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A novel aphid resistance locus in cowpea identified by combining SSR and SNP markers

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Abstract

The utility of combining simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) marker genotyping was determined for genetically mapping a novel aphid (*Aphis craccivora*) resistance locus in cowpea breeding line SARC 1-57-2 and for introgressing the resistance into elite cultivars by marker-assisted backcrossing (MABC). The locus was tagged with codominant SSR marker CP 171F/172R with a recombination fraction of 5.91% in an F_2 population from 'Apagbaala' x SARC 1-57-2. A SNP-genotyped biparental recombinant inbred line population was genotyped for CP 171F/172R, which was mapped to position 11.5 cM on linkage group (LG) 10 (physical position 30.514 Mb on chromosome Vu10). Using CP 171F/172R for foreground selection and a KASP-SNP-based marker panel for background selection in MABC, the resistance from SARC 1-57-2 was introduced into elite susceptible cultivar 'Zaayura'. Five BC_4F_3 lines of improved 'Zaayura' that were isogenic except for the resistance locus region had phenotypes similar to SARC 1-57-2. This study identified a novel aphid resistance locus and demonstrated the effectiveness of integrating SSR and SNP markers for trait mapping and marker-assisted breeding.

KEYWORDS
Aphis craccivora, backcrossing, cowpea, marker-assisted selection, polymorphism, *Vigna unguiculata*

1 | INTRODUCTION

In cowpea cultivation, attack by insect pests represents an important constraint to economic yields (Blade, Shetty, Terao, & Singh, 1997; Mortimore, Singh, Harris, & Blade, 1997). In the savanna regions of West Africa where the bulk of the world's cowpea crop is produced, the cowpea aphid (*Aphis craccivora*) is the most important insect pest during the vegetative phase of the crop (Obeng-Ofori, 1998, 2007; Singh, Jackai, Dos Santos, & Adalia, 1990). The pest primarily infests the seedlings of cowpea and causes direct damage by sucking phloem sap, resulting in distorted leaves, stunting and plant death. As the crop matures, flowers and pods can be attacked if the insect is not controlled (Jackai & Daoust, 1986). Although cowpea aphid growth, development and fecundity are influenced by the weather, female fecundity is typically high and both adults and nymphs attack the crop. Heavy feeding frequently kills young seedlings (Ofuya, 1995) and may induce delayed flowering and reduced seed set in plants that survive attack (Bohlen, 1978; Jackai & Daoust, 1986). The cowpea aphid also causes indirect damage by transmitting aphid-borne cowpea mosaic viruses (Singh & Jackai, 1985).

Although a wide range of legume species can host the cowpea aphid, cowpea promotes the highest growth and reproduction of the insect (Hamid, Shah, & Anwar, 1977). As many as five biotypes of *A. craccivora* have been observed (Saxena & Barrion, 1987). The insect colonizes wild legume species in the five to six dry months

following the rainy season across the main regions of cowpea production (Ofuya, 1988) and readily infests young cowpea seedlings early in the cropping season (Ofuya, 1991). Research at the International Institute of Tropical Agriculture (IITA) identified sources of *A. craccivora* resistance in cowpea, with antibiosis indicated as the main basis for resistance (Ansari, 1984; Singh, 1977). The breeding line IT84S-2246 is the main source of resistance identified at IITA (Singh, 1977), and from this, a number of breeding lines with aphid resistance were developed at IITA and distributed to cowpea breeding stations worldwide (Bata, Singh, Singh, & Ladeinde, 1987; Ofuya, 1997). Field tests in many locations including Ghana showed that the resistance from IT84S-2246 was not effective against local biotypes of the aphid in many locations (Kusi, Obeng-Ofori, Asante, & Padi, 2010; Messina, Renwick, & Barmore, 1985). Resistance tests in Ghana with IT97K-499-35, bred with the IITA source of resistance, for example, have proven to be highly susceptible to *A. craccivora* (Kusi et al., 2010). Other sources of resistance have been reported, including the line IT97K-556-6, which was found to carry two cowpea aphid resistance quantitative trait loci (QTLs) that were mapped to cowpea linkage groups 1 and 7 (Huynh et al., 2015).

Ongoing research at the CSIR-Savanna Agricultural Research Institute (CSIR-SARI) to identify additional sources of aphid resistance in cowpea revealed a number of advanced breeding lines, including SARC 1-57-2, with high levels of resistance (Kusi et al., 2010). In these tests, lines with the IT84S-2246 source of resistance were not more resistant than the susceptible check, 'Apagbaala'. As such, these CSIR-SARI lines represent valuable sources of resistance for developing cowpea cultivars with resistance to *A. craccivora*.

Owing to susceptibility in existing cultivars, insecticides currently are the only management tactic available for controlling cowpea aphid infestation in Ghana. High reproduction rates increase aphid population densities, outnumbering their natural enemies, which also tend to be susceptible to the same chemicals used to control aphids (Ofuya, 1997). This leads to aphid outbreaks, requiring multiple insecticide applications to achieve reasonable control.

Applying marker-based selection could enhance the efficiency of selection for aphid resistance because phenotypic screening is laborious, expensive and dependent on favourable environmental conditions. Discovery of markers tightly linked to the resistance trait will therefore facilitate early generation selection, reducing the effective size of breeding populations and enhancing the overall efficiency of cultivar development. The current study aimed to genetically map a novel aphid resistance locus and to identify a codominant PCR-based SSR marker associated with the aphid resistance for use in simple foreground selection. Coupled with SNP-based genomewide markers for genetic mapping of the resistance-linked SSR marker and for background selection of the recurrent parent, we tested the combined use of SSR and SNP marker genotyping in this genetic mapping approach and for marker-assisted backcrossing to improve an elite cowpea variety with aphid resistance.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Cowpea cultivars 'Padi Tuya', 'Zaayura', 'Songotra' and 'Bawutawuta' and progeny of the cross between the Ghanaian cowpea cultivar 'Apagbaala' (Padi et al., 2004) and an advanced breeding line SARC 1-57-2 were used in the study. SARC 1-57-2 is an inbred line (F_8) selected from the cross between 'Apagbaala' and a line with exotic pedigree, UCR 01-11-52 (Padi & Ehlers, 2008). SARC 1-57-2 was observed to be resistant to *A. craccivora* under both screen-house and field conditions among a large number of test lines (Kusi et al., 2010). One hundred and sixty-nine (169) F_2 lines of the cross between 'Apagbaala' and SARC 1-57-2 were tested for their reaction to aphid infestation in a screen-house facility using standard protocols (Bata et al., 1987; Kusi et al., 2010). As soon as individual lines could be unambiguously classified into resistant or susceptible classes, they were sprayed with lambda cyhalothrin (Lambda Super®) to control the aphids. Recovered plants were maintained to generate F_3 seeds for progeny testing. DNA was obtained by the CTAB method (Dellaporta, Wood, & Hicks, 1983) from each of the field-grown F_2 plants and refrigerated until needed. Each F_2-derived F_3 family ($F_{2:3}$) was tested further for phenotypic reaction to the aphid at 3-4 days after emergence using 20 seedlings per family.

2.2 | Identification of markers associated with aphid resistance

Fifty previously confirmed SSR primer pairs (Cowpea Genomics Knowledge Base (CGKB); http://cowpeagenomics.med.virginia.edu/CGKB) randomly distributed across the cowpea genome were used in this study (Table S1). The primers were tested for their ability to generate reproducible banding patterns in the parents of the mapping population, and those which were non-polymorphic were discarded. PCR conditions consisted of denaturing at 94°C for 3 min, annealing at 56°C for 30 s and extension at 72°C for 30 s. This cycle was repeated 35 times followed by a final extension at 72°C for 10 min. The subset of primers that were polymorphic between the parents and produced clear reproducible bands were tested by bulked segregant analysis (BSA) on two groups of five resistant and five susceptible F_2 individuals based on phenotypic classification of aphid-infested F_2 plants. Primer pairs that showed polymorphism between the two bulks of lines following denaturing polyacrylamide gel electrophoresis (h-PAGE 81-2325 by Galileo Biosciences, dimension of tank: 32 cm $W \times$ 37.5 cm $L \times$ 10.5 cm H; dimension of plate: 24.5 cm $W \times$ 27.5 cm L) were then run on all 169 individuals to test for their association with aphid resistance.

2.3 | Genetic mapping and physical location of the SSR marker

The SSR marker CP 171F/172R associated with aphid resistance was assayed on parents of 10 recombinant inbred line (RIL) mapping

populations (Lucas et al., 2011) to identify populations segregating for this SSR. DNA from these parents and the selected RIL mapping population CB27 x IT97K-556-6 was extracted and concentrated as described in Muchero et al. (2009). The RIL parents and selected RIL population were genotyped for CP 171F/172R using PCR conditions as described earlier. The PCR products were electrophoresed on 3% agarose gel at 150 V for 150 min. The gels were photographed under UV light using a Bio-Rad Universal Hood II (Bio-Rad laboratories Inc., www.bio-rad.com) imaging machine. SNP genotype data for the RIL population were obtained from Lucas et al. (2011) generated from the 1536-SNP cowpea GoldenGate assay (Muchero et al., 2009). Linkage maps were constructed using QTL IciMapping 4.1 software (http://www.isbreeding.net) using the Kosambi function, RECORD ordering algorithm and map orientation using the cowpea consensus genetic map available at HarvEst:Cowpea (http://harvestweb.org/). The physical location of each SNP marker was determined from the cowpea genome V.1.0 (Lonardi et al., 2017; https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er). The physical location of the SSR marker CP 171F/172R was determined by blasting the marker primer sequences against the sequence flanked by the two SNPs 1_0501 and 1_0140.

2.4 | Selection of recurrent parent

The SSR marker CP 171F/172R associated with aphid resistance (foreground marker) was assayed on Ghana cowpea cultivars 'Padi Tuya', 'Zaayura', 'Songotra' and 'Bawutawuta', which had been shown previously to be susceptible to the cowpea aphid. DNA samples were taken from leaf tissue of the four cultivars plus the standard resistant (SARC 1-57-2) and susceptible ('Apagbaala') genotypes. The samples were taken 2 weeks after planting using FTA cards, which were washed by standard procedure, and the PCR was run using the CP 171F/172R primers. The PCR products were run on a non-denaturing h-PAGE II gel. The banding patterns of the two parents and four susceptible cultivars were analysed to determine which of the cultivars was polymorphic with SARC 1-57-2 for the target SSR marker for use as the recurrent parent in marker-assisted backcrossing (MABC). As described in the results section, 'Zaayura' was chosen as the recurrent parent for MABC.

2.5 | Marker-assisted backcrossing

A cross was made between SARC 1-57-2 and 'Zaayura', and the F_1 was backcrossed to 'Zaayura' as the recurrent parent to generate 20 BC_1 lines. All individuals were genotyped to select plants heterozygous for the region of the foreground marker CP 171F/172R. These plants were phenotyped in a screen-house facility to confirm their resistance reaction to the aphid. Three resistant BC_1 plants were used for backcrossing to the recurrent parent to generate BC_2 individuals. This cycle of crossing, identification of lines heterozygous for the foreground marker and screen-house confirmation of resistance was repeated until BC_4 plants were obtained.

Dried leaf samples of the parents SARC1-57-2 and 'Zaayura' were sent in zip-lock bags containing desiccant packs to University of California—Riverside for SNP genotyping with the Kompetitive allele-specific polymerase chain reaction (KASP) assay (LGC Genomics Ltd., Hoddesdon, UK) (Semagn, Babu, Hearne, & Olsen, 2014). A subset of 137 SNP markers that were polymorphic between the two parents and spaced at least 2 cM apart across 11 linkage groups of the cowpea consensus genetic map (Lucas et al., 2011) were used to genotype 81 BC_4F_1 plants with the KASP assay.

A BC_4F_1 plant heterozygous for the region of the marker and carrying the highest proportion of 'Zaayura' background was selfed to generate 100 BC_4F_2 lines. These lines were genotyped with the foreground marker CP 171F/172R to select plants that were homozygous for the SARC 1-57-2 resistance-linked allele. The lines were also phenotyped in aphid-infested screens to confirm resistance and then bulked for larger scale field testing.

