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Research Article

Screening for resistance to cowpea aphids (*Aphis craccivora* Koch.) In mutation derived and cultivated cowpea (*Vigna unguiculata* L. Walp.) Genotypes.

Siyunda AC[1]*, N Mwila[2], M Mwala[2], KL Munyinda[2], K Kamfwa[2] and D Nshimbi[1]

[1]*Department of Crop Science, Natural Resources Development College (NRDC), Lusaka, Zambia*

[2]*Department of Plant Science, School of Agricultural Sciences, University of Zambia (UNZA), Lusaka, Zambia*

***Corresponding Author:** Siyunda AC, Department of Crop Science, Natural Resources Development College (NRDC), Lusaka, Zambia.

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Abstract

Research studies aimed at screening cowpea genotypes against *Aphis craccivora* K. commonly known as black aphids, under the no-choice infestation condition, were conducted in the greenhouse at Natural Resource Development College (NRDC), Lusaka, Zambia in 2021. The main objective was to discern the cowpea genotype that is resistant to *Aphis craccivora* K that would aid the reduction of yield losses encountered in cowpea production. The materials consisted of 21 cowpea genotypes advanced from the open field screening method. These 21 that were selected from an initial set of 110 genotypes exhibited the resistance trait during the open field screening method and thus were advanced to validate their resistance levels. The cowpea genotypes evaluated included 1 landrace variety, 2 mutation derived cultivated varieties, 15 advanced mutation derived varieties, and 3 pure line genotypes from IITA. The experimental design used was a Completely Randomized Design (CRD) with 3 replications. The variables collected were aphid score, aphid population build-up, plant vigour scores, and plant survival rate. Significant differences were seen among the genotypes for all the parameters considered in the investigation. The most resistant genotypes (BB10-4-2-3-2 and BBBT1-11) across all parameters investigated recorded an average aphid score of 1.0 and 1.68, respectively compared to LT11-3-3-12 which recorded the highest score of 7.0. The aphid population build-up of BB10-4-3-2 and BBBT1-11 was the lowest at 0.9 and 1.0 respectively compared to the highest recorded by LT11-5-1-1-4 at 3.54. The highest plant vigour of 3.0 was recorded by BB10-4-3-2, BBBT1-11 and LT11-5-1-1-4 and the lowest was recorded by Sanzi (1.2). Both BB10-4-3-2 and BBBT1-11 recorded a survival percentage of 100% at 21DAI compared to the lowest recorded by TVu 2027 (20%). Genotypes BB10-4-2-3-2 and BBBT1-11 could further be assessed for the possible presence of biochemical and traits, that could explain the basis of the observed differences among the genotypes in their reaction to *Aphis craccivora* K. Besides, BB10-4-2-3-2 and BBBT1-11 can also be incorporated in the breeding program with the target of breeding cowpea varieties that are resistant to aphid infestation. Furthermore, BB10-4-2-3-2 and BBBT1-11 may be released to address the yield losses experienced in cowpea production as a result of A. craccivora K.

Keywords: *Aphis craccivora* Koch; Infestation; Cowpea; genotype

Abbreviations

IAEA: International Atomic Energy Agency, ZARI: Zambia Agricultural Research Institute, RUFORUM: Regional University Forum, NRDC: Natural Resources Development College, UNZA: the University of Zambia, CD: Critical Difference.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an annual legume, mostly cultivated and considered important cash and nutritional security grain legume in the semi-arid regions of sub-Saharan Africa [4, 12, 19, 25]. It is an important component of cropping systems in the Semi-arid regions covering part of the Middle East, Asia and Oceania, Africa, Southern Europe, and central and south America [22]. However, grain yield in cowpea production remains low, rarely exceeding500 kg per ha in the traditional production system [13, 19]. Cowpea production is affected by several biotic stress factors, which includes several insect pests that damage the crop by infesting it at all developmental stages, in the field as well as at postharvest.

