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Genetic analysis of Fusarium wilt resistance in cowpea (*Vigna unguiculata* Walp.)

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Abstract

This study investigated the inheritance of resistance to *Fusarium oxysporum* f.sp. *tracheiphilum* (*Fot*) in cowpea lines. Resistant and susceptible cowpea lines were crossed to develop F_1, F_2 and backcross populations. Reaction to *Fot* was evaluated in 2015 and 2016 using seed soak and modified root-dip inoculation methods. The expression of resistance reaction in the F_1 and segregation in F_2 generations indicated the role of dominant gene controlling *Fot* in cowpea. These results were further supported by the result of backcross ($BC_1P_1F_1$ and $BC_1P_2F_1$) progeny tests. The backcross of F_1 with the resistant parent produced progeny that were uniformly resistant, whereas backcross of F_1 with the susceptible parent produced progeny that segregated into 1:1 ratio. The F_2 segregation ratio in the reciprocal cross showed no evidence of maternal effect in the inheritance of the resistance. Allelism test suggests that the gene for resistance in TVu 134 was the same in TVu 410 and TVu 109-1. We also identified an SSR marker, C13-16, that cosegregated with the gene conferring resistance to *Fot* in cowpea.

KEYWORDS
cowpea, *Fusarium oxysporum tracheiphilum*, Fusarium wilt, polymerase chain reaction, resistance, resistant, simple sequence repeat, susceptible

1 | INTRODUCTION

Cowpea is an important food legume crop in Africa. It plays a vital role in food security, human nutrition and subsistence agriculture because it is consumed by humans, serves as fodder for livestock, supports soil conservation, compatible with integrated farming systems and facilitates symbiotic nitrogen fixation (Reddy et al., 2005). Cowpea has been called the poor man's meat in Africa because of its high protein content, which ranges from 23% to 25% (Bressani, 1985). An estimated 14.5 million hectares of land is planted to cowpea annually worldwide. Global production of dried cowpeas in 2016 was 6.9 million metric tons, and 94% of this production was accrued to Africa (FAOSTAT, 2016). The top producers are the West and Central African subregions which contribute to about 64% of the global production. Nigeria, the largest producer and consumer, accounts for over 64% of production in Africa and 60% worldwide (FAOSTAT, 2016).

In the developing countries where soil infertility is high, rainfall is limiting and most of the cowpea is grown without the use of fertilizers and plant protection measures (i.e., pesticides or herbicides), a wide variety of biotic and abiotic constraints greatly limit growth and yield of cowpea (Singh, 2005; Timko, Ehlers, & Roberts, 2007). Fusarium wilt (FW) caused by the fungal pathogen, *Fusarium oxysporum* f. sp. *tracheiphilum* (*Fot*), is one of the diseases that pose a major threat to cowpea production worldwide. FW disease can be problematic in many areas where cowpeas are grown. The disease causes substantial yield losses ranging from 30% to 100% (Reddy et al., 1990). In the United States, high plant mortality with severe overall

yield loss has also been reported (Pottorff, Li, Ehlers, Close, & Roberts, 2014).

The occurrence and epidemic spread of this soil-borne disease are influenced by factors such as soil nutrient levels, temperature, moisture stress and resistance of varieties (Steven, Krishna, Davis, & Turini, 2003). The optimum temperature for growth of *F. oxysporum* has been reported to be between 25 and 28°C (Cook & Baker, 1983). The fungal pathogen *Fusarium oxysporium* has a wide host range, encompassing plants in the Leguminosae, Malvaceae and Solanaceae causing vascular wilt (Beckman, 1987). The pathogen enters the plant through the root system and invades the vascular tissue. Infected plants exhibit leaf chlorosis, wilting, vascular discoloration and death with severe overall yield loss. Broad irregular patches of affected plants are visible in infested cowpea fields (Armstrong and Armstrong 1981). The disease is widespread and causes substantial crop losses in most of the major cowpea producing areas of the world. The yield loss largely depends upon the stage at which the plants wilt and can reach up to 100% when wilt occurs at the pre-pod stage (Okiror, 2002). The outward symptoms typically become evident at the seedling stage or during flowering and early pod development, resulting in high mortality in the affected areas.

Fot is soil-borne and seed-borne fungus that is difficult to manage through fungicide applications alone. Continuous use of broad-spectrum biocides to fumigate soil before planting, particularly methyl bromide, is environmentally damaging and may lead to development of resistant strains of the pathogen. Also, the chlamydospore that forms a thick-walled asexual spore can survive in organic soil residue for several years (Nelson, 1981). These factors make crop rotation and use of fungicide an incomplete control measure. The most cost-effective and environmentally safe control is the use of resistant cultivars when they are available (Fravel, Olivan, & Alabouvette, 2003). At present, four races of *Fot* have been characterized according to differential interactions on several cowpea genotypes (Hare, 1953; Patel, 1985; Smith, Helms, Temple, & Frate, 1999). While *Fot* race 3 is currently the most widely distributed, race 1 is predominant in Nigeria (Armstrong & Armstrong, 1980; Smith et al., 1999). In Nigeria, the first report of FW, caused by *Fusarium oxysporum* in cowpea (Oyekan, 1975) indicated 50%–100% crop damage on susceptible cultivars under suitable conditions. The *Fot* isolate was later identified as race 1 (Armstrong & Armstrong, 1980), and further research has documented more isolates, from Ife Brown, TVu 4557 and Prima with disease incidence of 21, 15 and 55 per cent, respectively (Aigbe & Fawole, 2009). Despite the devastating effects of the pathogen, breeding research towards the development of Fusarium-resistant cowpea cultivars has been minimal in Nigeria.

To develop an effective breeding strategy for the introgression of FW resistance genes in cowpea, a detailed knowledge of the inheritance of FW resistance in cowpea is important (Zhang, Hwang, Gossen, Chang, & Turnbull, 2007). Some researchers (Lv et al., 2011; Rubio, Hajj-Moussa, Kharrat, Moreno, & Millan, 2003) suggest that resistance to FW follows the gene-for-gene concept described by Flor (1971), whereby single major resistant (R) genes recognize and

respond to pathogen avirulence (Avr) genes. However, in some cases oligogenic resistance to *F. oxysporum* has been reported (Beckman & Roberts, 1995). Although the genetic basis is unknown in most cases, it is common for different host varieties to possess different levels of resistance to Fusarium vascular disease. While inheritance studies on FW resistance have been well documented in other crops (Augustine, Paul, Narla, Buruchara, & James, 2010; Rubio et al., 2003; Scott & Jones, 1989), very limited studies have been undertaken to elucidate the genetic basis of FW resistance in cowpea, especially in Nigeria cowpea germplasm.

In cowpea, a major resistance gene has been identified and mapped in the United States (US) germplasm for races 3 and 4, but limited information is available in any published research on the mode of resistance gene(s) for race 1. Rigert and Foster (1987) reported a single dominant resistance gene for both races 2 and 3 in California cowpea cultivars '7964' and 'CB3'. Further, the authors noted that the race 3 gene in 'CB3' also conferred incompletely, dominant resistance to race 2, while the race 2 gene in '7964' conferred incompletely, dominant resistance to race 3. Pottorff et al. (2014) reported that two independent loci confer resistance to *Fot* race 4 in some cowpea RIL population derived from IT93k-503-1 × CB46, CB27 × 24-12-125B-1 and CB27 × IT82E-18/Big Buff. The *Fot* race 4 with gene symbol *Fot4-1* was positioned on linkage group 5, *Fot4-2* on linkage group 3, while race 3 with gene symbol *Fot3-1* was positioned on linkage group 6 (Pottorff et al., 2014). Knowledge gap exists regarding the genetic basis of *Fot* resistance in cowpea to race 1 in Nigeria, such information is important for genetic improvement of the local germplasm.

In history, phenotypic screening has been used to identify resistant cultivars, while conventional breeding strategy has been adopted to transfer resistance gene(s) into susceptible varieties in West Africa. Breeding crop cultivars for resistance to disease often requires a decade (or more) to develop and release a new cowpea cultivar because it involves screening and identifying appropriate resistant germplasm sources and then introgressing the resistance trait. Molecular tools, including marker-assisted selection, have the potential to accelerate and improve the efficiency and effectiveness of breeding for disease resistance in many crops. These genomic resources have been integrated using single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) genotyping platforms. *Fot* resistance determinants have been mapped in the cowpea genome, and several cowpea accessions have been SNP genotyped by the University of California Riverside (UCR) cowpea group. The utility of SNP markers in discriminating Fusarium wilt resistant and susceptible sources will facilitate cultivar improvement using marker-assisted breeding. However, genetic diversity study using amplified fragment length polymorphism (AFLP) to determine the relationship between US cowpea germplasm and the lines developed in West Africa revealed a distinct overlap between the US germplasm and from the West Africa breeding lines. This indicates that there is no pedigree relationship between the US germplasm and the resistant lines developed in Africa. Several markers have also been developed for Fusarium wilt resistance in other crops such as soybean (Ellis et
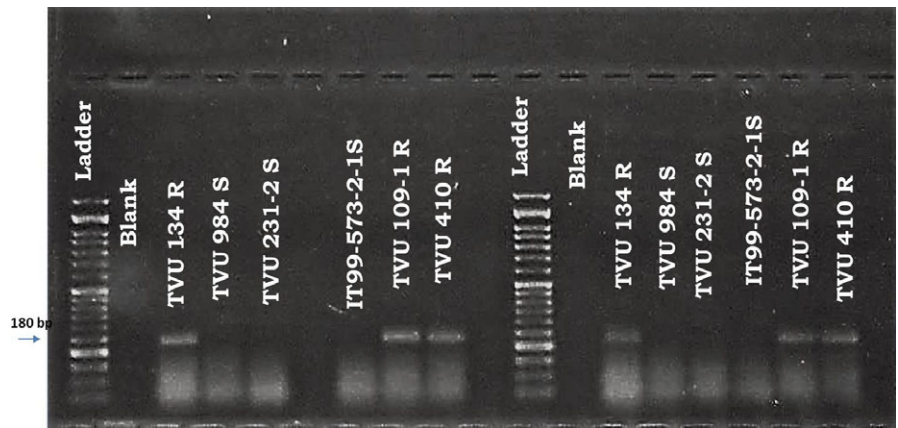
FIGURE 1 Polymerase chain reaction (PCR) banding pattern of the SSR marker C13-16 linked with Fot resistance gene. DNA ladder 100 bp. Validation of marker on parental cultivars. R = resistant, S = susceptible

al., 2012), chickpea (Varshney et al., 2014) and pigeon pea (Deepu et al., 2016). In recent times, some promising markers identified for race 1 were validated in this study (UVA cowpea group, unpublished data).

The objectives of this present study were to (a) characterize available cowpea germplasm for resistance to Fot (b) determine the mode of inheritance of Fot resistance in cowpea and (c) validate the molecular marker recently identified to be tightly linked to Fot race 1 resistance gene. Identification of closely linked marker will contribute to future use in marker-assisted breeding to enhance cowpea for Fusarium wilt resistance in Nigeria.

2 | MATERIALS AND METHODS

2.1 | Plant material

Sixty cowpea genotypes, obtained from the International Institute of Tropical Agriculture (IITA), were screened for FW resistance using the seed soak method described by Aigbe and Fawole (2010) with slight modification. The slight modification to this method was as follows: The plates used were incubated for 7 days in the laboratory as opposed to the 4 days originally described by Aigbe & Fawole, 2010;. Genotypes were classified according to their resistance response to the fungus and, six genotypes from the two extreme ends (highly susceptible and highly resistant) of the phenotypic spectrum were selected for further analysis. Three Fot susceptible cowpea genotypes namely TVu 984, TVu 231 and IT99K-573-2-1 and three Fot-resistant genotypes namely TVu 134, TVu 410 and TVu 109-1 were selected for this study based on their reaction to FW. The six parental lines were further screened using the root-dip and seed soak inoculation methods to validate their reaction to Fusarium wilt and validated using molecular marker identified previously to be linked to resistant gene (Figure 1). Based on the reaction of the lines to FW and grouping for resistance and susceptibility, the resistant and susceptible genotypes were sown in crossing block in the screenhouse at the University during the 2015 cropping season. The different biparental cross-combinations were developed to obtain F_1 hybrids. The true F_1 plants were identified based on linked molecular markers. The F_1 along with the parents was grown in crossing blocks

TABLE 1 Number of F_1, F_2 and backcross progeny developed and evaluated

Populations	Generation	Progeny
TVu 984 × TVu 134	F_1	50
TVu 984 × TVu 134	F_2	249
F_1 × TVu 134	BC_1P_1	50
F_1 × TVu 984	BC_1P_2	46
IT99K-573-2-1 × TVu 410	F_1	52
IT99K-573-2-1 × TVu 410	F_2	246
F_1 × IT99K-573-2-1	BC_1P_1	42
F_1 × TVu 410	BC_1P_2	50
TVu 231-2 × TVu 109-1	F_1	50
TVu 231-2 × TVu 109-1	F_2	176
F_1 × TVu 231-2	BC_1P_1	46
F_1 × TVu 901-1	BC_1P_2	44
TVu 134 × TVu 984	F_1	50
TVu 134 × TVu 984	F_2	200
F_1 × TVu 984	BC_1P_1	50
F_1 × TVu 134	BC_1P_2	46

during rainy season of 2015 and 2016, and each F_1 plant was allowed to self-pollinate and set seed in the screenhouse.

2.2 | Population development

The cowpea lines used in this study were chosen based on their contrasting reaction to Fot (unpublished data). Plant emasculation and pollination were carried out either in the morning or in the evening when the temperature is relatively low, and humidity is high to increase the chances of success. Matured pods resulting from successful crossing were harvested at maturity for F_1 seeds, and resulting F_1 plants were grown in the screenhouse to produce the F_2 seeds. F_1s were crossed to the original parents in the screen house to obtain $BC_1P_1F_1$ and $BC_1P_2F_1$ progeny. The backcross progeny were harvested as individual pods. The total number of progeny developed from the different crosses, F_1, F_2 and backcross, is presented in Table 1.

2.3 | Isolation and culture of *Fusarium oxysporum*

Samples of *Fot* which originated from infected cowpea plants in Makurdi, Benue State, Nigeria, were used for inoculation culture. Diseased and wilted plants collected from the field were examined in the laboratory for symptoms of Fusarium wilt infection. Plant roots were washed carefully to remove soil debris, and then, a cut was made on the diseased plant to observe the discoloration on the stem. Plants showing reddish brown vascular discoloration on the roots and stem were separated for isolation of the pathogen. The pathogen was isolated and cultured on potato dextrose agar (PDA) with streptomycin and chloramphenicol peptone agar. Serial dilution was used to produce single spore cultures of *Fot*. Germinating spores were isolated and transferred onto fresh PDA/streptomycin-plated Petri dishes using a sterilized wire loop. Spore suspension from single spore cultures was used as source of inoculum for all disease assays. Potato dextrose agar was used for inoculum production. Single spore cultures were grown by incubating them at room temperature for 10 days. The culture surface was flooded with 10 ml of sterile distilled water (SDW) containing Tween 80 (at the rate of one drop of Tween 80 to 1000 ml SDW) to ensure uniform spore distribution. The agar surface was scraped with a sterile glass rod and the spore suspension filtered through two layers of sterile cheesecloth (Aigbe, 2008). A spore concentration of 2×10^6 was used for all disease assays.

2.4 | Disease assay

Parents, F_1, F_2 and BC_1 progeny of all crosses were evaluated for Fusarium wilt resistance using seed soak method developed by Aigbe and Fawole (2010). Seeds of parent and their F_1, F_2 and backcross progeny were surface-sterilized for 1 minute in 10% NaOCl suspension mixture, rinsed three times with sterile distilled water and soaked in spore's concentration of 2×10^6 per ml of *Fot*. Seeds were removed from the inoculum after 6 hr. Twenty seeds of each parent were placed on sterile filter papers well-spaced contained in a 9-cm-diameter Pyrex dish replicated three times, and the different populations using the appropriate population size were evaluated in Pyrex dish. The total number of healthy seedlings, total seed rot, total number of infected seedlings and total seedling mortality were recorded for each population, separately grown by incubating them at temperature of 28°C for 10 days. Slides of hyphae/spores formed on rotted seeds/ seedlings were identified under the compound microscope to be *Fot*. Inoculation techniques and cultural conditions have been reported to affect the expression of FW resistance in cowpea (Sarfatti, Abu-Abied, Katan, & Zamir, 1991). Therefore, progeny of the cross between TVu 984 × TVu 134 were also evaluated for resistance using a modified root-dip inoculation method described by Rigert and Foster (1987) for comparison. The parents, F_1, F_2 and backcross progeny of the different populations were planted in plastic pots measuring 20.5 diameter × 19.5 cm depth filled with sterilized soil (sand (80%) and loam soil (20%) mixture) treated with 2 g of diammonium phosphate fertilizer per pot. All experiments were laid out in a completely randomized design replicated three times. At 7 days after planting (when the primary leaves are fully expanded), seedlings were gently uprooted. The roots were washed in running tap water and trimmed with scissors. Seedlings with trimmed roots were dipped in a spore suspension of 2×10^6 spores ml^{-1} for 1 hr.

2.5 | Disease evaluation

Plants were evaluated for reaction to *Fot* based on phenotypic vascular discoloration by uprooting the entire plant and then slicing the stem vertically to evaluate the extent of disease damage. The severity of the disease was evaluated on a 0–5 scale described by Pottorff et al., 2012. This was evaluated by approximating the percentage of wilting or stunting on the entire plant. A score of zero indicated a healthy plant with no signs of disease, 1 = approximately 10% of the plant showing symptoms of disease, 2 = approximately 25% of the plant showing symptoms of disease, 3 = approximately 50% of the plant showing symptoms, 4 = approximately 75% of the plant showing symptoms and 5 = 100% of the plant showing disease symptoms. The rating was carried out at 7 weeks postinoculation. Seedlings with disease ratings of 2 never developed other disease symptoms and usually recovered from stunting. For genetic hypotheses, a disease rating of 1 or 2 was considered resistant (R) and disease rating of 3, 4 or 5 was considered susceptible (S). A single spore initiated isolate of each *Fot* race was used in all tests. Allelic relationship among the resistance parents was also determined.

2.6 | Primer screening

A set of cowpea SSR primer combinations based on cowpea gene space read (GSR) sequences annotated for disease and pest resistance genes (Timko et al., 2008) were downloaded from the Cowpea Genomics Knowledge Base (CGKB) (http://cowpeagenomics.med.vir ginia.edu/CGKB) website. About 2000 SSR markers were screened to identify closely linked markers for *Fot* race 1 in cowpea. Based on the screening, we identified C13-16 as utility marker that could be used to screen cowpea for resistance to *Fot*. This marker was employed for molecular analysis to screen the parental materials and the segregating populations.

2.7 | DNA amplification

Young leaf tissues from 14-day-old plantlets were collected from parents and their F_1, F_2 populations and stored at −20°C until DNA extraction. The genomic DNA was extracted using the Geneaid's Genomic DNA Mini Kit (Biochem Life Sciences, New Delhi, India). Polymerase chain reaction (PCR) amplification mixture (15 μl) consisted of 20–25 ng of genomic DNA, 200 μM dNTPs, 2 mM MgCl$_2$, 1-unit *Taq* DNA polymerase (MBI Fermentas, Hanover, USA), 1 × PCR buffer and 0.6 mM reverse and forward primers. DNA amplification was carried out in a Thermal Cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany) with a PCR profile which included an initial denaturation step at 94°C for 3 min followed by 35 cycles with a denaturing step at 94°C for 30 s, a primer annealing step at optimum

annealing temperature for 30 s and an extension step at 72°C for 1 min. After the last cycle, samples were kept at 72°C for 5 min for final extension. The amplification products were separated electrophoretically in 2% agarose gels containing 0.05 μg/ml ethidium bromide and prepared in 1 × TAE buffer. The amplification products were examined under UV light and photographed using a gel documentation system (Gel DocTM XR+, Bio-Rad Laboratories, Hercules, USA). SSR banding profile from only the genotype × primer combination, which gave consistent amplification for all the genotype and without any blank lane/unclear bands, was included in this study. The amplified fragments were scored as "+" for the presence of a band specific to Fusarium wilt susceptible check. The primer sequences of C13-16 marker used for this study were as follows: 5′-GTCAAAGC AATGGACTAA-3′ and 5-TGAATTTGATACACACACTACT-3′. The temperature (Tm) for the reaction was 55°C.

2.8 | Data analysis

The analysis of phenotypic data (disease severity scores) was performed in SAS system for Windows (SAS Institute 2014) using restricted maximum likelihood (REML). Significantly different means were separated using LSD proc mean test.

2.8.1 | Genetic analysis

Data from the replicates were pooled together. Chi-square (χ^2) test for independence analysis was conducted to assess the goodness of fit to appropriate genetic ratio for the estimation of number of gene (s) governing FW resistance. Significant difference was considered at 5% probability level.

The genetic distance which measures the average number of nucleotide difference per gene was determined using the formula described by Neil 1972. Recombination frequency = (number of recombinant progeny/total number of progeny) * 100.

Allelic relationship: segregation ratios for each resistant × resistant (R × R) progeny were computed. Genetic hypotheses were tested for significance using the chi-square goodness-of-fit test to determine the deviation of observed frequencies from the hypothesized ratios.

The F_1, F_2 and backcross populations from the reciprocal cross derived from TVu 134 × TVu 984 were also examined for maternal effect using the seed soak method.

3 | RESULTS AND DISCUSSION

3.1 | Phenotypic analysis

The Fot disease severity index (DSI) ratings among the parental lines were significantly different ($p \leq 0.0001$). More than 90% of the individuals evaluated for reaction to Fot had disease ratings of 1, 2 or 5; 5% had disease ratings of 4. TVu 134 had the lowest disease rating based on vascular discoloration/wilting score, but the genotype disease reaction was like TVu 410 and TVu 109-1 (Table 2). The DSI ratings also showed that TVu 134, TVu 410 and TVu 901-1 were

TABLE 2 Segregation ratios, expected ratios, χ^2 and probability (p) for reaction to *Fusarium oxysporum* f. sp. *tracheiphilum* in the progeny from three crosses among resistant cultivars and susceptible cultivars using the laboratory seed soak methods

| | | Observed | | | | | |
Parents and crosses	Total no of plants	R	S	Observed ratio	Expected ratios	χ^2-value	p-values
TVu 134 (P_1)	42	41	1	All R	All R		
TVu 984(P_2)	40	0	40	All S	All S		
TVu 984 × TVu 134(F_1)	50	59	1	1:0	All R		
TVu 984 × TVu 134(F_2)	249	186	63	3:1	3:1	0.012	0.913
BC_1P_1 (F_1 × TVu 134)	50	49	1	1:0	All R		
BC_1P_2(F_1 × TVu 984)	46	23	21	1:1	1:1	0.04	0.841
TVu 410 (P_1)	40	39	1	All R	All R		
IT99K-573-2-1(P_2)	40	2	38	All S	All S		
IT99K-573-2-1 × TVu 410(F_1)	52	51	1	1:0	All R		
IT99K-573-2-1 × TVu 410(F_2)	246	184	62	3:1	3:1	0.005	0.944
BC_1P_1(F_1 × IT99K-573-2-1)	42	22	20	1:1	1:1		
BC_1P_2(F_1 × TVu 410)	50	48	2	1:0	All R	0.048	0.827
TVu 109-1(P_1)	42	40	2	All R	All R		
TVu 231-2(P_2)	43	1	44	All S	All S		
TVu 231-2 × TVu 109-1(F_1)	50	48	2	1:0	All R		
TVu 231-2 × TVu 109-1(F_2)	176	131	45	3:1	3:1	0.03	0.863
BC_1P_1(F_1 × TVu 231-2)	46	24	21	1:1	1:1		
BC_1P_2(F_1 × TVu 901-1)	44	42	2	1:0	All R	0.04	0.841

Notes. Where I: No of infected seedling (intermediate resistance); R: No. of healthy seedling (resistant); S: No. of rotted seed (susceptible).

TABLE 3 Segregation for reaction to *Fusarium oxysporum* f. sp. *tracheiphilum* in the progeny from crosses among resistant cultivar TVu 134 and susceptible cultivar TVu 984 using the pot screening method

| Population | Generation | Total no of plants | No. of plants | | Genetic ratio | χ^2-value | *p* |
			R	S			
TVu 134	Parent 1	45	43	2			
TVu 984	Parent 2	44	1	43			
TVu 134 × TVu 984 (F$_1$)	F$_1$	50	48	2	All R		
TVu 134 × TVu 984 (F$_2$)	F$_2$	200	145	55	3:1	0.67	0.413
BC$_1$P$_1$ (F$_1$ × TVu 984)	BC$_1$P$_1$F$_1$	50	26	24	1:1	0.4	0.527
BC$_1$P$_2$(F$_1$ × TVu 134)	BC$_1$P$_2$F$_1$	46	44	2	All R		

resistant, whereas TVu 984, IT99K-573-2-1 and TVu 231-2 were susceptible to *Fot*. The wide variability observed in the genetic materials based on resistance and susceptibility to FW justifies the use of the parental lines for the inheritance study.

F$_1$ and F$_2$ progeny derived from crosses between resistant and susceptible lines (TVu 410 × TVu 134, TVu 410 × TVu 109-1 and TVu 134 × TVu 109-1) were all resistant (Table 2). This suggests that the genes controlling *Fot* resistance in TVu 410, TVu 134 and TVu 109-1 are allelic. We propose that the genes symbol conferring resistance to *Fot* race 1 in "cowpea" should be designated as *Fot1-1* in accordance with the previous designation established for races 3 and 4 by Pottorff et al. (2014).

3.2 | Genetics of Fusarium wilt resistance

All the F$_1$ plants resulting from the crosses, involving TVu 984 × TVu 134, IT99K-573-2-1 × TVu 410 and TVu 231-2 × TVu 109-1, were resistant. The expression of resistance reaction in F$_1$ generation is an indication of the role of dominant gene in controlling *Fot* in cowpea. The nondetection of differences in disease rating between F$_1$ plants may be attributed to better combining ability of the parent (heterosis) plants. A bimodal distribution of resistant and susceptible reactions among F$_2$ plants was observed in both populations of R × S crosses, and no intermediate resistance levels were observed. This justified separation of disease rating classes into R or S classes. Segregation of the F$_2$ population derived from the cross between TVu 984 × TVu 134 gave 186 resistant: 63 susceptible lines; segregation of F$_2$ population derived from the cross between IT99K-573-2-1 × TVu 410 gave 184 resistant: 62 susceptible lines, while segregation of F$_2$ population derived from the cross between TVu 109-1 × TVu 231-2 also gave 131 resistant: 45 susceptible lines. F$_2$ segregation for resistance and susceptibility in the three F$_2$ populations fits a 3:1 (R/S) ratio ($p \leq 0.05$; Table 3). This ratio suggested that a single dominant gene conferred resistance to *Fot* in these crosses. Segregation within 25 BC$_1$P$_1$ families derived from resistant F$_1$ and susceptible parents fits a 1:1 (segregating) progeny ratio (Table 2). This segregation pattern further confirmed that a single dominant gene conferred resistance to Fusarium wilt in the cowpea lines used in this study. The F$_1$ data were consistent with this hypothesis because all the F$_1$ progeny

were completely resistant. This result agrees with previous findings by different authors who reported that *Fot* resistance is governed by a single dominant resistant gene (Rigert & Foster, 1987; Brick, Ogg, Schwartz, Byrne, & Kelly, 2004; Augustine et al., 2010; Rubio et al., 2003; Zink et al., 1990). Furthermore, Deepu et al. (2016) had also reported a single dominant gene resistance to Fusarium wilt disease in pigeon pea.

3.3 | Checking for maternal effect in the transmission of Fusarium wilt resistance gene

Maternal effects are generally considered "troublesome" sources of error in the sense that it reduces the precision of genetic studies. The reciprocal cross produced the same patterns of phenotypic variation observed from the straight cross (Table 3). The segregation of F$_2$ population for Fusarium wilt resistance gave 145 resistant: 55 susceptible lines. This segregation pattern fits the 3:1 genetic ratio indicating that resistance is conferred by a single major dominant gene. The segregation in the F$_2$ and backcross progeny indicated that there were no maternal effects in the transmission of the Fot resistance gene. This implies that either of the parents can be used as male or female in the crossing plan without fear of maternal linkage in genetic studies.

To test for allelic relationship among the resistance cultivars, segregation ratios for each resistant × resistant (R × R) progeny were computed (Table 4). Allelic relationship test of resistance to Fot in the three sources of resistant cultivars indicated that the resistance gene present in TVu 134 is the same in TVu 410 and TVu 109-1.

3.4 | Identification of an SSR marker for Fusarium wilt resistance

From a screening of cowpea SSRs available on the CGKB database, a set of cowpea SSR primer combinations were tested for their segregation with *Fot* resistance (data not shown) and one SSR marker, designated C13-16, was found to be closely associated with the *Fot* resistance gene. Moreover, when the six parental lines used in this study were genotyped with the SSR C13-16, the resistant parents showed the band for resistance with SSR C13-16 marker, whereas no band for susceptible parents was observed. The marker

TABLE 4 Segregation ratios of F1 and F2 progeny derived from crosses between the resistant cultivars

Populations			Disease reaction						
	Generation	Total no. of plants	R	S	I	Observed ratio	Expected ratio	χ^2-value	p-value
TVu 134	P1	40	39	1	0	1:0	All R	–	–
TVu 410	P2	40	40	0	0	1:0	All R	–	–
TVu 109-1	P3	38	37	1	0	1:0	All R	–	–
TVu 134 × TVu 410	F1	40	38	2	0	1:0	All R	–	–
TVu 134 × TVu 109-1	F1	40	39	1	0	1:0	All R	–	–
TVu 410 × TVu 109-1	F1	36	36	0	0	1:0	All R	–	–
TVu 134 × TVu 410	F2	100	99	1	0	1:0	All R	–	–
TVu 134 × TVu 109-1	F2	110	110	0	0	1:0	All R	–	–
TVu 410 × TVu 109-1	F2	120	119	1	0	1:0	All R	–	–

FIGURE 2 Polymerase chain reaction (PCR) banding pattern of the SSR marker C13-16 associated with FW resistance in a cross between TVu 134 × TVu 984. B = blank, L = 100-bp DNA ladder, 1–26 (Cowpea genotypes as listed in Table 4) P1 = resistant parent, P2 = susceptible parent

score was consistent with the phenotypic data of these cultivars (Figure 1). The marker produces an amplicon of 180 bp in the resistant genotypes, whereas the susceptible lines had no band. The marker results further affirmed the phenotypic screening that finds the genotypes different in terms of resistance and susceptibility to *Fot*. The marker also cosegregates with one of the segregating populations and discriminated between resistant and susceptible cultivars (Figure 2). The purpose of using the markers was to test its utility for precision in discriminating between resistant and susceptible lines. The marker mapped a genetic distance of 11.8 cM from the *Fot* resistance gene. The scores used to compute the genetic distance are contained in the supplementary information Table S1. In previous studies, only SNP markers have been reported in discriminating Fusarium wilt resistant and susceptible lines in cowpea (Pottorff et al., 2014). Identification of an SSR-linked marker to *Fot* in this study is a landmark that will help the low budget laboratory breeders to do the marker-assisted selection (MAS) using SSR markers to shorten the breeding cycle in developing cowpea cultivars for Fusarium wilt resistance. This marker will also help for precision in discriminating between resistant and susceptible genotypes when screening large parental genotypes for *Fot* in cowpea breeding programme. SSR markers had also been found to be efficient in screening for Fusarium wilt susceptible and resistant genotypes in pigeon pea (Singh, Rai, Chand, Singh, & Singh, 2013; Deepu et al., 2016) and marker introgression of Fusarium wilt resistance in chickpea (Varshney et al., 2014).

4 | CONCLUSIONS

The findings of this study have established that single dominant gene confers resistance to *Fot* in cowpea. Therefore, breeding programmes can incorporate resistant gene(s) to *Fot* susceptible cultivars through any selection method, where Fusarium wilt is a problem. The single dominant gene possessed by TVu 134, TVu 410 and TVu 109-1 indicated that any of these genotypes would be a good choice of parent for breeding resistance cultivars to *Fot* because major genes are easy to transfer. Allelic relationship indicated that the resistance gene present in TVu 134 is the same in TVu 410 and TVu 109-1.

Utility of C13-16 marker, previously identified to be linked with FW resistance, was also validated in the parental cultivars and F2 segregating population. The marker was found to have a strong utility for discriminating between resistance and susceptibility in segregating populations and parental genotypes in cowpea Fusarium wilt resistance breeding programmes. The identification of an SSR marker closely linked to *Fot* in this study offers opportunity for us in the developing world who do not have opportunity to use SNP markers in low budget laboratories. The F2 segregation in the reciprocal cross indicated no maternal effect in the inheritance of Fusarium wilt resistance in cowpea. At last, the information on genetics of *Fot* resistance generated from this study would be helpful in a breeding programme designed to introgress *Fot* resistance into susceptible cowpea cultivars through marker-assisted selection.

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CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

AUTHORS CONTRIBUTION

Dr Lucky Omoigui initiated the research topic, design, genetic analysis and interpretation. Miss Catherine Danmaigona carried out the laboratory and greenhouse inoculation, data collection and analysis. