37091* on chromosome 1, and *Vigun01g234900* on chromosome 1 were involved in elongating the insect developmental period.

4 | DISCUSSION

The study demonstrated the existence of candidate genes conferring resistance to bruchid which could be incorporated into farmers preferred but susceptible cowpea cultivars. The existence of genomic regions conferring resistance to bruchid was previously reported on cowpeas (Fatokun, 2000,2002), rice bean (Venkataramana et al.,

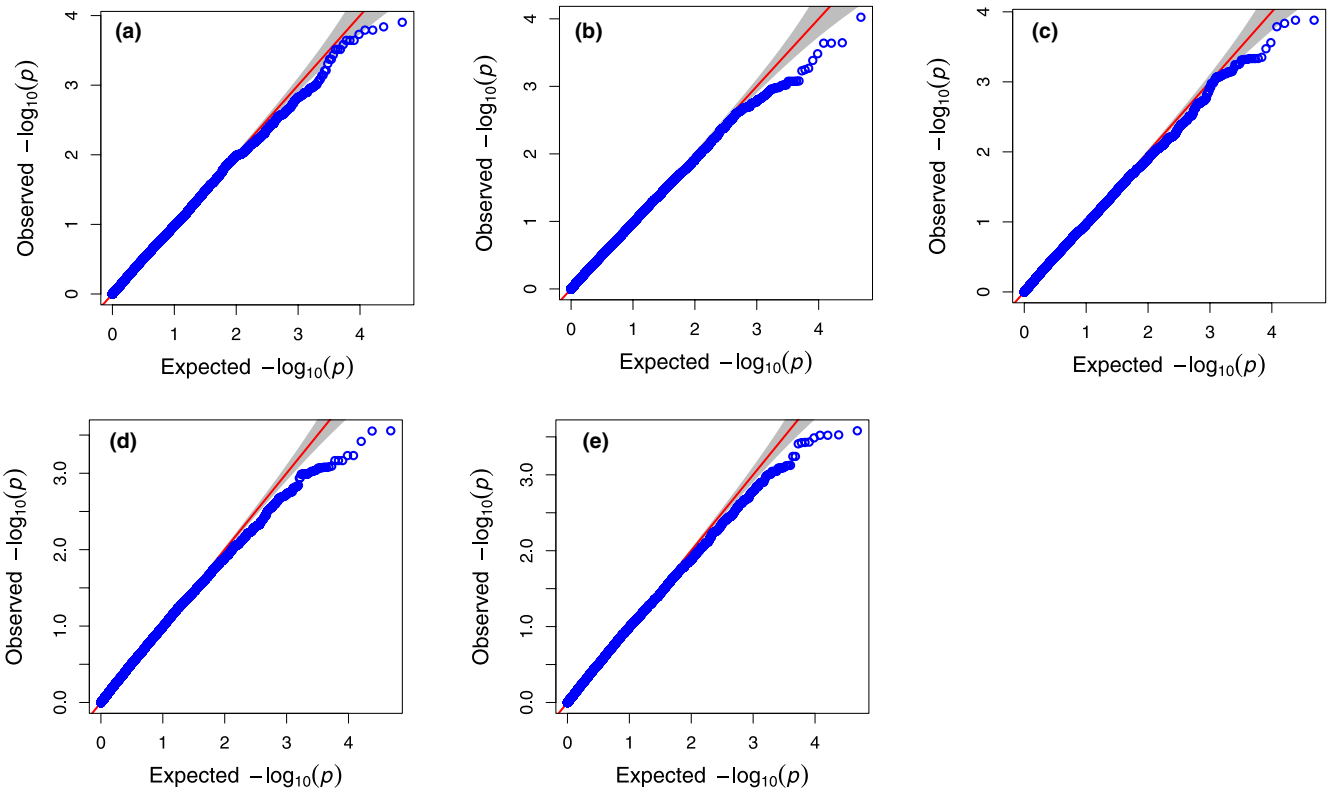

FIGURE 2 Quantile-quantile (QQ) plots of GWAS for ANE (a), ANI (b), ANH (c), MDP (d) and DSI (e) evaluated in a cowpea association mapping panel

TABLE 4 Bruchid resistance QTL with LOD >3.5 and their effects on resistance traits

Trait	SNP marker	Chromosome	Position (cM)	Allelic[a] effects	R^2 (%)	LOD	Favorable allele	Alternative allele
ANE	*2_20268*	8	30,339,110	−1.5	9.1	3.8	T	C
ANH				−0.7	10.7	3.9		
ANI				−0.7	8.5	3.6		
ANE	*2_36050*	8	33,041,542	−6.5	9.2	3.8	T	C
ANE	*2_37236*	6	17,949,201	−6.4	8.8	3.6	T	C
MDP	*2_15764*	2	28,200,370	1.8	7.1	3.5	A	G
MDP	*2_32564*	1	40,651,151	2.1	7.4	3.6	C	G
MDP	*2_37091*	1	37,818,038	5.1	7.4	3.6	G	T
ANI	*2_36627*	7	19,304,251	−2.1	9.3	4.0	G	A
ANE	*2_38306*	7	12,810,789	−6.2	9.4	3.9	G	A
ANE	*2_38338*	9	40,479,817	−3.1	9	3.7	T	A
DSI	*2_54737*	7	19,387,024	−1.4	10.6	3.6	C	A
DSI	*2_35230*	7	19,399,548	−1.5	10.5	3.5	G	A

ANE, average number of eggs; ANH, average number of holes; ANI, average number of emerged insects; DSI, Dobie susceptibility index; MDP, median development period.
[a]Marker additive effect, all significance at $p > 0.001$.

TABLE 5 Identified candidate genes and their molecular and biological functions

Trait	SNP marker[a]	Chromosome[a]	Candidate genes[b]	Molecular function[b]	Biological function[b]
ANE ANH ANI	*2_20268*	8	*Vigun08g132300*	Chloroplast envelope transporter (Tic110)	Biosynthesis of carbohydrates and proteins
ANE	*2_36050*	8	*Vigun08g158000*	Interacting selectively and non-covalently with any protein or protein complex	Cellular transport processes
ANE	*2_37236*	6	*Vigun06g053700*	WRKY DNA -binding domain (WRKY)	Regulation of transcription
MDP	*2_15764*	2	*Vigun02g131000*	Alpha/beta hydrolase fold-containing protein (ABH)	Glycerol biosynthesis
MDP	*2_32564*	1	*Vigun01g234900*	Glutamate-prephenate aminotransferase	The chemical reactions and pathways resulting in the formation of substances; typically, the energy-requiring part of metabolism in which simpler substances are transformed into more complex ones
MDP	*2_37091*	1	*Vigun01g201900*[c]	Any molecular function by which a gene product interacts selectively and non-covalently with DNA (deoxyribonucleic acid)/PTHR	Transcription factor activity

ANE, average number of eggs; ANH, average number of holes; ANI, average number of emerged insects; MDP, median development period.
[a]Downstream gene. [b]Data from TASSEL version 5.2.15. [c]Cowpea reference genome annotation accessible through Phytozome.

2015) and in mungbean (Mei et al., 2014; Wanga et al., 2016). The identification of 11 genomic regions associated with resistance to bruchid may also indicate the predominance of additive gene action in conferring resistance to bruchid (Miesho et al., 2018).

Five significant genomic regions associated with the average number of eggs were identified on chromosome 6, 7, 8 and 9 (Table 4). The highest allelic effect was recorded from SNP *2_36050* (−6.5) and the lowest from *2_20268* (−0.7). The negative allelic effect indicated the involvement of the alternative alleles (cytosine and adenine) in reducing the average number of eggs (Burridge et al., 2016) thereby enhancing resistance. Similarly, SNPs *2_20268* and *2_36050* which are co-localized on chromosome 8 accounted for 18.3% of the variation in the average number of eggs highlighting the need to target this chromosome to discourage oviposition. The other SNPs

distributed on chromosomes 6, 7 and 9 contributed 27.2% of the total phenotypic variation. The results also suggested that SNP *2_20268* is in linkage disequilibrium with *2_36050*, and indeed the two SNPs cover a narrow interval (around 30,338,080–33,041,839 bp) flanked by SNPs on chromosome 8 (Table 4), and within this region, there was one chloroplast envelope transporter Tic110 gene (*Vigun08g132300*) and one PTHR gene (*Vigun08g158000*) (Table 5) involved in reducing oviposition through their ability to control carbohydrate (Block, Douce, Joyard, & Rolland, 2007) and functional protein biosynthesis (Lindemose, O'Shea, Jensen, & Skriver, 2013). In addition, WRKY DNA-binding domain (WRKY) was closely associated to SNP *2_37236* and encoded by candidate gene *Vigun06g053700* involved in reducing eggs through its ability to modulate transcription (Pedra et al., 2003). Negative allelic effect of the SNPs associated with the average number of eggs was also another evidence for their involvement in resistance (Burridge et al., 2016). Similarly, gene analogue to *Vigun06g053700* in soybean (*Glyma.08G320200*) was involved in a wide range of developmental and physiological processes, particularly in the plant response to biotic and abiotic stresses (Yu, Nan, Ruibo, & Fengning, 2016).

Genomic regions associated with the average number of emerged insects identified on chromosome 7 and 8 (Table 4) are of particular interest because of their importance in reducing insect emergence. SNP *2_20268* on linkage group (LG) 8 which is linked to chloroplast envelope transporter (*Vigun08g132300*) gene is co-located with SNP for the average number of holes and eggs in which the SNP haplotype confers a reduced number of insects. The strong correlation between the traits (Table 3) is an evidence for the existence of a common gene among them (Miesho et al., 2018).

Chloroplast envelope transporter (Tic110) was involved in enhancing resistance to bruchid through its ability in reducing insect emergence, holes, oviposition and elongating insect development period. The chloroplast envelope membranes are the permanent membrane structure of the different types of plastids (proplastids, chloroplasts, chromoplasts, etioplast) and an important structure for the integration of plastid metabolism within the cell (Block et al., 2007). Chloroplasts are crucial for photosynthesis and are the sites of carbon dioxide reduction and its assimilation into carbohydrates, amino acids, fatty acids,and terpenoid compounds (Block et al., 2007). The higher the carbohydrate assimilated by the plant, the higher will be seed carbohydrate leading to increased seed hardness (Ajeigbe, Ihedioha, & Chikoye, 2008), thereby making seed penetration by the insect difficult, resulting into reduced insect population and average number of holes, and elongated insect development period (Miesho et al., 2017). Similarly, the higher the amino acid biosynthesis, the higher will be the seed inhibitory enzymes and the better the seed resistance to bruchid (Westermann & Craik, 2010). The involvement of inhibitory enzymes in enhancing seed resistance to bruchids was previously reported by Lattanzio et al. (2005) and by Miesho et al. (2017) on cowpea and by Wisessing, Engkagul, Wongpiyasatid, and Choowongkomon (2010) on mung beans. In beans, for example, α-amylase inhibitor is found only in the seeds (Moreno, Altabella, & Chrispeels, 1990). This is because of more efficient glycosylation in the seeds than other parts of the plant (Obiro, Zhang, & Jiang, 2008). The better carbohydrate biosynthesis will increase glycolysis that leads to a more efficient synthesis of α-amylase inhibitor. The higher concentration of α-amylase inhibitors reduce the average number of eggs, insect emergence and average number of holes and the longer insect development period (Miesho et al., 2017). This is because α-amylase inhibitors target α-amylase enzyme in the insect guts and the insect will suffer from reduced availability of carbohydrates that serve as energy resource (Westermann & Craik, 2010).

Two candidate genes (*Vigun01g234900* and *Vigun02g131000*) and one downstream gene (*Vigun01g201900*), 1482bp far from SNP *2_37091*, associated with median development period were identified (Table 5). Aspartate transaminase is encoded by a candidate gene *Vigun01g234900* involved in extending insect development period through its ability to synthesize carbohydrate and different essential amino acids (Torre et al., 2014). Alpha/beta hydrolase family (ABHs), encoded by the candidate gene *Vigun02g131000*, is associated with housekeeping roles and participates in the breakdown and recycling of cellular metabolites and processing of external nutrients and detoxification of xenobiotics (Long & Cravatt, 2011). It is also involved in increased insect development period (Chang & Hartman, 2017) through its regulatory roles in metabolism and modulating protein lifetime, function and turnover (Van der Hoorn, 2008). The positive allelic effect of the SNPs on the median development period is also another evidence for their involvement in resistance (Burridge et al., 2016). Lin et al. (2016) reported *At2g22250* gene, a homologue to *Vigun02g131000*, in *Arabidopsis thaliana* involved in contributing resistance against biotic stress. Similarly, the downstream candidate gene, *Vigun01g201900*, encoding transcriptional factors were also involved in extending insect development period through its ability to modulate protein synthesis. Chang and Hartman (2017) reported the involvement of genes, encoding transcription factors, for the resistance of soybean to potato leafhopper and soybean looper. Similarly, in arabidopsis, genes with the same function were also reported to enhance insect resistance (Misra et al., 2010). Therefore, the identified gene (*Vigun*01g201900) could also be involved in enhancing cowpea seed resistance to bruchid.

5 | CONCLUSION

The identified candidate genes (*Vigun08g158000*, *Vigun06g053700*, *Vigun02g131000*, *Vigun01g234900* and *Vigun01g201900*) could be used for developing new cowpea varieties by introgressing into farmers preferred but susceptible cowpea genotypes to bruchid. The SNP *2_20268* which was associated with the main resistance traits could be used for marker-assisted selection of cowpea genotypes for resistance to bruchids. The information generated from this study could be used as a tool for analysing the inheritance of the resistance genes, for monitoring transmission of the resistance genes or genomic regions from parents to progeny, and for map-based cloning of the genes. Further work on molecular cloning and

functional analysis of the candidate genes will suggest their roles in bruchid resistance.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts regarding this publication.

AUTHORS' CONTRIBUTION

BM, SK, PR, PG and RE designed the field experiment; BM, MH, and UM conducted the experiment; BM, BA, MGM and POO conducted the statistical analyses and drafted the manuscript. All authors contributed in the interpretation of the data and editing of the manuscript.

ORCID

Belay Miesho https://orcid.org/0000-0002-2297-6077

Awio Bruno https://orcid.org/0000-0001-5269-9149

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