2.6 | Data analysis

Chi-square tests were performed to test the goodness of fit of observed data (segregation among F_2 plants and $F_{2:3}$ families) to a single dominant gene model. Similarly, the segregation pattern of SSR markers was tested for goodness of fit to that of a single locus model. Segregation among $F_{2:3}$ families was analysed after classifying each family as homozygous resistant (all plants showing the same vigour as non-infested controls), homozygous susceptible (all plants dead by 10 days after infestation) or heterozygous (both resistant and susceptible plants identified within a family).

3 | RESULTS

3.1 | Inheritance of aphid resistance in SARC 1-57-2

Only two parental phenotypes ('Apagbaala', susceptible; SARC 1-57-2, resistant) were observed in the F_2 population of 'Apagbaala' × SARC 1-57-2. On resistant plants, aphid colonies increased in numbers slowly on inoculated trifoliates within the first 10 days, such that it was easy to count the total number of aphids per plant. Susceptible infested plants were overcrowded with aphids and death of seedlings began 10 days after inoculation. The observed segregation ratio was 123:46 (resistant: susceptible plants), which fit a 3:1 ratio ($\chi^2 = 0.44$; $p = .505$) expected for the segregation of a single dominant gene. After spraying the plants with insecticide to kill the aphids, only 108 resistant plants (88% recovery) and 20 susceptible plants (43% recovery) established successfully in the field and produced a minimum of 20 progeny. Due to genetic distortion caused by high mortality of susceptible F_2s, only 108 $F_{2:3}$ families derived from the 108 resistant F_2 plants were tested further. Among the 108 $F_{2:3}$ families, 35 were uniformly resistant and so were classified as being derived from homozygous resistant F_2 plants, while 73 segregating families were considered as having being derived from heterozygous F_2 plants, based on the response of 20 individuals from each $F_{2:3}$ family. This $F_{2:3}$ ratio of 35:73 fit

the 1:2 resistant (homozygous)/segregating (heterozygous) ratio expected for the segregation of a single dominant gene ($\chi^2 = 0.042$; $p = .838$).

3.2 | Identification of markers associated with aphid resistance

Of the 50 SSR markers tested, 31 amplified from a template of DNA bulked from either five resistant or five susceptible individuals, and produced reproducible banding patterns following denaturing PAGE. However, only four primer pairs (CP171F/CP172R, MS50F/MS50R, Y31F/Y31R and CP573F/CP573R) showed polymorphism between the two classes of resistant and susceptible lines. Of these, only CP 171F/172R (left sequence: 5′-CATAGTAATATGGTATGTCAGTA-3′; right sequence: 5′-CAACCGATGTAAAAAGTGGACA-3′) displayed a segregation pattern consistent with the phenotypic scores obtained following aphid infestation of the 169 lines (Figure S1). The expected product size of 176 bp based on information in the cowpea genomics database (http://cowpeagenomics.med.virginia.edu/CGKB/) was observed following PAGE (Figure 1). CP 171F/172R behaved as a codominant marker and segregated in the expected 1:2:1 fashion ($\chi^2 = 0.25$; $p = .856$) in the F_2 population. Based on the SSR marker and phenotypic data of 169 $F_{2:3}$ lines, there were 10 cases where either a resistant plant carried the 'Zaayura' CP 171F/172R allele or where a susceptible plant carried the SARC 1-57-2 (resistant parent) allele. These segregants represented recombinants between marker CP 171F/172R and the resistance locus.

3.3 | Genetic mapping of SSR marker CP 171F/172R

Among RIL populations that were segregating for SSR marker CP 171F/172R, the population CB27 x IT97K-556-6 (87 RILs), which was used to map QTL for aphid resistance in California (Huynh et al., 2015), was selected for the genetic mapping of CP 171F/172R. The SSR marker was mapped on linkage group (LG) 10 of the CB27 x IT97K-556-6 genetic map at position 11.5 cM and flanked by SNP markers 1_0501 and 1_0140 spanning from 10.3 cM to 12.1 cM (Figure 2). The physical location of SSR marker CP 171F/172R was at 30.514 Mb (Figure 2). The physical distances of the LG 10 SNP markers also are indicated on Figure 2. LG 10 corresponds to Vu10 in the new cowpea pseudomolecules designation (Lonardi et al., 2017; see https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er). QTL mapping using phenotypic data from Huynh et al. (2015) confirmed that CP 171F/172R was not associated with the two aphid resistance QTLs identified on LG 1 (Vu05) and LG 7 (Vu02) in the African cowpea genotype IT97K-556-6, which confer resistance to cowpea aphid populations in California.