Cowpea aphid, *A. craccivora* Koch (Hemiptera: Aphididae), is one of the main insect pests that affect cowpea production, in that it causes significant yield losses [6, 11, 14, 20]. This species of cowpea aphid feeds on phloem and has been a leading pest in cowpea yield losses at a pre-post stage, in America, Asia, and Africa [17-19]. Besides, causing direct damageto cultivars that are susceptible by sucking sap, modifying the metabolism and by extracting nutrients from plants, *A. craccivora* K is also responsible for the indirect damage, by acting as a vector in phytopathogenic virus transmission, in which a wide range of viruses are transmitted i.e., cucumber mosaic virus (CMV), cowpea severe mosaic virus (CPSMV), cowpea aphid-borne mosaic virus (CABMV) etc. [7, 9, 14, 17, 28]. Singh and Allen (1980) cited by Souleymane et al, (2013) have reported several insecticides that have proved to be effective against aphids, but insecticides are often not accessible to small-scale farmers who are mostly involved in cowpea production. The use of resistant varieties offers the best option to small-scale farmers owing to its low cost (8). Host Plant Resistance is an efficient, cheap and environmentally friendly way of controlling *A. craccivora* in an integrated control system. Different cowpea genotypes have been screened, in different parts of the African continent, and different levels of resistance to aphids have been identified [2, 12, 19]. In spite, of greater efforts in discovering the aphid resistance genotypes, resistance to aphids (*A. craccivora* K.) of most of the identified cowpea cultivars at IITA has recently broken down, due to the occurrence of resistance-breaking down biotypes, in various plant-aphid systems (Fatokun personal correspondence) as cited by [25].

Thus, there is a need to identify new sources of resistance to *A. craccivora*. The main objective of the current study was to identify cowpea cultivars with resistance to *A. craccivora* to contribute to the improvement of cowpea productivity. The specific objectives of the current study were, firstly, to investigate differences among genotypes in terms of the degree of infestation of aphids, secondly, to find out vital parameters for genotype classification and, lastly to classify genotypes based on the resistance level of aphids.

Materials and Methods

The study was conducted in the greenhouse at Natural Resource Development College (NRDC), Lusaka, Zambia. The materials consisted of 21 cowpea genotypes advanced from the open field screening method. These 21 that exhibited the resistant trait during the open field screening method were selected from an initial set of 110 genotypes and thus were advanced to validate their resistance. The aphids used for this study were collected from the University of Zambia field station, during the open field screening of cowpea genotypes. The aphids were maintained on a susceptible cowpea cultivar TVx 3236, in the greenhouse using the insect-proof cage away from parasites and/or other predators at NRDC.

The experimental design used in the present study was a Completely Randomized Design (CRD) with 3 replications [26]. Seeds of 21 cowpea genotypes were sown on July 28, 2021, in plastic polyethene pots filled with sterilized topsoil and kept in insect-proof cages with fine mesh. Each pot contained three plants of the same accession. Seven days after planting, each seedling was infested with five aphids, this was done by placing them with a camel hair brush [14]. The pots remained in the insect-proof cages for 21 days after infestation during which the plants were assessed using different variables. Dead plants were regarded as susceptible while those still alive, with first trifoliate leaves developing, as resistant [14, 25].

The variables collected from this study were aphid score, aphid population build-up, plant vigour scores, and plant survival rate. The number of insects was counted using a visual score of 1 to 9, where 1 =0-4 aphids, 3=5-20 aphids, 5=21- 100 aphids, 7= 101-500 aphids and 9= >500 (high aphid colonisation and plant death) [15, 25]. Plant vigour scores were calculated using a scale of 1-3, where 1 denotes senescence (weak), 2 indicates medium growth and 3 denotes survival (more vigorous)[15]. Aphid population build-up rating score was calculated using a scale of 0 to 5, where 0=no aphids, 1= a few individual aphids, 2= few small individual colonies, 3= several small colonies, 4=large individual colonies and 5= large continuous colonies [15, 25]. Aphid score (Aphids per plant), Aphid population build-up, plant vigour and plant survival rate were counted at 5, 9, 13, 17 and 21 days after infestation. Plants that survived aphid infestations were allowed to grow and produce seeds [25].

Data Analysis

The data collected from the study were subjected to analyses of variance (ANOVA) for the test of significance at 5%.The average number of aphids per accession was calculated and the mean level of infestation scores of each accession was determined. Variables that display significant differences were separated using Turkey's HSD test. Treatment means were considered significant when P≤ 0.05 at the 5% level.

Results and Discussion

Average Aphid Score

Table 1 presents the average aphid score for the genotypes at different intervals as defined by DAI. The cowpea genotypes showed significant differences from each other at a 5% level of significance, in terms of the number of aphids per plant. The cowpea lines LT4-2-4-1-1, LT11-3-3-12 and Sanzi, recorded the highest aphid score of 4.3 at 5 DAI. These lines were not significantly different from cowpea lines; Lunkwakwa (3.7), LT 11-5-1-1-4 (3.7), TVu 2027 (3.0), Namuseba (3.0), BB7-9-7-5-1 (3.0) and BB7-9-7-5-4 (3.7), Lukusuzi (3.7), BB10-4-2-3-1 (3.0) and BB10-4-2-3-3 (3.0), LT3-8-4-1 d (3.0), and MS 1-8-2-6-8 (3.0). The cowpea line BB10-4-2-3-2 recorded the lowest aphid score of 1.0 which was at par with BBBT1-11, BBBT1-5 and LTBT1-5 with aphid scores of 1.7, 2.3 and 2.3 respectively. At 9, 13, 17, and 21 DAI, cowpea line BB10-4-2-3-2 recorded the lowest aphid scores of 1.0, 1.0, 1.0 and 0.7 respectively. However, the lower aphid scores recorded by BB10-4-2-3-2 were not significantly different from the aphid score of BBBT1-11 on 13(1.7), 17(1.0) and 21(1.0) DAI and also for Namuseba (1.0) and BB10-4-2-3-1(1.7) on 21 DAI. At 9 DAI, cowpea lines LT4-2-4-1-1, LT4-2-4-1-2, Lukusuzi and LT11-5-1-1-4 had the highest aphid score of 7.0 which was significantly different at 5% level of significance, from BB10-4-2-3-2 (1.0), BBBT1-11(1.7), BBBT1-5 (2.3) and LTBT1-5(2.3). On 13, 17 and 21 DAI, LT11-3-3-12 recorded the highest aphid scores of 7.0, 9.0 and 9.0 respectively.

The multiplication of aphids on cowpea lines that showed susceptibility and resistance was rapid. Some of the plants were fully colonized within four to nine days after infestation. Significant differences at a 5% level of significance, occurred at 5 DAI and onwards for the cowpea lines. The findings are consistent with Arturo et al (1988), in which the researcher reported significant differences in the cumulative number of progenies per aphid female on the fourth day and onwards in a cross between the susceptible line and the two resistant lines. Ofuya (1993) and Souleymane et al (2013), also reported significant differences in the number of aphids in susceptible and resistant varieties. The leaves of cowpea lines that showed susceptibility in the present study turned yellow, became stunted and started dying at 9 DAI onwards, this agrees with Bata et al (1987) and Souleymane et al (2013). The present study had some genotypes that indicated tolerance against aphids, this is in conformity with Souleymane et al (2013), who also reported the genotype IT97K-556-6 having some level of tolerance as it withstood the aphid pressure. The constant resistance observed in the cowpea lines BBBT1-11 and BB10-4-2-3-2, maybe due to resistance which is expressed through antibiosis and antixenosis compounds that can disturb the functioning of aphids, and also the growth and development of aphids [1, 3, 18, 24, 27]. Therefore, cowpea lines BBBT1-11 and BB10-4-2-3-2 suggest high levels of antixenosis and antibiosis. These lines were the least favourable to aphid infestation and LT11-3-3-12 was the most favourable. According to Teetes (2007) as cited in Ouedraogo et al, (2018), stated that antixenosis and antibiosis activities often result in the rise in insect mortality or reduced longevity and reproduction of most insects.

Line	Genotype	Days After Infestation(DAI)				
		5	9	13	17	21
L1	BBBT 1-11	1.7	3.0	1.7	1.0	1.0
L2	BB10-4-2-3-2	1.0	1.0	1.0	1.0	1.0
L3	Namuseba	3.0	3.7	3.7	3.0	1.0
L4	BB7-9-7-5-4	3.7	5.0	4.3	3.0	3.0
L5	BB10-4-2-3-1	3.0	4.3	3.7	3.0	1.7
L6	Lukusuzi	3.7	7.0	7.0	5.7	3.7
L7	BB7-9-7-5-1	3.0	6.3	6.3	5.0	4.3
L8	LT4-2-4-1-2	3.0	7.0	6.3	5.0	5.0
L9	MS1-8-2-6-8	3.0	6.3	6.3	5.0	5.0
L10	BBBT1-7	3.0	5.0	7.0	5.7	5.0
L11	LTBT1-5	2.3	5.7	6.3	5.0	5.0
L12	LT3-8-4-1-4	3.0	5.0	7.0	7.0	5.7
L13	BB10-4-2-3-3	3.0	5.0	6.3	5.7	5.0
L14	IT07K205-8	3.0	5.0	5.7	7.7	4.3
L15	BBBT1-5	2.3	5.0	7.0	5.7	5.0
L16	TVU 2027	3.0	5.0	5.0	5.0	3.7
L17	LT11-5-1-1-4	3.7	7.0	5.7	7.7	7.7
L18	Lunkwakwa	3.7	5.7	6.3	5.0	5.0
L19	Sanzi	4.3	5.7	5.7	7.0	7.0
L20	LT11-3-3-12	4.3	5.7	7.0	9.0	9.0
L21	LT4-2-4-1-1	4.3	7.0	6.3	7.7	7.0
P-value		0.01	0.00	0.00	0.02	0.01
CD at 5%		1.33	1.39	1.88	1.49	1.68

Table 1: Average Aphid Score.

Aphid Population Build-up Score

Table 2, presents the rate of aphid population build-up on seedlings of some cowpea genotypes. Aphid population build-up was very rapid and the plants were fully colonized within 7 to 9 days after infestation (DAI). At 5 DAI, there were fewer differences in terms of aphid population build-up among cowpea lines. 12 cowpea lines recorded the lowest aphid population build-up score of 1.0 at 5 DAI, this included cowpea lines; BBBT 1-11, BBBT1-5, BB10-4-2-3-1, BB10-4-2-3-2, and BB10-4-2-3-3, BB7-9-7-5-1, LT4-2-4-1-2, MS1-8-2-6-8, LTBT1-5, LT3-8-4-1-4, and IT07K205-8. However, these cowpea lines differed significantly from cowpea lines LT11-3-3-12 and LT11-5-1-1-4 which had the highest score of 2.0. Nevertheless, LT11-3-3-12 and LT11-5-1-1-4 recorded the aphid population build-up score, not significantly different at a 5% level of significance from the score of cowpea lines BBBT1-7, Lunkwakwa and Sanzithat recorded 1.5, 1.7 and 1.7 respectively. At 9, 13, 17, and 21 DAI, cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, and Namuseba recorded the lowest scores that were not significantly different. At 9 DAI, the highest score was recorded by cowpea line LT4-2-4-1-2 with a score of 4.0, at 13 DAI the highest score was recorded by Lukusuzi with a score of 4.0, at 17 the highest score was recorded by LT3-8-4-1-4 with the score of 4.0 and lastly at 21 DAI, LT11-5-1-1-4 recorded the highest score of 4.7. It is worth acknowledging, that differences were significantly increasing as DAI was increasing. A high score of aphid population build-up was observed between 9 and 13DAI. However, between 13 and 21DAI, the rate of aphid population build-up was lower compared to that between 9 and 13 days. Cowpea line LT 11-5-1-1-4, unlike other cowpea lines that showed a decreasing trend in aphid population build-up after the 13th day from infestation, this line showed an increasing trend in the aphid population build up score and recorded its highest score at 21 DAI.

Line	*Genotype*	*Days After Infestation (DAI)*				
		5	*9*	*13*	*17*	*21*
L1	BBBT 1-11	1.0	1.7	1.0	1.0	0.3
L2	BB10-4-2-3-2	1.0	1.0	1.0	1.0	0.7
L3	Namuseba	1.0	1.3	1.0	1.0	1.0
L4	BB7-9-7-5-4	1.3	2.7	2.0	2.3	1.3
L5	BB10-4-2-3-1	1.0	1.7	1.7	1.0	1.0
L6	Lukusuzi	1.3	4.0	4.0	3.3	1.3
L7	BB7-9-7-5-1	1.0	3.7	3.7	3.0	2.0
L8	LT4-2-4-1-2	1.0	4.0	3.7	3.0	2.7
L9	MS1-8-2-6-8	1.0	3.7	3.7	3.0	2.7
L10	BBBT1-7	1.5	3.0	4.0	2.5	2.5
L11	LTBT1-5	1.0	3.3	3.7	3.0	2.0
L12	LT3-8-4-1-4	1.0	3.0	4.0	4.0	2.7
L13	BB10-4-2-3-3	1.0	3.0	3.7	3.0	2.3
L14	IT07K205-8	1.0	2.7	3.7	3.7	2.3
L15	BBBT1-5	1.0	2.3	4.0	3.0	2.0
L16	TVU 2027	1.7	3.0	3.0	2.3	1.7
L17	LT11-5-1-1-4	2.0	3.0	4.0	4.0	4.7
L18	Lunkwakwa	1.7	2.7	3.7	2.7	2.7
L19	Sanzi	1.7	2.7	3.7	3.3	2.3
L20	LT11-3-3-12	2.0	3.0	4.0	4.0	3.3
L21	LT4-2-4-1-1	1.3	2.7	3.7	3.7	3.3
P-value		0.00	0.04	0.03	0.01	0.00
CD at 5%		0.55	0.81	0.92	0.93	1.11

Table 2: Aphid Population Build-up Score.

In the present study, cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, and Namuseba recorded the lowest scores of aphid population build-up consistently, which was not significantly different at the 5% level of significance. These lines may be classified as resistant varieties against *A. craccivora* as indicated by Giga (2001), who stated that resistance to aphid attack can be characterized by an isolated and lower insect population density. As observed in the current study, differences in the spread of aphid colonies and variations in the insect population density on different cowpea lines may be attributed to varying levels of sweetness of different cowpea lines (3). That is, without antixenosis and antibiotic compounds in the plant tissues that can disturb the functioning of aphids, and also growth and development [3, 27, 24, 1, 18].

Plant Vigour Analysis at 21 DAI

Table 3 presents the results obtained, showing that the plant vigour of different cowpea varieties was significantly different ($P<0.05$). Cowpea lines BBBT1-11, BB10-4-2-3-2, and LT11-5-1-1-4 scored the highest plant vigour of 3.0. Though not significantly different from cowpea lines BB10-4-2-3-1 (2.3), Namuseba (2.3) and LTBT1-5 (2.7). Cowpea line BBBT1-7 recorded the lowest score of 1.0. However, BBBT1-7 was not significantly different at 5% level of significance, from cowpea lines LT4-2-4-1-1 (1.3) and LT4-2-4-1-2 (1.7), LT11-3-3-12 (1.7), Sanzi (1.2), TVU 2027 (1.3), BBBT1-5 (1.3), IT07K205-8 (1.3), LT3-8-4-1-4 (1.7). Unlike other cowpea lines that recorded a lower score of aphids per line, cowpea line LT11-5-1-1-4 recorded a significant high aphid score of 7.7 at 21DAI but yet its plant vigour score was 3 (vigorous growth). 9 cowpea lines out of 21 lines showed low performance (poor growth) and 6 cowpea lines indicated medium promising vigour. The remaining 6 cowpea lines out of 21 lines showed good performance. Thus, the cowpea lines with medium to good performance, according to Arturo (1988), managed to tolerate the severity of an aphid attack. The present findings are also supported by the findings of Giga (2001) who reported that resistant varieties can grow vigorously with no or fewer damage symptoms compared to susceptible varieties, regardless of the insect pressure. Singh et al (1996), further stated that resistance in plants, can be seen by the development of new leaves (trifoliate) even under attack and thus the crop continues to flower and form pods.

Line	*Genotype*	*Average Score*
L1	BBBT 1-11	3.0
L2	BB10-4-2-3-2	3.0
L3	Namuseba	2.3
L4	BB7-9-7-5-4	2.0
L5	BB10-4-2-3-1	2.3
L6	Lukusuzi	2.0
L7	BB7-9-7-5-1	2.0
L8	LT4-2-4-1-2	1.7
L9	MS1-8-2-6-8	2.0
L10	BBBT1-7	1.0
L11	LTBT1-5	2.7
L12	LT3-8-4-1-4	1.7
L13	BB10-4-2-3-3	2.0
L14	IT07K205-8	1.3
L15	BBBT1-5	1.3
L16	TVU 2027	1.3
L17	LT11-5-1-1-4	3.0
L18	Lunkwakwa	2.0
L19	Sanzi	1.2
L20	LT11-3-3-12	1.7
L21	LT4-2-4-1-1	1.3
P-value		0.03
CD at 5%		0.99

Table 3: Plant Vigour Analysis at 21 DAI.

