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New sources of aphids [*Aphis craccivora* (Koch)] resistance in cowpea germplasm using phenotypic and molecular marker approaches

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Abstract This study sought to identify new sources of resistance to cowpea aphids (CPA) using molecular and phenotypic approaches and the inheritance pattern. Sixty cowpea genotypes were phenotyped for resistance to CPA using insect proof cages and further confirmed using markers linked to aphid resistance. Result revealed that among the cowpea genotypes, TVu 2897 and TVNu 1158 supported lowest number of aphids and plant damage scores. The seedlings of these genotypes also had high level of survival rates and were completely healthy with normal growth. This indicates that these genotypes are resistant to aphid attacks. However, the resistance in TVNu 1158 did not seem strong compared to the genotype TV_U 2897 that was confirmed to be resistant to multiple aphid biotypes. The mechanism of resistance in TVu 2897 and TVNu 1158 were expressed as a hypersensitive response at the site of infestation on the leaves. The other genotypes especially Aloka local and keffi local supported the highest number of aphids, damage score and low level of survival rate, suggesting that they are susceptible to aphid attack. The cowpea genotype IT84S-224-6 previously reported to be resistant to aphids supported high number of aphids and was marked by stunted growth and high mortality rate. Molecular and phenotypic screening revealed that TVu-2876 has a strong resistance to cowpea aphid and should be a good source of resistance gene that can be used in breeding to develop new aphid resistant cowpea cultivars. Although, the results of phenotypic tests and molecular marker detection agreed in most cases, molecular markers detection was found more reliable in identifying genotypes for resistance to CPA. The segregation in F_2 and BC1 populations derived from the cross between TVNu 2876 and Keffi local indicated that resistance to cowpea aphids in TVu-2876 is controlled by a single dominant gene. Allelism test revealed that resistance gene in TVNu 2876 is non-allelic with the gene that confers resistance in SARC 1-57-2 and TVNu 1158.

Keywords Cowpea · *Aphis craccivora* · Molecular markers · Genetic resistance · Control

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important food and forage legume crops in subtropical and tropical regions of the world, primarily in sub-Saharan Africa. It is a versatile crop cultivated between Latitude 35°N–30°S of the equator, covering Asia, Africa, southern Europe and some parts of southern America (Bata et al.1987). Cowpea is grown on 11.32 million ha worldwide, with an annual grain production of about 5.72 million tons (FAO 2014). Of this amount, Africa accounts for 94% of grain production. Nigeria is the largest cowpea producer in the world and accounts for over 2.5 million tons grain production from an estimated 4.9 million ha (FAO 2014). It is mainly cultivated for the seeds; however, the sale of the fodder as animal feed during the dry season also provides vital income for farmers (IITA 2009). More importantly, the unique ability of cowpea to fix nitrogen even in poor soils makes it compatible as an intercrop with cereals and root crops. Despite the importance of the crop, the productivity of cowpea in sub-Saharan Africa is low, less than 500 kg/ha due to a number of factors such as old varieties and poor agronomic practices, insect pests, diseases and parasitic weeds. Insect pests have been shown to constitute a serious setback in the cultivation of cowpea especially in the drier regions of the tropics. Every stage in the life cycle of cowpea has at least one major insect pest that can cause serious damage and impact yield negatively (Fatokun 2002).

The cowpea aphid *(Aphis Craccivora* Koch.) has been described as one of the most important pest of cowpea [*Vigna unguiculata* (L.) Walp.], causing significant yield losses when either young seedlings or the pods of adult plants are attacked (Annan et al. 1996). Cowpea aphid is a sap-sucking insect pest of cowpea (Gunilla 1985; Singh et al. 1990). It causes damage in susceptible cultivars, directly by modifying plant metabolism and ingesting plant nutrients and in many cases indirectly, through the transmission of plant-pathogenic viruses (Blackman and Eastop 2000). In addition to sucking sap from the young leaves stem tissues and pods, Aphids also act as vectors in the transmission of cowpea aphids-borne mosaic virus even at a low population density (Atiri et al. 1986; Kitch et al. 1999). At high infestation levels, honeydew released by CPA can block plant respiration and stimulate development of black mold, thereby reducing photosynthesis. Cowpea aphids are well distributed throughout the tropics, colony expand very quickly in hot and dry weather and have numerous hosts but primarily on legumes. Cowpea aphids produce eggs that develop within the adult aphid which gives birth to nymphs alive. Nymphs mature into reproductive adults between 2 and 3 days causing a high severity of infestation as their population density increase rapidly (Schreiner 2000). Aphids primarily attack young cowpea seedling, however large populations also infest flower buds, flowers and pods, and cause direct damage to the plants by sucking its sap (Singh et al. 1996). Little damage may be seen on plant with small population of the insect. However, large population which results in heavy feeding kills young plant through the distortion of leaves, stunting of plant, thus affecting the overall plant vigour. There is also the delay in flower initiation, significant reduction in the nodulation of the root system as well as reduced pod set in plants which survive attack (Singh et al. 1996).

A number of aphid control measures (cultural, chemical and bio-control measures) have been suggested for the control of *A. craccivora* to prevent its impact on cowpea yield and spread of different viruses but are of limited value to subsistence farmers. Among the control options, the use of resistant varieties appears to be more viable and economical for resource poor farmers. Biological control alone is not adequate because natural enemies often appear when CPA infestation is already high and causing serious damage. Applying pesticides early in the season prevents CPA infestation and colonization but beneficial insects can be destroyed, leading to outbreaks of other insect pests. In fact, pesticide application is not a common practice in low-input farming systems in Africa (Souleymane et al. 2013) because of high cost and unavailability. Improving cultivars by adding in resistance genes through breeding promises a sustainable strategy for aphid control not only in cowpea but also in many other crop species (Huynh et al. 2013; Smith and Chuang 2014). The cumulative effect of several different mechanisms often provides effective resistance to insect by deterring, delaying or tolerating attack, feeding and reproduction. Even if the cumulative phenomena of resistance are not inherited together, individual mechanisms are often simply inherited and can be transferred in a stepwise manner into susceptible varieties (Bidinger 1992).

