Received: 22 October 2020 | Revised: 6 September 2021 | Accepted: 17 September 2021

DOI: 10.1002/leg3.127

ORIGINAL ARTICLE

Legume Science Open Access WILEY

Determination of inheritance of aphid resistance in cowpea genotypes and identification of single sequence repeat markers linked to resistance genes

Adelaïde P. Ouedraogo[1] | Agyemang Danquah[2] | Jean-Baptiste Tignegre[1,3] | Leandre S. Poda[1] | Joseph B. Batieno[1] | Isaac K. Asante[2] | Jeremy T. Ouedraogo[1,4] | Jonathan N. Ayertey[2] | Kwadwo Ofori[2]

[1]Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso

[2]West Africa Centre for Crop Improvement (WACCI), Accra, Ghana

[3]AVDRC-The World Vegetable Center, Tainan, Taiwan

[4]African Biosafety Network of Expertise (ABNE), Dakar, Senegal

Correspondence
Adelaïde P. Ouedraogo, Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso.
Email: opingdewinde@gmail.com

Abstract

An understanding of the mode of inheritance is fundamental to plant breeding. The choice of an appropriate selection method considers the number of genes involved and their modes of action. In plant breeding, markers can overcome the false resistance that may arise from greenhouse screening. The goal of this study was to facilitate the better use of cowpea genotypes resistant to aphids. Specifically, we sought to determine the number of genes involved in the ability of cowpeas to survive aphid infestation and identify single sequence repeat (SSR) markers linked to aphid resistance genes. Two pairs of parents (Tiligre × KVX295-124-2-99 and Tiligre × IT97K556-6) were used to generate two F_2 populations. Each of these populations was screened with aphids to determine the mode of inheritance of resistance to aphids in cowpeas. SSR markers were used to screen parent, F_1, and F_2 individuals to locate polymorphic markers and markers linked to the genes involved in resistance to aphids. The results showed that the ability to survive under aphid infestation in KVX295-2-124-99 and IT97K556-6 is controlled by two nonallelic genes. The markers MA61 and MA70 were found linked to aphid resistance in cowpea.

KEYWORDS
aphids, cowpeas, polymorphic markers, SSR

1 | INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is a legume with significant economic importance worldwide as human food and animal feeding. Its cultivation is quite economical given its low input requirement and rapid maturity (Badhe et al., 2016). However, plant biotic and abiotic stresses pose serious threats to cowpea production. Insects are considered largely responsible as 90%–100% yield reduction may be caused by them (Jackai & Daoust, 1986; Murdock, 2002). *Aphis craccivora* Koch has been described as one of the most important pests of cowpeas causing significant yield losses from the seedling to adult stages (Annan et al., 1996). It is also a major vector of plant viruses. Several insecticides are effective against insects such as leafhoppers (*Empoasca* sp.), aphids, and whiteflies (*Bemisia tabaci*) (Oyewale & Bamaiyi, 2013). However, occasionally, farmers must spray their farms as many as eight to ten times during the growing

season (Omongo et al., 1998). This results in environmental pollution, toxicity to mammals, and hazards to users and consumers (Alabi et al., 2003). Pest resurgence, resistance to insecticides, and lethal effects to nontargets have also been observed. Therefore, the use of resistant varieties appears to be a good alternative, because it is more viable and economical for resource-poor farmers and compatible with other methods of pest control (Ofuya, 1997). Several sources of resistance to aphids have been identified (Boukar et al., 2018; Kusi et al., 2010; Omoigui et al., 2017; Ouédraogo et al., 2018; Souleymane et al., 2013). For better use of these genetic resources, the mode of inheritance must be determined. In cowpeas, investigations have been conducted to determine the mechanisms of resistance to aphids, and genetic studies have revealed that a single dominant gene controls resistance in cowpeas (Ombakho et al., 1987; Singh & Ntare, 1985). However, Pathak (1991) observed that although resistance to certain pests can be controlled by a single major gene, the expression of the major gene can be influenced by changes that could improve or delay its expression. Githiri et al. (1996) suggested that two genes may be involved in the expression of aphid resistance. Recently, Huynh et al. (2015) used molecular markers to identify two quantitative trait loci (QTLs) associated with genetic response of cowpea varieties to aphid infestation. For efficient breeding, the mode of inheritance of the trait of interest must be determined. The choice of an appropriate selection method considers the number of genes involved and their modes of action (Sharma & Vaishampayan, 2009). It is also important to develop diagnostic markers for marker-assisted selection (MAS) to ensure resistance diversity (Xu et al., 2014). The use of DNA markers linked to resistant genes is also a means to overcoming false resistance that may arise from greenhouse screening (Hittalmani et al., 2000). MAS can be a very efficient tool in breeding, especially for traits that are labor intensive to the phenotype (Gepts et al., 2005; Ordon et al., 2009). The goal of this study was to increase the use of cowpea genotypes resistant to aphids and the use of molecular markers in the breeding process. The specific objectives were to:

- Determine the number of genes involved in the ability of cowpeas to survive aphid infestation.
- Identify SSR markers linked to gene(s) involved in aphid resistance.