Percentage survival of seedlings from 5 to 21 days after infestation

Table 4, indicates that the plant survival rate of different cowpea varieties was not significantly different (P>0.05) at 5 and 9 DAI as shown in Table 5. However, the cowpea lines were significantly different (P<0.05), beginning at 13 DAI to 21 DAI and the trend of significance was progressive. At 21 DAI, TVu 2027 and IT07K205-8 recorded the lowest percentage survival rate of 20 and 44.4 respectively. Cowpea line IT07K205-8 which recorded 44.4 was not significantly different from the mutant lines BBBT1-7 at 50, BB7-9-7-5-4 at 55.5 and MS1-8-2-6-8 at 62.5. Cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, BB7-9-7-5-1, LT4-2-4-1-2, LT11-5-1-1-4 recorded a survival percentage rate of 100%, which is a good indication of tolerance.

Line	*Genotype*	*Days After Infestation*				
		5	*9*	*13*	*17*	*21*
L1	BBBT 1-11	100	100	100	100	100
L2	BB10-4-2-3-2	100	100	100	100	100
L3	Namuseba	100	100	100	100	87
L4	BB7-9-7-5-4	100	88	88	66	55
L5	BB10-4-2-3-1	100	100	100	100	100
L6	Lukusuzi	100	100	100	87	87
L7	BB7-9-7-5-1	100	100	100	100	100
L8	LT4-2-4-1-2	100	100	100	100	100
L9	MS1-8-2-6-8	100	100	87	87	62
L10	BBBT1-7	100	100	100	100	50
L11	LTBT1-5	100	100	100	100	66
L12	LT3-8-4-1-4	100	100	100	100	87
L13	BB10-4-2-3-3	100	100	100	88	77
L14	IT07K205-8	100	100	77	77	44
L15	BBBT1-5	100	100	100	100	88
L16	TVU 2027	100	100	80	80	20
L17	LT11-5-1-1-4	100	100	100	100	100
L18	Lunkwakwa	100	100	100	100	88
L19	Sanzi	100	100	100	100	88
L20	LT11-3-3-12	100	100	88	77	77
L21	LT4-2-4-1-1	100	100	100	100	77
P-value		0.28	0.12	0.00	0.00	0.00
CD at 5%				21.99	18.97	19.46

Table 4: Plant Survival Rate.

The present findings are supported by the study of Ouedraogo et al., (2018), who recorded the lowest average survival percentage of 33% and the degree of infestation equal to 3.6 in the genotype N°2300 and also the highest survival rate of 93.3% and the degree of infestation equal to 1.4 in the genotype IT97K-556-6. A lower average survival percentage may be an indication of susceptibility to aphid attack in cowpea plants, which might be a result of lower levels of antibiosis activities that failed to reduce the development and multiplication of aphids [1, 18, 21, 27]. Thus, TVu 2027 and IT07K205-8 exhibited lower levels of antibiosis activities while cowpea lines BBBT1-11, BB10-4-2-3-1, BB10-4-2-3-2, BB7-9-7-5-1, LT4-2-4-1-2, LT11-5-1-1-4exhibited high levels of antibiosis activities, that enabled the plants to develop new leaves (trifoliate) even under aphid attack [8, 21].

Conclusion

The most resistant genotypes in the present study were BB10-4-2-3-2 and BBBT1-11 based on all parameters investigated. BB10-4-2-3-2 and BBBT1-11 could be assessed for the possible presence of biochemical and traits that could explain the basis of the observed differences among the genotypes in their reaction to *Aphis craccivora* K. Besides, BB10-4-2-3-2 and BBBT1-11 can also be incorporated in the breeding program with the target of breeding cowpea varieties that are tolerant to aphid infestation. Furthermore, BB10-4-2-3-2 and BBBT1-11 may be released to address the yield losses experienced in cowpea production as a result of *A. craccivora* K.

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Conflict of Interest

The authors did not declare any conflict of interest.

Data Availability

Data is available upon request, by writing to the corresponding author.

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