Many cowpea cultivars grown in the West African sub-region are susceptible to CPA and require pesticide treatments during early vegetative and flowering stages. Breeding resistant cowpea cultivars must rely on African cowpea genotypes that can act as aphid resistant donors (Hall et al. 2003). To successfully breed for resistance to cowpea aphids; suitable sources of resistance are a basic requirement. Resistance sources can be obtained from cultivated varieties, germplasm collection, wild species as well as by induced mutation. Sources of resistance to the cowpea aphids have been identified in some varieties of cowpea. However, the problem of biotype occurrence still persists as most resistance has broken-down. This is because high level resistance found in some wide relative and cultivated genotypes are conditioned by single dominant gene, which is not durable. For example, in Nigeria, there have been reports of breakdown of aphid resistance in previously resistant cowpea cultivars (Singh personal communication). This, therefore, call for the need to conduct screening to identify new sources of resistance to the cowpea-aphids.

Previous cowpea screening for aphid resistance was done by visual rating and counts which is difficult, complex, and often unreliable. There has been instance in which some lines that were earlier found to be resistant based on phenotyping in one location turned out to be susceptible in another location. The recent availability of molecular markers that are linked to aphid resistance can improve efficiency and precision in selecting cowpea genotypes for aphid resistance (Mayers et al. 1996). In the past, molecular markers have been used in cowpea to identify traits such as *Striga* resistance (Omoigui et al. 2012, 2016). However, progress in utilizing molecular markers associated with aphid has been limited due to the lack of reliable marker for the trait. Fortunately, recent advances in crop genomics have facilitated the identification of molecular markers associated with aphid resistance (Kusi et al. 2010). Integration of molecular markers in *aphid* resistance screening will greatly enhance the selection efficiency since field screening is difficult, complex, and often unreliable.

Aliyu and Ishiyaku (2013) reported differential reaction among seven (7) cowpea genotypes screened for resistance to cowpea aphids using conventional method. The differential reaction was observed in fecundity, larval development, adult longevity as well as the intrinsic rate of increase and multiplication of aphid. In a similar study, Obopile and Ositile (2010), reported differences in aphid fecundity among resistant and susceptible cowpea varieties. Laamari et al. (2008) reported antibiosis-mediated resistance to aphids on some landraces of broad bean, which was evidently observed in reduced fertility, multiplication rate and duration of reproductive life as well as in the observed longer duration of larval development. Kamphuis et al. (2012) reported the involvement of antibiosis, antixenosis and tolerance to CPA resistance in accessions of Barrel clover *Medicago truncatula*. In testing cowpea for resistance to aphid, Souleymane et al. (2013) screened some cowpea lines for aphid resistance and reported resistance by tolerance in TVNu-1158 (a wild cowpea line) compared to the cultivated lines. High flavonoid content has also been reported to be responsible for aphid resistance in cowpea (Cardinali et al. 1995) which is mediated by gene. This therefore, suggests the possibility of breeding resistant cowpea varieties against aphid attack. The different methodologies developed in screening cowpea germplasm and breeding lines, have provided the building blocks for the development of resistant varieties to insect pests (Singh et al. 1996). Some of the known resistant cowpea lines and germplasm have been found to be susceptible (Timko personal communication) either due to different biotypes or breakdown in aphid resistance or due to the approach used for screening for resistance to aphid attack. Most researchers (references) have used the conventional phenotyping approach to screen cowpea genotypes for resistance to aphis attack which may not be efficient. Another possible source for the different biotype could be the unrestricted movement of research materials from one part of the country to another and beyond.

There is the need to screen the previously reported resistant germplasm lines alongside new collections to revalidate resistant status and identify strong sources of resistance to aphids that will serve as resistant donors in breeding programs. Although phenotypic screening has been used in the past in selecting cowpea for aphid resistance (Souleymane et al. 2013; Ofuya 1993), it is a difficult process and often unreliable because of the strong influence of the environment on its expression. However, selection efficiency can be greatly improved by integrating molecular markers

with the phenotyping screening for precision in selecting for aphid resistance.

Over the last 8 years many efficient markers for aphid resistance genes have been described. Kusi et al. (2010) reported CP171/172 a PCR based marker linked to aphid resistance while Huynh et al. (2015) reported a SNP marker for aphid resistance in cowpea. Some of these markers are being used in marker-assisted selection (MAS) and to identify resistance genes in varieties and lines where the genetic background is unknown.

In this study, we screened cowpea germplasm collections to identify new sources of resistance to aphid infestation in cowpea using phenotypic data collected from germplasm materials grown under aphid kept in insect-proof cages with fine saran mesh and molecular markers validation. Gene-associated PCR markers (Kusi et al. 2010) and newly identified marker (unpublished data) were used to distinguish between resistant and susceptible genotypes.

Materials and methods

The experiment was conducted at the University of Agriculture, Makurdi, Benue State, Nigeria. The cowpea genotypes used for this study consisted of 60 cowpea genotypes obtained from the Genetic Resources Unit (GRU) of the International Institute of Tropical Agriculture (IITA) and the Molecular Biology Laboratory of the University of Agriculture Makurdi (UAM) (Table 1). The experiment was carried out during the months of April and June 2014 and 2015. Temperature profile at this time ranges between 23 and 30 °C with daily relative humidity ranging between 65 and 70%. The climatic profile was collected from our nearby weather station. The cowpea aphids used in this study were collected from Ukange Local Government area of Benue State. The aphid culture was maintained on a highly susceptible cowpea cultivar, TVx-3236 planted in an insect proof cage as previously described (Souleymane et al. 2013).