2 | MATERIALS AND METHODS

2.1 | Plant and aphid genetic materials

In this study, lines KVX295-2-124-99 and IT97K556-6, both resistant to aphids, were crossed with Tiligre (KVx775-33-2G) (a commercial but aphid susceptible line) to generate two F_2 populations. The F_1 individuals from the cross Tiligre × KVX295-124-2-99 and those from Tiligre × IT97K556-6 were self-pollinated to produce 179 and 197 F_2 individuals, respectively. These F_2 populations were screened using the Kamboinse aphid biotype. Parental lines, F_1 and F_2, were also used to identify markers associated with aphid resistance. Aphids used for screening were collected in September 2018 at Kamboinse and reared on a susceptible variety (KVX 396-4-5-2D).

2.2 | Green house screening process

The screening was conducted using see-through plastic pots. There was one plant per pot. Seven-day-old cowpea seedlings were infested. Ten aphids were collected with a camel hair brush and deposited on individual plants. Water was supplied to the plant directly at the foot to prevent aphid stalling. Plants remained under aphid infestation throughout the test. For this purpose, each pot was covered with aphid-proof tissue held by small wooden beams. Elastic ribbons were used to close the set hermetically. To avoid water stagnation, the bottoms of the pots were provided with apertures.

2.3 | Molecular analysis

DNA extraction was conducted using FTA (Flanders Technology Associates) technology. FTA cards are media for storage and transport of DNA, wildly used for plant as well as animal DNA extraction (Kusi et al., 2018; Perozo et al., 2006; Stangegaard et al., 2011). To purify the DNA, a 1 cm^2 piece of the card containing macerated leaves was isolated using punch tool and placed in a tube (Eppendorf). The piece was washed with specific buffers according to the following procedure: (i) two successive washes of 5 min each with 200 µl of alcohol (70°C) per piece; (ii) two successive rinsing of 5 min each with 200 µl of Tris EDTA (TE) per piece; (iii) drying at room temperature for 2 h. Polymerase chain reaction (PCR) was performed in an Eppendorf Master Cycler Gradient thermocycler. It consisted of DNA denaturation at 94°C during 3 min, hybridization 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 PCR products were electrophoresed on 3% agarose gel (3 g of agarose flour for 100 ml of Tris-Borate-EDTA [TBE] 0.5% concentrate) containing 5 µl of ethidium bromide (BEt). The migration lasted 1 h under 75 V at 400 mA. The gels were photographed with a camera (Canon Power Shot A620, 7.1 Mega Pixels). Markers with DNA bands, showing evidence of polymorphism between the resistant and susceptible parents, were regarded as candidate markers linked to the resistance gene(s) and were further investigated. This investigation consisted of screening candidate markers on 12 of the most susceptible and 14 of the most resistant F_2 individuals. Phenotypic and genotypic data from this screening were subjected to a chi-squared (χ^2) test for association. Then, all F_2 individuals from each of the two populations were screened with the validated marker. The genotypic data generated from this screening were combined with the phenotypic data and analyzed to confirm the mode of inheritance using a chi-squared test for goodness-of-fit. The sequences of the validated markers were BLAST aligned to identify the chromosomes on which the genes of interest were located.

2.4 | Data collection and analysis

Observations were made every 3 days. In this study, susceptible plants were defined as those that died on the first day after screening up to 1 month after screening. The resistant plants were those that survived. Chi-squared tests for goodness-of-fit were used to evaluate deviations in the observed data from the expected segregation ratios. These expected ratios were as follows: 3:1 (one dominant gene or two linked genes), 15:1 (two duplicated genes), 9:7 (two complementary genes), and 63:1 (three independent genes). Chi-squared tests for association were used to determine whether there was an association between the genotypic and phenotypic data. R version 3.5.1 was used to conduct the tests and the BLAST function of Phytosome v13 (https://phytozome-next.jgi.doe.gov/) was used to determine the location of genes.

3 | RESULTS

For Population 1 (Tiligre × KVX295-2-124-99) and population 2 (Tiligre × IT97K556-6), all *P* values were less than 0.05 (Table 1). However, these results could be grouped into two categories. The first category concerns tests where the *P* value was lower than 0.001, and the second category concerned tests where the *P* value was higher than 0.001. Thus, for Population 1, in the case of one dominant gene or two linked genes without crossing over (3:1), the difference between the observed and expected values was highly significant ($\chi^2 = 20.03$; *P* value $= 7.6 \times 10^{-6}$). In the same population, for two duplicated genes (15:1), the difference between the observed and expected values was not significant at the 0.01 threshold ($\chi^2 = 5.69$; *P* value $= 0.017$). In the same population, the observed and expected values were significantly different for cases where there were two complementary genes and three genes ($\chi^2 = 80.59$ and 94.65, respectively; *P* values $= 2.2 \times 10^{-16}$ for both).

For Population 2 (Tiligre × IT97K556-6), in the case of one dominant or two linked genes, the observed and expected data were significantly different ($\chi^2 = 20.10$; *P* value $= 7.34 \times 10^{-6}$). In the case of two duplicated genes, the χ^2 value was 8.13, and the *P* value was 0.004. In this case, the *P* value was higher than 0.001. In the cases involving two complementary genes and three genes, the difference between the observed and expected values was significant ($\chi^2 = 84.98$ and 118.16, respectively; *P* value $= 2.2 \times 10^{-16}$ for both).

3.1 | Polymorphism test and markers validation

3.1.1 | Polymorphism test

Of 157 SSR markers used to screen the parents for resistance to aphids, five polymorphic markers were located: MA70, MA61, MA112, MA118, and VM68. Among these polymorphic markers, two were selected as candidate markers for validation: MA61 for Population 1 and MA70 for Population 2 (Table 2 and Supporting Information). For marker MA61, the band for the resistant parent (MA61R) had 150 bp, and the band of the susceptible parent (MA61S) had 280 bp (Figure 1). For marker MA70, the band of the resistant parent (MA70R) had 180 bp, and the band of the susceptible parent (MA70S) had 280 bp (Figure 2).

3.1.2 | Marker validation

For each F_2 population (Tiligre × KVX295-2-124-99 and Tiligre × IT97K556-6), 14 resistant individuals and 12 susceptible

TABLE 2 Sequences of markers MA61 and MA70

Marker name	Sequences F–R (5′-3′)
MA61	GATGTTATACACAGCAGCAAC
	GGGAATCGAAAACAGACGCTA
MA70	GACTAGTGCAAGTTCCCAACC
	GAAGCAGAACCCAAAGAATCT

TABLE 1 Test for genetic ratios

Parents	Assump.	Ratio	N.P.	O.V. R	O.V. S	E.V. R	E.V. S	df	dfi	χ^2 value	*P* value
Tiligre × KVX295-2-124-99	1 dominant gene or 2 linked genes	3:1	180	161	19	135	45	1	179	20.03***	7.6×10^{-6}
Tiligre × KVX295-2-124-99	2 duplicat. genes	15:1	180	161	19	169	11	1	179	**5.69***	**0.017**
Tiligre × KVX295-2-124-99	2 Compl. genes	9:7	180	161	19	101	79	1	179	80.59***	$<2.2 \times 10^{-16}$
Tiligre × KVX295-2-124-99	3 genes	63:1	180	161	19	177	3	1	179	94.64***	$<2.2 \times 10^{-16}$
Tiligre × IT97K556-6	1 dominant gene	3:1	197	175	22	148	49	1	196	20.10***	7.3×10^{-6}
Tiligre × IT97K556-6	2 duplicat. genes	15:1	197	175	22	185	12	1	196	**8.13****	**0.004**
Tiligre × IT97K556-6	2 Compl. genes	9:7	197	175	22	111	86	1	196	84.98***	2.2×10^{-16}
Tiligre × IT97K556-6	3 genes	63:1	197	175	22	194	3	1	196	118.16***	2.2×10^{-16}

Abbreviations: Assump., assumptions; Compl., complementary; df, degree of freedom of ration categories; dfi, degree of freedom of individuals; duplicat., duplicate; E.V., expected value; N.P., total number of plants; O.V., observed value; R, resistant; S, susceptible.

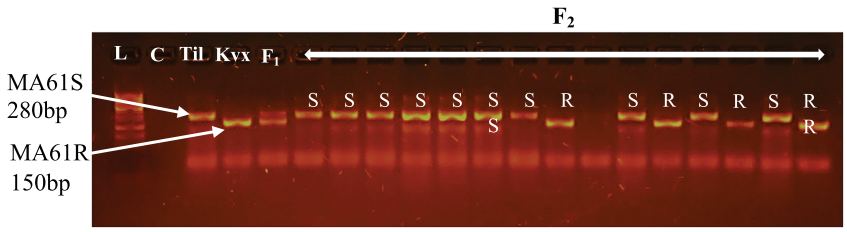

FIGURE 1 Agarose gel profile of parental lines, F_1 and $15F_2$. L = DNA lader; C = Control; Kvx = Kvx295-2-124-99; Til = Tiligre

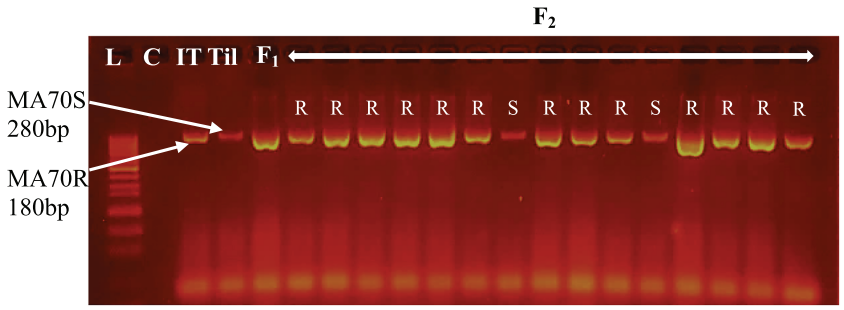

FIGURE 2 Agarose gel profile of parental lines, F_1 and $15F_2$ screened with marker MA70. L = DNA lader; C = Control; IT = IT97K556-6; Til = Tiligre

TABLE 3 Distribution of most susceptible and most resistant F_2 plants screened with MA61 per genotype

	Unmatched	GR	GS	Total
PR	3	7	4	14
PS	4	0	8	12
Total	7	7	12	26

Abbreviations: GR, genotypically resistant; GS, genotypically susceptible; PR, phenotypically resistant; PS, phenotypically susceptible.

TABLE 4 Distribution of most susceptible and most resistant F_2 plants screened with MA70 per genotype

	GR	GS	Total
PR	10	4	14
PS	2	10	12
Total	12	14	26

Abbreviations: GR, genotypically resistant; GS, genotypically susceptible; PR, phenotypically resistant; PS, phenotypically susceptible.

individuals were screened with the two polymorphic markers. MA61 was used to screen Population 1 (Tiligre × KVX295-2-124-99), and MA70 was used to screen Population 2 (Tiligre × IT97K556-6). Genotypic and phenotypic data obtained from row data (Supplementary Information) are presented in Tables 3 and 4. These contingency tables were used to compute association tests. The results of these tests revealed a relationship between genotype and phenotype for marker MA61 ($\chi^2 = 5.56$; P value = 0.018) (Table 5). The test of association for marker MA70 also showed a relationship between phenotype and genotype ($\chi^2 = 5.75$; P value = 0.016) (Table 5).

Markers MA61 and MA70 were also screened with F_1 and F_2 individuals. The band of the resistant parent and that of the susceptible parent were both detected in F_1 individuals in both populations (Figures 1 and 2). The sequences of the two markers indicated that marker MA61 was located on chromosome number 3 (Vu03) with an E-value of $1.36e^{-2}$, and the percentage of identity ranged from 96% to 100%. Marker MA70 was located on chromosome number 7 (Vu07) with the percentage of identity ranging from 89% to 100% with E-value of $1.11e^{-3}$ (Table 6).

TABLE 5 Association between markers and phenotypic characters

Markers	X^2	df	dfi	*P* value
MA61	5.56	1	25	0.0184*
MA70	5.75	1	25	0.0165*

3.1.3 | Mode of inheritance based on phenotyping and genotyping

The results of the chi-squared test for goodness-of-fit of combined phenotyping and genotyping data are shown in Table 7. Based on the results in Table 1, the test was limited to two duplicated genes; the rest of the assumptions (one dominant gene, two linked genes, two complementary genes, and three genes) were unlikely.

With a test ratio equal to 15:1 for both, Population 1 (Tiligre × KVX295-2-124-99) and Population 2 (Tiligre × IT97K556-6), the difference between the observed and expected data were not significant ($\chi^2 = 3.78$; P value = 0.052 for Population 1 and $\chi^2 = 0.58$; P value = 0.44 for Population 2) (Table 7).

TABLE 6 Marker locations on chromosomes

Marker name	Location	Species	E-value	% identity
MA61	Vu03	*V. unguiculata* V1.1	1.36e-2	96
	Vu03	*V. unguiculata* V1.1	1.36e-2	100
MA70	Vu07	*V. unguiculata* V1.1	1.11e-3	89
	Vu07	*V. unguiculata* V1.1	1.36e-2	100

Abbreviation: *V. unguiculata, Vigna unguiculata*.

TABLE 7 Determination of number of genes based on F_2 individuals' genotypes and phenotypes

Couple of parents	Assumptions	Test ratio	N. P.	O.V.		E.V.		df	dfi	χ^2 value	*P* value
Tiligre × KVX295-2-124-99	2 dup. genes	15:1	173	156	17	162	11	1	172	3.78^{ns}	0.052
Tiligre × IT97K556-6	2 dup. genes	15:1	182	170	14	172	12	1	181	0.58^{ns}	0.44

Abbreviations: df, degree of freedom of ration categories; dfi, degree of freedom of individuals; dup., duplicated; E.V., expected value; N.P., total number of plants; O.V., observed value.

4 | DISCUSSION

4.1 | Phenotyping and mode of inheritance

To determine the genetic ratio that best fit the observed data, a threshold of 0.001 was used. This threshold is often used for statistical tests in plant breeding (Benjamin et al., 2018; Betensky, 2019; Mwadzingeni et al., 2017). Phenotypic data showed that two duplicate genes were involved in the ability of cowpea to survive aphid infestation. Population 1 (Tiligre × KVX295-2-124-99) had a *P* value of 0.017 ($\chi^2 = 5.69$). Population 2 (Tiligre × IT97K556-6) had a *P* value equal to 0.004, indicated that the duplicate genes were involved in the ability of the two lines (IT97K556-6 and KVX295-2-124-99) to survive aphid infestation. The remaining ratios tested were less likely to fit the observed data. In the case of one dominant gene or two linked genes without crossing over, the *P* values for populations 1 and 2 were 7.6×10^{-6} and 7.3×10^{-6}, respectively. This result indicated that the two genes were not allelic, because at the F_2 generation, ratios for one dominant gene or two dominant and allelic genes without crossing over are similar (3:1). In the case of two complementary genes and three genes, the *P* values were even lower. They were 2.2×10^{-16} for both Populations 1 and 2. These results indicated that two duplicate genes were involved in the ability of KVX295-2-124-99 and IT97K556-6 to survive aphid infestation. QTLs controlling different aspects (antibiosis, antixenosis, and tolerance) of aphid resistance in cowpeas and other species have been identified (Guo et al., 2012; Kamphuis et al., 2013). Huynh et al. (2015), using SNP markers, discovered two QTLs involved in aphid resistance of IT97K556-6: a major QTL, QAc-vu7, and a minor QTL, QAc-vu1.1. However, monogenic aphid resistance in cowpeas has also been reported (Kusi et al., 2018; Omoigui et al., 2017). Boukar et al. (2018) reported that the single dominant gene conferring aphid resistance in cowpeas and incorporated in several improved breeding lines and varieties has become ineffective.

4.2 | Markers validation

Marker validation of MA61 and MA70 consisted of testing for the existence of a relationship between each marker and the trait (survival under aphid infestation) from generation to generation. For marker MA61, the band of the resistant parent (MA61R) with 150 bp and the band of the susceptible parent (MA61S) with 280 bp were both found in F_1. This indicates that the character was transmitted from parents to the first generation. The transmission of the character to the second generation was also tested. This test showed that there was a relationship between the phenotype and genotype ($P = 0.018$). Thus, resistant F_2 individuals exhibited the band of the resistant parent and the susceptible F_2 individuals had the band of the susceptible parent. Therefore, the character was transmitted from the parents to the first generation (F_1) and from the first generation to the second generation (F_2). Marker MA70 exhibited the same trend. The band of the resistant parent (MA70R) had 180 bp, and the band of the susceptible parent (MA70S) had 280 bp. Both MA70R and MA70S were found in F_1 individuals. The association between phenotype and genotype was significant ($P = 0.016$). This indicated that the character was transmitted to the second generation (F_2) and that the marker illustrated this. The results established that both markers MA61 and MA70 were able to pass the gene involved in the expression of the character from one generation to the next, respectively, in line KVX295-2-124-99 and IT97K556-6. Markers MA61 and MA70 were linked genes responsible for the ability of cowpea to survive aphid infestation. SSR marker validation using segregating populations is widely used (Carletti et al., 2016; Kongjaimun et al., 2012; Okogbenin et al., 2008; Omoigui et al., 2017). Marker MA61 is located on chromosome 3 and marker MA70 on chromosome 7. Despite the shortness of the sequences of the two markers, the E-values were very low, and the percentages of identity were high. This indicated that the two genes involved in aphid resistance in cowpeas were located on chromosomes 3 and 7.

These markers can be used in MAS to incorporate one or more genes into susceptible lines. Given that these markers are codominant,

they can also be used to detect F_1 false positives in crosses that involve line KVX295-2-124-99 and line IT97K556-6. Furthermore, they can be used to follow up on the source of resistance they signal. Markers linked to aphid resistance have also been reported in other studies. For instance, Kusi et al. (2018) and Omoigui et al. (2017) working with cowpeas found two markers (CP171/172 and KAD61) linked to genes involved in aphid resistance.

4.3 | Mode of inheritance based on phenotyping and genotyping

The screening of the F_2 individuals of Population 1 with aphids and marker MA61 confirmed the result of phenotyping. For a genotypic ratio of 15:1, the *P* value was not significant, confirming that two duplicate genes were involved in the expression of this character. Similarly, the combined data of genotyping and phenotyping of Population 2 showed that two duplicate genes were involved in the expression of the character. This result is in agreement with the work of Huynh et al. (2015). Omoigui et al. (2017) also found two dominant genes involved in aphid resistance in cowpeas. However, one of these genes is found in variety TVNu 2876 and the other in variety Sarc1-57-2, suggesting monogenic resistance. Aphid resistance has three components: tolerance, antibiosis, and antixenosis (Emden, 2002; Koch et al., 2016; Peterson et al., 2017). One or more genes may be involved in the expression of each component. Depending on the screening method, the expression of one or several resistance components may be captured. Both antibiosis and antixenosis involve a plant response and a pest response, but in the case of tolerance, only a plant response is involved (Smith, 2005). In host plant resistance to insects, antixenosis and antibiosis may be involved simultaneously, but in different proportions (Annan et al., 1997; Obopile & Ositile, 2010). The survival rate under aphid infestation is an important indicator for assessing aphid resistance. Plant survival under aphid infestation is part of tolerance. Given the number of genes involved in the expression of this trait, it is controlled by QTLs. Antibiosis and antixenosis are also important for host plant resistance. For instance, plants produce a number of metabolites in response to insects, pathogens, and other stress factors (Sharma & Vaishampayan, 2009; Simmonds, 2003). They act as antifeedants and affect insects by reducing their growth and development (Morimoto et al., 2000; War et al., 2013). Each of the components of host plant resistance to aphids (insects) has a genetic basis.

5 | CONCLUSION

The ability of cowpeas (KVX295-2-124-99 and IT97K556-6) to survive aphid infestation is controlled by two QTLs. Results based on phenotypic and genotypic data established that two duplicated genes are involved in the expression of this characteristic. These two genes were not allelic. Two SSR markers (MA61 and MA70) were found linked to genes involved in resistance to aphids in cowpeas. These markers can be used in MAS for breeding purposes. Because these markers are codominant, they can also be used to detect F_1 false positives in crosses involving Tiligre, IT97K556-6, and KVX295-2-124-99. The two genes involved in resistance to aphids in cowpeas are located on chromosomes 3 and 7.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed significantly and in different ways in the writing of this paper.

ETHICS STATEMENT

We hereby certify that the information set above is true and complete to the best of our knowledge.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available.

ORCID

Adelaïde P. Ouedraogo https://orcid.org/0000-0001-7718-9583
Agyemang Danquah https://orcid.org/0000-0003-3840-3799

REFERENCES

Alabi, O. Y., Odebiyi, J. A., & Jackai, L. E. N. (2003). Field evaluation of cowpea cultivars (*Vigna unguiculata* (L) Walp.) for resistance to flower bud thrips (*Megalurothrips sjostedti* Trybom) (Thysanoptera: Thripidae). *International Journal of Pest Management*, *49*(4), 287–291. https://doi.org/10.1080/0967087031000123706

Annan, I. B., Tingey, W. M., & Schaefers, G. A. (1997). Population dynamics and clonal comparisons of *Aphis craccivora* (Homoptera: Aphididae) on resistant and susceptible cowpea cultivars. *Environmental Entomology*, *26*, 250–255. https://doi.org/10.1093/ee/26.2.250

Annan, I. B., Schafers, G. A., & Tingey, W. M. (1996). Impact of density of aphids craccivora (Aphididae) on growth and yield of susceptible and resistant cowpea cultivars. *The Annals of Applied Biology*, *128*, 183–193. https://doi.org/10.1111/j.1744-7348.1996.tb07315.x

Badhe, P. L., Raut, D. M., Magar, N. M., Borole, D. N., & Pawar, V. Y. (2016). Diallel analysis in cowpea (*Vigna unguiculata* (L.)Walp.). *Electronic Journal of Plant Breeding*, *7*(2), 291–302. https://doi.org/10.5958/0975-928X.2016.00037.5

Benjamin, D., Berger, J., Johannesson, J. O., Nosek, M., Wagenmakers, B. A., Berk, E. J., Bollen, R., Brembs, K. A., Brown, B., Camerer, L. C., & Cesarini, D. (2018). Redefine statistical significance. *Nature Human Behaviour*, *2*(6), 6–10. https://doi.org/10.1038/s41562-017-0189-z

Betensky, R. A. (2019). The P-value requires context, not a threshold. *American Statistician*, *73*(sup1), 115–117. https://doi.org/10.1080/00031305.2018.1529624

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M. L., Olufajo, O., & Fatokun, C. (2018). Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding*, *138*(4), 415–424. https://doi.org/10.1111/pbr.12589

Carletti, G., Carra, A., Allegro, G., Vietto, L., Desiderio, F., Bagnaresi, P., Gianinetti, A., Cattivelli, L., Valè, G., & Nervo, G. (2016). QTLs for woolly poplar aphid (*Phloeomyzus passerinii* L) resistance detected in an inter-specific *Populus deltoides* x *P. nigra* mapping population. *PLoS ONE*, *11*(3), 1–18. https://doi.org/10.1371/journal.pone.0152569

Emden, H. (2002). Mechanisms of resistance: Antibiosis, antixenosis, tolerance, nutrition. In D. Pimentel (Ed.), *Encyclopedia of pest management*. Marcel Dekker, Inc.

Gepts, P., Beavis, W. D., Brummer, E. C., Shoemaker, R. C., Stalker, H. T., Weeden, N. F., & Young, N. D. (2005). Legumes as a model plant family. Genomics for food and feed report of the cross-legume advances through genomics conference. *Plant Physiology*, *137*(4), 1228–1235. https://doi.org/10.1104/pp.105.060871

Githiri, S. M., Ampong-Nyarko, K., Osir, E. O., & Kimani, P. M. (1996). Genetics of resistance to Aphis craccivora in cowpea. *Euphytica*, *89*(3), 371–376. https://doi.org/10.1007/bf00022295

Guo, S., Kamphuis, L. G., Gao, L.-L., Klingler, J. P., Lichtenzveig, J., Edwards, O. R., & Singh, K. B. (2012). Identification of distinct quantitative trait loci associated with defence against the closely related aphids *Acyrthosiphon pisum* and *A. kondoi* in Medicago truncatula. *Journal of Experimental Botany*, *63*, 3913–3922. https://doi.org/10.1093/jxb/ers084

Hittalmani, S., Parco, A., Mew, T. V., Zeigler, R. S., & Huang, N. (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theoretical and Applied Genetics*, *100*, 1121–1128. https://doi.org/10.1007/s001220051395

Huynh, B. L., Ehlers, J. D., Ndeve, A., Wanamaker, S., Lucas, M. R., Close, T. J., & Roberts, P. A. (2015). Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. *Molecular Breeding*, *35*(1), 35–36. https://doi.org/10.1007/s11032-015-0254-0

Jackai, L. E. N., & Daoust, R. A. (1986). Insect pests of cowpeas. *Annual Review of Entomology*, *31*, 95–119. https://doi.org/10.1146/annurev.en.31.010186.000523

Kamphuis, L. G., Lichtenzveig, J., Peng, K., Guo, S., Klingler, J. P., Siddique, K., Gao, L. L., & Singh, K. B. (2013). Physiology and genetics of resistance to spotted alfalfa aphid (*Therioaphis trifolii*) in Medicago truncatula. *Journal of Experimental Botany*, *64*, 5157–5172. https://doi.org/10.1093/jxb/ert305

Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., & Sarath, G. (2016). Plant tolerance: A unique approach to control hemipteran pests. *Frontiers in Plant Science*, *7*, 1363. https://doi.org/10.3389/fpls.2016.01363

Kongjaimun, A., Kaga, A., Tomooka, N., Somta, P., Shimizu, T., Shu, Y., Isemura, T., Vaughan, D. A., & Srinives, P. (2012). An SSR-based linkage map of yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. unguiculata Sesquipedalis Group) and QTL analysis of pod length. *Genome*, *55*(2), 81–92. https://doi.org/10.1139/g11-078

Kusi, F., Obeng-Ofori, D., Asante, S. K., & Padi, F. K. (2010). New sources of resistance in cowpea to the cowpea aphid (*Aphis craccivora* Koch) (Homoptera: Aphididae). *Journal of the Ghana Science Association*, *12*, 95–104. https://doi.org/10.4314/jgsa.v12i2.62811

Kusi, F., Padi, F. K., Obeng-Ofori, D., Asante, S. K., Agyare, R. Y., Sugri, I., Timko, M. P., Koebner, R., Huynh, B. L., Santos, J. R., & Close, T. J. (2018). A novel aphid resistance locus in cowpea identified by combining SSR and SNP markers. *Plant Breeding*, *137*(2), 203–209.

Morimoto, M., Kumeda, S., & Komai, K. (2000). Insect antifeedant flavonoids from *Gnaphalium affine* D. Don. *Journal of Agricultural and Food Chemistry*, *48*(5), 1888–1891. https://doi.org/10.1021/jf990282q

Murdock, L. (2002). Cowpea (*Vigna unguiculata*) and crop genetic transformation in general. *A BIOTECH, Bulletin d'information*, *7*. www.aab.org.d2

Mwadzingeni, L., Shimelis, H., Rees, D. J. G., & Tsilo, T. J. (2017). Genome-wide association analysis of agronomic traits in wheat under drought- stressed and non-stressed conditions. *PLoS ONE*, *12*, 1–13. https://doi.org/10.1371/journal.pone.0171692

Obopile, M., & Ositile, B. (2010). Life table and population parameters of cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) on five cowpea *Vigna unguiculata* (L. Walp.) varieties. *Journal of Pest Science*, *83*(1), 9–14. https://doi.org/10.1007/s10340-009-0262-0

Ofuya, T. I. (1997). Control of the cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), in cowpea, *Vigna unguiculata* (L.) Walp. *Integrated Pest Management Reviews*, *2*, 199–207. https://doi.org/10.1023/A:1018461320137

Okogbenin, E., Marin, J., & Fregene, M. (2008). QTL analysis for early yield in a pseudo F2population of cassava. *African Journal of Biotechnology*, *7*(2), 131–138. https://doi.org/10.5897/AJB2008.000-5017

Ombakho, G. A., Tyagi, A. P., & Pathak, R. S. (1987). Inheritance of resistance to the cowpea aphid in cowpea. *Theoretical and Applied Genetics*, *74*(6), 817–819.

Omoigui, L. O., Ekeuro, G. C., Kamara, A. Y., Bello, L. L., Timko, M. P., & Ogunwolu, G. O. (2017). New sources of aphids [*Aphis craccivora* (Koch)] resistance in cowpea germplasm using phenotypic and molecular marker approaches. *Euphytica*, *213*(8), 1–15. https://doi.org/10.1007/s10681-017-1962-9

Omongo, C. A., Adipala, E., Ogenga-Latigo, M. W., & Kyamanywa, S. (1998). Insecticide application to reduce pest infestation and damage on cowpeas in Uganda. *African Plant Protection*, *4*, 91–100.

Ordon, F., Habekuss, A., Kastirr, U., Rabenstein, F., & Kühne, T. (2009). Virus resistance in cereals: Sources of resistance, genetics and breeding. *Journal of Phytopathology*, *157*(9), 535–545. https://doi.org/10.1111/j.1439-0434.2009.01540.x

Ouédraogo, A. P., Batieno, B. J., Traore, F., Tignegre, J., Huynh, L., Roberts, P. A., Close, T., & Ouédraogo, J. T. (2018). Screening of cowpea (*Vigna unguiculata* (L.) Walp.) lines for resistance to three aphids (*Aphis craccivora* Koch) strains in Burkina Faso. *African Journal of Agricultural Research*, *13*(29), 1487–1495. https://doi.org/10.5897/AJAR2018.13241

Oyewale, R. O., & Bamaiyi, L. J. (2013). Management of cowpea insect pests. *Scholars Academic Journal of Biosciences*, *1*(5), 217–226.

Pathak, R. S. (1991). Plant genetics in pest management. *International Journal of Tropical Insect Science*, *12*(5-6), 553–564. https://doi.org/10.1017/s1742758400013023

Perozo, F., Villegas, P., Estevez, C., Alvarado, I., & Purvis, L. B. (2006). Use of FTA® filter paper for the molecular detection of Newcastle disease virus. *Avian Pathology*, *35*(2), 93–98. https://doi.org/10.1080/03079450600597410

Peterson, R. K. D., Varella, C. A., & Higley, L. G. (2017). Tolerance: The forgotten child of plant resistance. *PeerJ*, *5*, e3934. https://doi.org/10.7717/peerj.3934

Sharma, A. K., & Vaishampayan, S. M. (2009). Evaluation of light trap as eco friendly control tool in the management of insect pest species of paddy ecosystem. *JNKVV Research Journal*, 187p.

Simmonds, M. S. J. (2003). Flavonoid-insect interactions: Recent advances in our knowledge. *Phytochemistry*, *64*, 21–30. https://doi.org/10.1016/S0031-9422(03)00293-0

Singh, B. B., & Ntare, B. R. (1985). Development of improved cowpea varieties in Africa. In Singh, S. R. & Rachie, K. O. (Eds.), *Cowpea research, production and utilization* (pp. 105–115). New York: Wiley.

Smith, C. (2005). *Plant resistance to arthropods: Molecular and conventional approaches*. Springer.

Souleymane, A., Ova, M. E. A., Fatokun, C. A., & Alabi, O. Y. (2013). Screening for resistance to cowpea aphid (*Aphis Craccivora* Koch) in wild and cultivated cowpea (*Vigna Unguiculata* L. Walp.) accessions. *International Journal of Science, Enviroment*, *2*(4), 611–621.

Stangegaard, M., Ferrero-Miliani, L., Børsting, C., Frank-Hansen, R., Hansen, A. J., & Morling, N. (2011). Repeated extraction of DNA from FTA cards. *Forensic Science International: Genetics Supplement Series*, *3*(1), e345–e346. https://doi.org/10.1016/j.fsigss.2011.09.035

War, A., Paulraj, R., Hussain, M. G., Buhroo, B., Ignacimuthu, A. A., & Sharma, S. H. (2013). Effect of plant secondary metabolites on legume pod borer, *Helicoverpa armigera*. *Journal of Pest Science*, *86*, 399–408. https://doi.org/10.1007/s10340-013-0485-y

Xu, H., Zhang, J., Zhang, P., Qie, Y., Niu, Y., Li, H., Ma, P., Xu, Y., & An, D. (2014). Development and validation of molecular markers closely linked to the wheat stripe rust resistance gene YrC591 for marker-assisted selection. *Euphytica*, *198*(3), 317–323. https://doi.org/10.1007/s10681-014-1108-2

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Ouedraogo, A. P., Danquah, A., Tignegre, J.-B., Poda, L. S., Batieno, J. B., Asante, I. K., Ouedraogo, J. T., Ayertey, J. N., & Ofori, K. (2022). Determination of inheritance of aphid resistance in cowpea genotypes and identification of single sequence repeat markers linked to resistance genes. *Legume Science*, *4*(2), e127. https://doi.org/10.1002/leg3.127