3.4 | Test for polymorphism

Among the four susceptible cultivars that were tested as a possible recurrent parent for MABC, SSR marker CP 171F/172R showed polymorphism between 'Zaayura' and the resistant cultivar SARC 1-57-2 (Figure S2).

3.5 | Validation of F_1 plants

Results from genotyping with marker CP 171F/172R the F_1 plants generated from crossing SARC 1-57-2 and 'Zaayura' prior to backcrossing are presented in Figure S3. Four samples of susceptible parent 'Zaayura' and three samples of resistant parent SARC 1-57-2 were included as checks. All the putative F_1s were heterozygous for the codominant marker CP 171F/172R, confirming that each was a genuine hybrid.

3.6 | Genotyping to select heterozygotes from the backcross populations

Examples of results from individual plants from successive backcross populations (BC_1 to BC_4) genotyped for their CP 171F/172R marker banding pattern are presented in Figure S4. Based on chi-square tests for goodness of fit, the segregation ratios fit the expected 1:1 ratio for heterozygous and homozygous susceptible individuals ($\chi^2 = 0.138$; $p = .710$).

3.7 | SNP genotyping for background selection

Results of SNP genotyping for background selection are presented in Table S1, showing the individual plants and the percentage of the

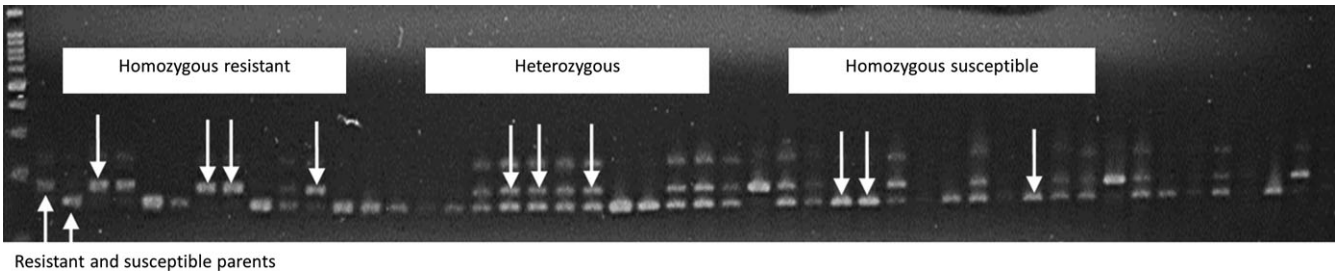

FIGURE 1 h-PAGE showing the DNA banding patterns of F_2 plants derived from Apagbaala × SARC 1-57-2 amplified by SSR marker CP 171F/172R. The faster migrating banding is from susceptible Apagbaala, and the slower banding is from resistant SARC 1-57-2 (the third band in the heterozygous bands should be ignored)

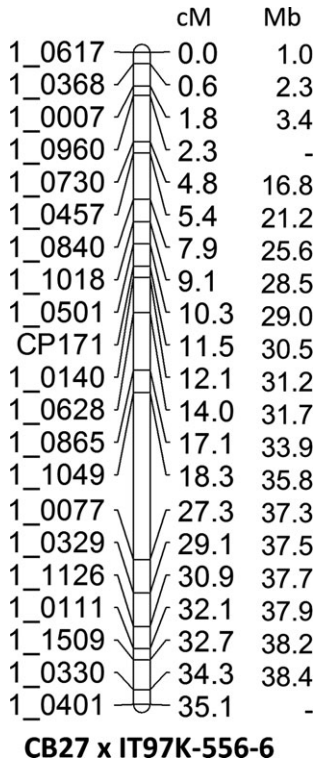

FIGURE 2 Position of the SSR marker CP171F/172R at 11.5 cM (physical position at 30.514 Mb) on cowpea linkage group 10 of the RIL mapping population CB27 x IT97K-556-6, equivalent to Vu10 of the cowpea pseudomolecules (SNP physical positions are given in Mb next to genetic positions in cM). The physical location of each marker was determined from the cowpea genome V.1.0 (Lonardi et al., 2017; https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Vunguiculata_er)

background of 'Zaayura' recovered. Individual plant number 148, which expressed resistance to aphid, had recovered 95% of the 'Zaayura' background. Based on marker profiles of 81 BC_4F_1 individuals, there was no significant linkage between CP 171F/172R and any of the 137 SNPs, including the SNP markers 1_0906 and 1_0755 that flank a major QTL region for aphid resistance on LG 7 (Vu02) in a different mapping population (Huynh et al., 2015).

3.8 | Selection of BC_4F_2 homozygous lines

The selected individual (plant number 148) that was shown to be resistant and had recovered 95% of the recurrent parent background was selfed to generate a BC_4F_2 population, which was genotyped to select individual plants homozygous for the resistance-associated marker allele. A subset of the BC_4F_2 SSR marker genotypes is given in Figure S5. For the 60 BC_4F_2 plants, the CP 171F/172R marker segregation was 13:31:16 (homozygous resistant: heterozygous: homozygous susceptible) which fit the expected ratio of 1:2:1 for a single dominant gene ($\chi^2 = 0.37$; $p = .83$). The BC_4F_2 plants homozygous for the SARC 1-57-2 allele at marker CP 171F/172R were phenotyped in aphid screens to confirm that they were resistant to aphids. Five plants showing an aphid resistance phenotype were selected for multiplication and were bulked for field evaluation.

4 | DISCUSSION

The discovery of aphid resistance in a local × exotic cowpea cross ('Apagbaala' × UCR 01-11-52, Kusi et al., 2010) provided the impetus to initiate breeding for resistance to this insect pest to improve existing cultivars. Previously reported sources of resistance were found ineffective in Ghana (Singh, 2004) and because 'Apagbaala' is highly susceptible, the source of the resistance was identified to be UCR 01-11-52. UCR 01-11-52 is a breeding line developed at the University of California, Riverside, from a cross between California Blackeye No. 46 and a large seeded Brazilian cultivar 'Montiero', and it was selected due to its attractive large grain size and earliness to flowering (Padi & Ehlers, 2008). The 'CB46' cultivar is susceptible to cowpea aphid; thus, Montiero must have been the resistance donor into UCR 01-11-52. We used an F_8 inbred line, SARC 1-57-2, derived from the 'Apagbaala' × UCR 01-11-52 population as the resistance donor in the current study.

Due to the simple monogenic inheritance of the resistance found in SARC 1-57-2 and the ease of distinguishing resistant from susceptible plants in aphid resistance bioassays, breeders will be able to rapidly convert existing susceptible cowpea cultivars into aphid-resistant cultivars using efficient backcross breeding procedures. Despite the ease of distinguishing resistant and susceptible plants in phenotyping screens, conducting the entire resistance bioassay in large populations is tedious due to the need to maintain aphids on live plants and to use nymphs of the same age for infestation. Moreover, relative humidity and temperature influence the growth and survival of aphids under screen-house conditions, which can lead to inefficiencies in selection. Our discovery of a codominant SSR marker linked to the aphid resistance locus is an important facilitator of marker-assisted selection (MAS), which is simpler than phenotypic screening and saves time, resources and labour (Akhtar et al., 2010). Moreover, homozygous and heterozygous resistant plants cannot be distinguished by the phenotypic screening, requiring further progeny testing to select desirable plants. Considering that marker CP171F/172R is several centiMorgans in genetic distance from the resistance gene locus based on our finding of 10 of 169 recombinants in the segregating population, the most practical use of this marker may be its application in reducing the number of plants in a population prior to phenotypic screening.

To our knowledge, this is the first account of linkage between a codominant SSR marker and an unknown aphid resistance locus with strong effect on *A. craccivora* infestation in cowpea. Based on its location on linkage group 10 of the cowpea consensus genetic map (Figure 2), the CP171F/172R marker linked resistance in SARC 1-57-2 is independent from the two aphid resistance QTLs recently discovered on cowpea LG 1 (Vu05) and 7 (Vu02) (Huynh et al., 2015) of the CB27 x IT97K-556-6 RIL population when screened in California. Thus, an important finding from this study is the

occurrence of a novel aphid resistance gene that differs from other mapped aphid resistance loci in cowpea. As SARC 1-57-2 and IT97K-556-6 carry different cowpea aphid resistance genes, combining these sources in breeding programmes may enable development of cowpea lines with more broadly effective and durable resistance.

Due to the incomplete association between CP 171F/172R and aphid resistance in the BC_4F_1, mapping of CP 171F/172R and surrounding SNP markers within the target region of the genome is needed to identify markers more closely linked to the resistance locus. The identification of this single resistance locus and its position on the cowpea genetic map will facilitate the deployment of resistance as a component of integrated management of *A. craccivora* in West Africa.

The tests for polymorphism between the donor of the resistance locus (SARC 1-57-2) and the susceptible elite cultivars determined that only the cultivar 'Zaayura' was suitable as a recurrent parent for genetic improvement when employing the linked SSR marker. The sizes of the populations created and phenotyped for resistance in the screen-house studies in the various generations were reduced significantly by selecting only for segregants heterozygous for the resistance-linked marker locus. This reduced the total amount of time spent in selecting individuals that served as parents for the subsequent generation, and increased the overall efficiency of the backcross method of transferring the resistance locus. Segregation distortion, although common in mapping populations (Lorieux, Goffinet, Perrier, Gonzalez de Leon, & Lanaud, 1995), was not observed in the current study. At each generation, the segregation was consistent with the behaviour of a single gene.

At the BC_4F_1 generation where on average over 90% of the background of the recurrent parent was recovered and the aphid resistance gene was homozygous in most lines, SNP genotyping with a genomewide polymorphic set of 137 SNPs was used successfully for background selection. The plant sample identified with the resistance phenotype and with 95% recovery of the background of susceptible 'Zaayura' was selfed to generate BC_4F_2 individuals. Genotyping the BC_4F_2 population enabled selection of individuals having the aphid resistance gene in a homozygous resistant state. These individuals were subjected to further phenotypic selection based on the desirable features of the recurrent parent (vegetative characters, podding and seed traits), and the seeds of the improved individuals were subsequently multiplied. In a preliminary field evaluation of the improved 'Zaayura' carrying aphid resistance, it was determined that all the physical features of the original 'Zaayura' had been recovered. This indicated that advancement to the BC_4 generation was adequate to regain the background of the recurrent parent. Seeds of the improved 'Zaayura' have been multiplied and presented for assessment, and approval has been given to release the improved 'Zaayura' as a new cowpea variety in Ghana.

Despite the limitation of the SSR marker CP 171F/172R, it was deployed successfully in this study in combination with intermittent aphid resistance phenotyping screening and the use of genomewide SNP genotyping to optimize recovery of the recurrent parent background in a coordinated backcrossing programme. Development of improved cv. 'Zaayura' was achieved within 2 years using this approach, which would not be possible under a conventional backcrossing approach. Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progeny resulting from hybridization. It is often time-consuming as breeding a new variety in many crops including cowpea can take eight to twelve years, and even then the release of an improved variety is not guaranteed (Ibitoye & Akin-Idowu, 2010). Molecular marker-assisted selection offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in crop breeding (Ibitoye & Akin-Idowu, 2010). Thus, molecular markers bring a systematic basis to traditional breeding, enhancing its precision and expediting the process (Collard, Jahufer, Brouwer, & Pang, 2005; Kumar, 1999). The current study has shown that the SSR-SNP dual marker approach enabled the genetic mapping of the resistance locus and its identification as a novel resistance gene, unique from previously mapped resistance loci in the cowpea genome. In addition, we have shown the high efficiency in backcrossing of combining foreground and background marker-based selection using both SSR and SNP markers.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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