Experimental design

The experiment was laid out in completely randomized design (CRD) with three replications. Cowpea seeds were planted in wooden trays measuring 57.5 cm × 37.5 cm × 14.5 cm filled with top soil up to 10 cm depth and kept in insect-proof cages with fine saran mesh, small enough to allow passage of air but not insects. The seeds of each genotype were planted in single row, comprising five rows per tray, spaced 10 cm apart, while the distance between plant stands was kept at 5 cm. The plant growth conditions were as described previously by Bata et al. (1987), Githiri et al. (1996) and Kusi et al. (2010). Five 4th-instar nymphs (apterous adult) aphids were transferred on each plant at seven (7) days after planting using a camel's hair brush to reduce mechanical injury on the insect. Five plants each, of the genotypes were maintained per row. The plants were irrigated as at when necessary. The trays remained in the insect-proof cages for 21 days after which the plants were assessed for damage by aphids. Dead plants were regarded as susceptible while those still alive, with first trifoliate leaves developing, as resistant.

Counting of aphid and scoring for the aphid population per plant were used in screening of cowpea genotypes during the 21-day screening. Aphids per plant were counted at 5, 9, 13, 17 and 21 days after infestation. The aphid population build-up on each plant and the survival rate of CPA was measured at 21 days after infestation using a 1–5 rating scale based on visual and inspection counts and its symptoms given by Souleymane et al. (2013) and El-Defrawi and Bishara (1992) (Table 2).

Data collection

The following data were collected to assess the cowpea genotypes for resistance and susceptibility:

- number of aphids on individual plants was counted at 5, 9, 13, 17, and 21 days after infesting seedlings with aphids.
- visual score damages on each plant were recorded on 7 and 14 days after infestation.
- the survival rate of CPA was measured at 21 days after infestation.
- aphid population pressure on each plant was weighed using a 1–5 rating scale (1 = a few individual aphids, 2 = few small individual colonies, 3 = several small colonies, 4 = large individual colonies, 5 = large continuous colonies)

Table 1 Origin and description of the cowpea germplasm used for the study

S/no.	Germplasm	Reaction to aphids	Source	Genetic study (if any)
1	TVu 347	N/A	IITA	N/A
2	TVu 984	N/A	IITA	N/A
3	IT99K-216-24-2	N/A	IITA	N/A
4	IT84S-2246-4	R	IITA	N/A
5	TVu1016-1	N/A	IITA	N/A
6	IT82D-812	R	IITA	Breeding
7	TVNu 2876	R	IITA	Breeding
8	IT87S-1394	R	IITA	Breeding
9	TVu 1029	N/A	IITA	N/A
10	IT90K-76	R	IITA	Screening
11	IT90K-277-2	R	IITA	Breeding
12	TVu 1453	R	IITA	Screening
13	IT82E-16	R	IITA	Screening
14	BOSADP	N/A	Local	N/A
15	IT97K-499-35	R	IITA	Screening
16	TVX-3236	S	IITA	Screening
17	IT98K-131-2	R	IITA	Screening
18	IT98K-1092-1	R	IITA	Screening
19	IAR-48	S	IAR	Screening
20	TVu 4539	N/A	IITA	N/A
21	TVu 4540	N/A	IITA	N/A
22	TVu 6699	N/A	IITA	N/A
23	Ifebrown	N/A	OAU	N/A
24	TVu 57	N/A	IITA	N/A
25	TVu 134	N/A	IITA	N/A
26	TVu 157	N/A	IITA	N/A
27	TVu 231-2	N/A	IITA	N/A
28	IT82E-18	N/A	IITA	N/A
29	IT98K-1263	N/A	IITA	N/A
30	TVu 1000	N/A	IITA	N/A
31	TVu 16514	N/A	IITA	N/A
32	Golam white	R	Land race	Screening
33	UAM 1046-6-1	N/A	UAM	N/A
34	UAM 1051-1	N/A	UAM	N/A
35	UAM 1055-6	N/A	UAM	N/A
36	UAM 1046-6-2	N/A	UAM	N/A
37	UAM 1056-2	N/A	UAM	N/A
38	IT98K-573-1-1	R	IITA	Screening
39	IT98K-573-2-1	R	IITA	Screening
40	IT89KD-391	N/A	IITA	Screening
41	Aloka local	S	IITA	Screening
42	Kanannado	S	Land race	Screening
43	Danila	R	IITA	Screening
44	TVu 1092	N/A	IITA	N/A
45	TVNu 1158	R	IITA	Screening

Table 1 continued

S/no.	Germplasm	Reaction to aphids	Source	Genetic study (if any)
46	TVu 109-1	N/A	IITA	Screening
47	IT93K-503-1	N/A	IITA	N/A
48	SARC 1-57-2	R	SARI	Screening
49	IT90K-59	R	IITA	Breeding
50	Sierraleone 1	N/A	Land race	N/A
51	Sierraleone 2	N/A	Land race	N/A
52	Kano local	N/A	Land race	N/A
53	TVu 3000	R	IITA	Screening
54	TVu 36	R	IITA	Screening
55	TVu 62	R	IITA	Screening
56	TVu 408	R	IITA	Screening
57	TVu 410	R	IITA	Screening
58	TVu 801	R	IITA	Screening
59	TVu 2896	R	IITA	Screening
60	Tvu 2027	N/A	IITA	N/A

SARC 1-57-2 is also called F-Ghana

Reaction to aphids: *R* resistant, *S* susceptible, *N/A* information not available

Table 2 Aphid colony (population pressure) and visual plant damage score

Score	Description	Reactions
1	Few individual aphids (<20 aphids); no symptom of attack	Resistant
2	Few small individual colonies (21–50 aphids); plant showing little symptom (seedling slightly stunted)	Moderately resistant
3	Several small colonies (51–100 aphids); Plant showing symptoms of attack (seedling slightly stunted with slight yellowing of older leaves); no seedling damage	Moderately resistant
4	Large individual colonies (101–500 aphids); plant showing weak stem, leaves and seedling damage (seedling moderately stunted with yellowing of older leaves and curling of young leaves)	Susceptible
5	Large continuous colonies (>500 aphids). Severely stunted seedling with severely curled and yellow leaves, stem and leaves covered with sooty mould or dead seedling	Highly susceptible

A score of 1 was considered resistant (healthy/non-infested plants), 2 and 3 was considered intermediate (moderately healthy), 4 was considered moderately susceptible and 5 was considered susceptible to aphids (highly infested). Plant Resistance was also assessed by measuring differences in aphid population, number of dead plants and visual damages on the genotypes

taken at interval of 3, 6, 9 and 12 days after infestation.

- Total plant damage rating on scales 1–5 was measured at 16 days after aphid infestation, when aphid damage caused distinct phenotypic variation among cowpea plants based on crown damage and the extent of aphid occurrence applied to more than 60% of plants in each row number of dead plants was counted at 21 days after infestation. That is, seedlings killed or severely damaged by aphid infestation were regarded as susceptible while those still alive, with first trifoliate leaves developing, as resistant (Souleymane et al. 2013; Huynh et al. 2015).

Molecular marker analysis

Following insect cage proof phenotyping, DNA analysis was carried out to validate phenotypic data. For PCR assays, two SSR markers (CP171/172 and KAD61) previously reported to be linked to aphid resistance were used (Kusi et al. 2010; unpublished data). KAD16 has previously been validated in F_2

segregating populations derived from the cross between TVNu 2876 × Keffi local. KAD61 mapped 7.0 cm from the aphid resistance gene. Genomic DNA extracted from young leaves of parental plants was made ready for PCR as described previously (Omoigui et al. (2012). The sequences of the used primers and size fragment are present in Table 3.

DNA extraction

DNA was extracted from young leaf tissues of 14-day old plantlets from 60 cowpea germplasm and stored at −20 °C till DNA extraction. The genomic DNA was extracted using the CTAB extraction protocol (Doyle and Doyle 1987). Leaf samples collected in liquid Nitrogen were ground using the tissue-lyser until a fine powder was obtained. Pre-warmed 950 µl of C-TAB buffer and 2 µl of 2-mercaptoethanol was added into each sample before incubating at 60 °C for 30 min. The solution was mixed by inverting tubes gently at intervals of 10 min. 700 µl of 24:1 chloroform: isoamyl alcohol was added into the solution and incubated for 5 min at room temperature. The mixture was then centrifuged for 10 min at 7500 rpm, after which the supernatant was carefully transferred into newly labeled 1.5 ml tubes. 500 µl of ice cold isopropanol was added to the transferred supernatant to obtain a white precipitate and then stored at 20 °C for 30 min. The precipitate was then centrifuge for 20 min at 10,000 rpm to pelletize the DNA. To dissolve the DNA, 1× TE buffer was added to the pellet and stored at 4 °C. After dissolution, the extracted DNA was treated with RNase by incubating at 37 °C for 45 min. The concentrate DNA extract was stored at −20 °C until required.

Polymerase chain reaction (PCR) was performed using the Eppendorf master cycler gradient thermocycler in a total volume of 25 µl containing 2.5 µl 10× PCR buffer, 1.5 mM $MgCl_{2-}$, 0.5 µl dNTP's, 0.2 µl Taq polymerase, 18 µl of distilled water, 1 µl of each primer and about 50 ng of template DNA. Amplifications were performed at 94 °C for 10 min, followed by 40 cycles of 94 °C for 25 s, 55 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were separated in 2% agarose gel pre-stained with ethidium bromide and visualized using UV transilluminator. The image was photographed and gel images were used for scoring.

Data analysis

Data collected were analysed using descriptive statistics. Average number of aphids per genotype was calculated and means level of infestation scores of each genotype was determined. The count data collected was transformed using logarithm transformation. Data was subjected to analysis of variance (ANOVA) using the general linear model statistical procedures with the SAS system for Windows (SAS Institute 2014). Treatment means separation was done using the Student–Newman–Keuls (SNK) method.

Based on the result of screening cowpea germplasm for resistance/susceptibility, the mode of inheritance of the gene in TVu-2876 was determined using two genetically divergent parents that show clear differences for aphid resistance and susceptibility. Resistant germplasm (TVNu 2876) was crossed with the susceptible germplasm (Keffi local) to generate F_1. The seeds of the F_1 were selfed in the screenhouse to generate F_2 segregating population, while some were used in a backcross scheme to raise BC_1F_1 population for the inheritance study.

To test the allelic relationship among TVNu 2876 an aphid resistant germplasm identified in the study location, TVNu 1158 and F-Ghana (SARC 1-57-2) earlier reported to be resistant to aphid in Ghana, segregation ratios for each resistant × resistant (TVNu 2876 by F-Ghana (SARC 1-57-2) and TVNu 1158) progenies were computed. Genetic hypotheses were tested for significance using the Chi squared

Table 3 Details of the SSR markers used in the present study

Primer	Forward primer	Reverse primer
CP-171-172	5′-GTAGGGAGTTGCCCACGAATA-3′	5′-CAACCGATGTAAAAAGTGGAC-3′
KAD-61	5′-CTACGCTGGTTATTCTAGGGGA-3′	5′-GATAGAAGAAGAATGAGTAAGTAA-3′

goodness-of-fit test to determine the deviation of observed frequencies from the hypothesized ratios.

Results and discussion

There were significant ($P < 0.01$) differences among the cowpea genotypes for insect population pressure (PPI), plant damage (PTDMG), and number of dead plant (NDP), (Table 4). There was no genotype × year interaction effect for all the parameters measured, indicating that all the genotypes behaved the same in the different years. The rate of aphid population build-up on seedlings was very rapid and the plants were fully colonized within 6 and 9 days after infestation. Most of the genotypes showed severe stunting and wilting, and damage symptoms appearing as yellow patches or leaf chlorosis surrounding the aphid infestation sites within 15 days after infestation, with the exception of TVNu 2876. The cowpea genotypes, Aloka local and Keffi local had the highest number of aphids at 3, 6, 9 and 12 days after infestation based on the insect population pressure scored at these time intervals with mean scores of 4.5 and 4.6, respectively. The cowpea genotypes, TVNu 2876, TVNu 1158 and Sierra-Leone local had aphid population pressure of 2.2, 1.5 and 2.7, respectively that were significantly lower than other genotypes (Table 5). There were no significant differences among the genotypes for PP3DAI, PP6DA, and PP9DAI. However, TVNu 2876 had a strong resistance compared to the other two genotypes.

The strong differences in population pressure observed between susceptible and resistant cowpea genotypes tested corroborated the findings of Souleymane et al. (2013) who reported a rapid multiplication and full colonization on susceptible plants within 7 to 10 days after infestation. The aphid build up observed between 3 and 6 days after infestation for all the genotypes evaluated suggests that young plants are unable to elicit their resistance to prevent reproduction of aphid at the early stage of plant growth. However, the aphids' populations die out on the resistant plants as they grew older suggesting antibiosis resistance mechanism. This observation supports the findings of Alabi et al. (2012) who reported that all resistant and susceptible cowpea genotypes cluster in the same group with respect to the overall aphid build up in a

Table 4 Analysis of variance (ANOVA) for measured parameters of cowpea reaction to aphids combined over two years

SOV	df	PP3DAI	PP6DAI	PP9DAI	PP12DAI	PTDMG	NDP7DAI	NDP9DAI	NDP11DAI	NDP13DAI	NDPTMN
Variety	59	0.201ns	0.479ns	0.667ns	0.961**	1.314**	0.010ns	0.011ns	0.143ns	2.167*	5.124**
Year	1	39.609**	38.400**	33.004**	0.017ns	0.759ns	0.017ns	0.026ns	0.759*	26.334**	109.350**
Var*year	59	0.226ns	0.411ns	0.417ns	0.394ns	0.314ns	0.010ns	0.011ns	0.143ns	1.540ns	2.589ns
Error	120	0.174	0.504	0.433	0.475	0.468	0.010	0.011	0.132	1.314	2.927

PP3DAI population pressure three days after planting, *PP6DAI* population pressure six days after planting, *PP9DAI* population pressure nine days after planting, *PP12DAI* population pressure twelve days after planting, *PTDMG* plant damage, *NDP7DAI* number of dead plants seven days after infestation, *NDP9DAI* number of dead plants nine days after infestation, *NDP11DAI* number of dead plants eleven days after infestation, *NDP13DAI* number of dead plants thirteen days after infestation, *NDPTMN* number of dead plants at termination

Table 5 Mean aphid population score at 3, 6, 9 and 12 days after infestation of the top 15 extreme cowpea genotypes for resistance and susceptibility to aphids

Genotypes	PP12DAI
TVNu 1158	2.2
Sierra leone local 2	2.7
IT82D-812	3.4
TVNu 2876	1.5
TVu 134	3.9
IT84S-2246-4	4.0
IT97K-499-35	4.0
TVu 57	4.1
Aloka local	4.5
TVu 4539	4.3
IT98K-573-2-1	4.3
IT82E-16	4.4
IT90K-277-2	4.4
Keffi local	4.6
TVu 801	4.8
Mean	3.8
Lsd	1.2

Table 6 Mean number of dead plants at 21 days after infestation, of the top 14 cowpea genotypes for resistance and susceptibility to cowpea aphids

Genotypes	NDPTMN	Percentage survival (%)
IT82E-16	0.6	65
IT84S-2246-4	3.5	50
TVNu 2876	0.7	88
TVNu 1158	1.0	85
IT97K-499-35	0.9	66
Sierra-lone local 2	1.0	80
IT90K-277-2	2.0	28
Aloka local	2.9	44
IT99K-573-2-1	3.3	53
TVu 4539	3.4	23
TVu 57	3.5	50
TVu 36	3.6	48
TVu 134	4.5	23
Keffi local	10.0	0
Mean	1.1	
Lsd	0.9	

NDPTM—mean number of dead plants at 21 days after infestation

free-choice and no-choice tests at seedling stage. Similarly, antibiosis resistance has been reported to result in increased mortality or reduced longevity and reproduction of the insect (Teetes 2007). However, significant difference in population pressure was observed at twelve 12 days after infestation. This difference in time interval may be attributed to the innate ability of the plant to illicit the production of plant resistant chemicals (antibiosis) in response to aphid attack, a condition commonly referred to as hypersensitive reaction.

Although, aphid multiplication rate did not differ significantly in the evaluated genotypes at the early stage after infestation (within 3–6 days after infestation), the differential response of genotypes indicated by aphid population pressure at 12 days after infestation suggests an increase in fecundity. Fecundity increase was highest on TVu-801 with a mean score of 4.75 and lowest on TVNu-2876 with a mean score of 1.5 (Table 5).

Significant difference was also observed for a number of dead plants recorded at 16 days after infestation (Table 3). Keffi local had the highest mean number of dead plants followed by TVu 134 in comparison with TVNu 1158, TVNu 2876 and Sierra-Leone local that recorded high percentage survival rate (Table 6).

Ofuya (1993), also reported significant differences in number of aphids on susceptible and resistant varieties. The leaves of susceptible genotypes in the present study turned yellow and the plant became stunted and died between 15 and 21 days after infestation. The present study revealed that IT97K-556-6, a cultivated cowpea was tolerant to aphid. Despite the high number of aphids supported by this genotype, the genotype had high survival rate indicating that the genotype is tolerant to aphid. Resistance in TVNu 2786 and TVNu 1156 were expressed as hypersensitive reaction through the elicitation of antibiosis against the insect pest. Past studies have also showed that cowpea resistance against aphids was expressed through antibiosis and antixenosis in some cultivars (Arturo et al. 1988).

The mean plant damage score of cowpea genotypes is presented in Fig. 1. Among the 60 cowpea genotypes screened for resistance to cowpea aphids, two genotypes TVNu-1158 and TVu-2876, consistently expressed high resistance to cowpea aphid by

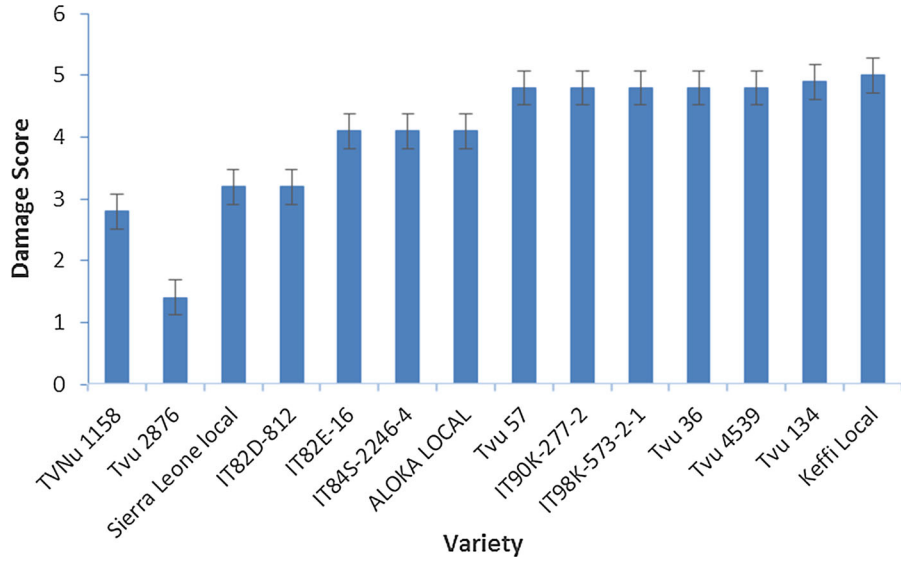

Fig. 1 Mean plant damage score of the top 14 cowpea genotypes for resistance and susceptibility to aphids

recording less damage scores ranging from 1.4 to 2.8. The most devastating effect caused by the cowpea aphid was observed on Keffi local, with a mean damage score of 5.0, most of the plants died before reaching 21 days after infestation (Fig. 1). This genotype is thus classified as highly susceptible to cowpea aphid. The other cowpea genotypes that suffered similar damage effects were TVu 134 (4.9), TVu 4539, TVu 36, IT98K-573-2-1, and IT90 K-277-2 had damage score of 4.8 each.

The result of the phenotyping screening identified TVu 2786 and TVNu-1158 as having strong sources of resistance to cowpea aphids. The genotype recorded lower population pressure, lower plant damage score, and lower number of dead plants than the other genotypes at 21 days after infestation. The ability of these parameters to distinctly discriminate between resistant and susceptible genotypes shows their potential for use in identifying differences in cowpea reaction to aphids.

High plant damage, increased CPA population pressure and high number of dead plants resulting from the direct feeding activities of cowpea aphids characterized the reactions of susceptible cowpea genotypes. Evidence of susceptibility on cowpea genotypes was prevalent on Keffi local, and Aloka local which were distinctly marked by stunted growth, increased secretion of honey dew, resulting in an early yellowing of older leaves and high plant mortality.

The cowpea genotype, TVNu-1158, a wild relative, also recorded the lowest mean plant damage score and mean population pressure score of 2.2 and 2.8 respectively. In addition to its high survival percentage (85%), TVNu-1158 also possesses resistant characteristics attributable to the plants innate ability not to support build-up of the insect population up to damaging levels. This present study agrees with the findings of Souleymane et al. (2013) who reported a slower aphid population build-up on the wild cowpea genotype and suggested antibiosis as the mechanism of resistance. Similarly, the current study reveals a survival rate of 85% which is close to 80% survival rate earlier reported for this genotype by Souleymane et al. (2013). One other resistant variety identified in this study that showed high percentage survival rate and low plant damage score include Sierra-Leone Local.

The F_2 segregating population derived from the cross between TVu-2876 (resistant parent) and Keffi local (susceptible parent) was used to determine the inheritance pattern. Segregation in the F_2 population segregated 168 resistant to 59 susceptible individuals (Table 7). The χ^2 analysis for goodness-of-fit revealed that the segregation pattern fits the 3:1 genetic ratio, indicating that resistance to cowpea aphids in TVNu-2876 is controlled by a single dominant gene. The backcross populations involving the F_1 plants and susceptible parents segregated into 1:1 ratio, which further confirmed that a single dominant gene confers resistance in TVNu 2876.

Marker assay

The thirty cowpea genotypes that were found to show some level of resistance or tolerance using phenotypic data were further screened for presence of markers conferring resistance to cowpea aphids with two different molecular markers linked to aphid resistance in cowpea. KAD16 has previously been validated in F_2 segregating populations derived from the cross between TVNu 2876 × Keffi local. KAD61 mapped 7.0 cm from the aphid resistance gene.

In contrast to the plant phenotype, there were some distinct differences in the genotypes based on the marker score. Some genotypes that were found to be resistant based on phenotypic data were found to be susceptible with the marker score. However, the two markers (CP171/172 and KAD61) gave reproducible and score-able bands with known resistant and susceptible genotypes (Figs. 1, 2). The two markers amplified the cowpea genotype, TVNu 2876, whereas TVNu 1158 was only amplified by KAD61. CP171/172 is a co-dominant marker developed for aphid resistance biotype in Tamale, Ghana while KAD61 a dominant marker was linked with aphid biotypes from Nigeria (Table 8). Since CP171/172 marker is linked with Ghana aphid biotype it is not surprising that this marker did not amplify TVNu 1158, indicating that TVNu 2876 could be resistant to different aphid biotypes in the study location. Also, cowpea genotype TVNu 2876 was highly diverse from TVNu1158 on the basis of marker assay and band size difference. The molecular weight amplified in TVNu 2876 was unique with a 100 bp while that in TVNu 1158 was 120 bp suggesting a novel gene in TVNu 2876. The result of this present study is interesting because most aphid resistant cowpea genotypes developed at IITA have been reported to be susceptible in other countries (Ofuya 1997). Kusi et al. (2010) recently reported high susceptibility of IITA lines and suggested the existence of cowpea aphid biotype in northern Ghana which is more virulent than the Nigerian biotypes. Identification of new genetic sources of resistance to aphid by TVNu 2876 will ensure gene pyramiding to guide against aphid biotypes. The marker score highly correlated with the phenotypic data for the susceptible genotypes as the marker presence were completely absent in those genotypes. The discrepancies that were observed were in relation to the moderately resistant

Table 7 Chi square analysis of segregation in the F_2 generation of single cross between Keffi local and TVu 2876

Cross	N	R	S	Genetic ratio	Calculated χ^2 value	Critical χ^2 value ($P < 0.05$)
TVu-2876	12	12	0			
Keffi local	12	0	12			
F_1	20	20	0	1:0		
F_2	227	168	59	3:1	0.09	0.09

N no of plants evaluated, *R* resistant, *S* susceptible

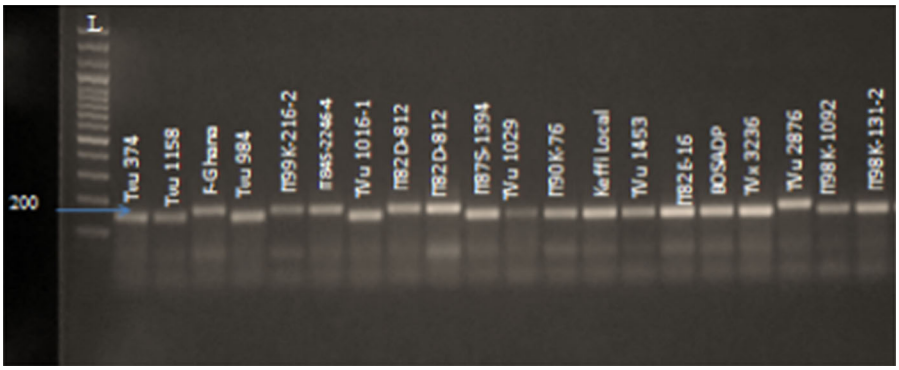

Fig. 2 Analysis of CP171/172 marker linked to CPA in cowpea genotypes. Shown is a representative picture of resolution of CP171/172 marker separated on 2% agarose gel. Presence of 200 bp product indicates the presence of resistance allele

Table 8 Presence of markers linked to gene conferring resistance to *Aphis craccivora* in 30 cowpea genotypes with known resistance and susceptible cultivars

Cowpea genotype	Dms[a]	Phen score	KAD61	CP-171/172
TVu 347	3.5	S	−	−
TVu 1158	2.0	R	+	−
F-Ghana	2.5	R	−	+
TVu 984	4.5	S	−	−
IT84-2246-4	4.5	S	−	+
TVu 1016-1	4.5	S	−	−
IT82D-812	2.5	R	−	+
IT87S-1394	4	S	−	−
TVNu 1029	4.5	S	−	−
IT90K-76	5	S	−	−
Keffi local	5	S	−	−
TVu 1453	3.5	S	−	−
IT82E-16	4	S	−	−
BOSADP	5	S	−	−
TVx 3236	4.5	S	−	−
IT98K-131-2	5	S	−	−
IT98K-1092	4	S	−	–
TVu 2876	1.5	R	+	+
IT97K-499-35	5	S	+	−
TVu 4539	4	S	−	+
TVu 4540	4	S	−	+
IAR 48	5	S	−	−
IFE brown	5	S	−	−
TVu 57	5	S	−	−
TVu 134	4	S	−	+
IT99K-216-2	2.5	R	−	+
TVu 231-2	3	R	−	−
IT82E-18	4.5	S	−	−
IT98K-1263	5	S	−	−
TVu 1000	5	S	−	+

[a] F_2 population

VD/WS wilting score, *RT* reaction type, *R* resistant, *VD* visual damage, *S* susceptible (+) presence of marker (−) absence of marker

genotypes. The presence of polymorphic markers between resistant and susceptible parents (Fig. 3) as demonstrated in this study reveals the possibility in tracking the gene for resistance to *A. craccivora* in cowpea.

Many of the cowpea genotypes that were identified as resistant in insect proof screening was confirmed susceptible by the markers applied. This result stresses the effectiveness of DNA marker characterizing genotypes for resistance and susceptibility to aphids rather than selecting cowpea for aphid resistance on the basis of phenotypic screening alone which may be influenced by the environment. Also, some of the genotypes that were earlier classified to be moderately resistant under phenotypic score were classified as susceptible when markers were applied (Table 8). Similar finding had been reported by Omoigui et al. (2016) who found SSR and SCAR markers to be effective in discriminating between *Striga* resistance and *Striga* susceptible cowpea genotypes. In many cases resistant genes can only be identified using molecular markers (Melchinger 1990). The utility of such findings is further authenticated by other studies, where the presence of rust resistance genes was confirmed with molecular marker (Li et al. 2017; Imbaby et al. 2014), Aphid resistance genes (Huynh et al. 2015; Kusi et al. 2010). Marker-assisted selection offers the opportunity to select desirable lines on the

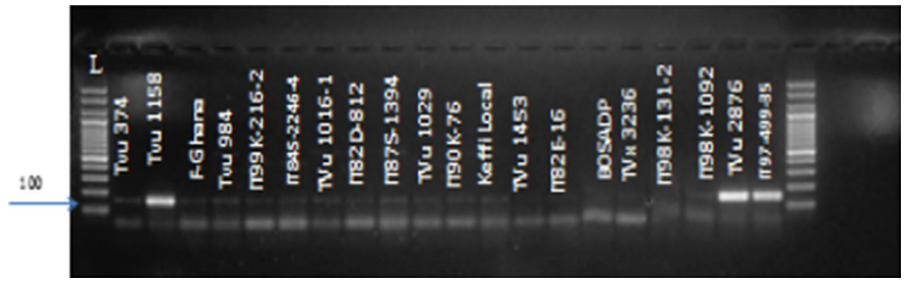

Fig. 3 Analysis of KAD16 marker linked to CPA in cowpea genotypes. Shown is a representative picture of resolution of KAD61 marker separated on 2% agarose gel. Presence of 200 bp product indicates the presence of resistance allele

basis of genotype rather than phenotype. In the current studies, the discrepancies observed in the reaction of the genotypes may suggest a gap in our present knowledge of genetics of aphid resistance in cowpea using phenotypic data alone.

KAD61 is a dominant marker, it amplifies resistant lines with a single band while susceptible lines had no band. Whereas, cp117/16 is a co-dominant marker and showed different bands size in resistant and susceptible parents. The limitation of a dominant marker is that it can only classify segregating population into two categories (resistant: susceptible), it cannot differentiate alleles of the same genotype. Unlike a co-dominant marker that can different alleles of the same genotype. A co-dominant marker will amplify both resistant and susceptible parents. When used in a segregating population, three possible genotypes are seen (AA, Aa, and aa). The advantage of this is that homozygous dominant (AA) can be distinguish from heterozygous dominant (Aa). Consequently, categorize the segregating population into three classes (homozygous resistant with single band: heterozygous resistant with double band: homozygous recessive susceptible with a single band), which is very critical for selection in F3 population. This limitation, however, does not mean that dominant marker cannot be used in marker-assisted backcrossing program. The marker can be employed in F_2 segregating population to quickly eliminate the susceptible lines, so that only resistant lines (homozygous resistant and heterozygous resistant lines) are carried forward to F3 for further screening and selection. Dominant markers can also be employed in successive backcrossing program to check each backcross if the resistant lines are carried forward. For each backcross, the successful backcross progenies being carried forward are heterozygous. The utility of dominant markers (C42B) in discriminating *Striga* resistant and susceptible lines had been successfully demonstrated in cowpea (Omoigui et al. 2012, 2016).

After the identification of TVNu 2876 as good source of resistant to aphid, allelic relationship was conducted to confirm if the gene that confer resistant in TVNu 2876 and F-Ghana (SARC 1-57-2) are the

Table 9 Segregation ratios of F_2 progenies derived from the crosses between resistant parents

Populations	Generation	Total no. of plants	Number of plants		Genetic ratio	χ^2-value	$Pr \leq \chi^2$
			Resistant	Susceptible			
TVNu 2876	P1	25	25	0	R		
F-Ghana (SARC 1-57-2)	P2	20	19	1	R		
TVNu 1158	P3	25	23	2	R		
SARC 1-57-2 × TVNu 2876	F_1	20	19	1	R		
TVu 1158 × TVNu 2876	F_1	25	23	2	R		
SARC 1-57-2 × TVNu 2876	F_2	125	115	10	15:1	2.9	92.25
TVu 1158 × TVNu 2876	F_2	120	111	9	15:1	4.2	96.25

R resistant, *S* susceptible

same. TVNu 2876 was crossed with SARC 1-57-2 and TVNu 1158 to obtain F_1. The F_1 plants were selfed to obtain F_2 populations. One hundred and twenty individual plants of the F_2 populations derived from the cross between TVNu 2876 and SARC 1-57-2 were planted and screened for aphids under artificially infested plants with aphids. Twenty-Five F_1 seeds from both the straight and reciprocal cross, twenty seeds of parents: TNVu 2876, and SARC 1-57-2 included as checks were assessed for aphid resistance.

In the F_2 cross, the segregation for aphid resistance in the allelism test showed that 115 plants were resistant and 10 plants were susceptible, which exhibited the action of dominant genes conferring resistance to TVNu 2876 and SARC 1-57-2 (Table 9). The Chi square values showed a good fit for a segregation ratio of 15 resistant to 1 susceptible, which demonstrates the presence of two independent dominant genes. This result supported the hypothesis that the gene conferring resistance to aphids in TNVu 2876 is independent, harboured in SARC 1-57-2. Similarly, in the cross between TNVu 2876 and TVNu 1158, segregation was observed in the F_2 population (110 resistant plants and 10 susceptible plants), which suggests that resistance gene in TVNu 2876 and TVNu 1158 are not the same. This finding also corroborates the marker data, which showed different banding pattern in the two cowpea genotypes.

Conclusions

This study identified new cowpea genotype TVNu 2876 as a strong source of resistance to CPA. The application of molecular markers in this study has revealed that phenotypic data alone is not always comprehensible in identifying genotypes for resistance to CPA. This inconsistency shows that markers should be used in conjunction with phenotypic data for selection of genotypes for resistance to aphids. The mechanisms of resistance in cowpea genotype, TVNu 2876 and TVNu 1158 suggest that of hypersensitive response. The susceptibility of IT84S-2246-4 indicates the possibility that there is a new variant of cowpea aphid that is capable of infesting known resistant sources or that the resistance gene in IT84S-2246-4 has broken down. With the frequent breakdown of single dominant gene in our extensive monoculture agricultural systems, it is important to pyramid multiple resistant genes into crops with major R-genes to help inhibit the occurrence of new virulent biotypes or against gene breakdown. The segregation in F_2 and BC1 populations derived from the cross between TVNu 2876 and Keffi local indicated that resistance to cowpea aphids in TVu-2876 is controlled by a single dominant gene. Allelism test revealed that resistance gene in TVNu 2876 is non-allelic with the gene that confers resistance in SARC 1-57-2 and TVNu 1158. The cowpea genotype, TVNu 2876 identified in this study has a great potential as source of additional resistant gene or for gene pyramiding against the cowpea aphid in Makurdi and its environs.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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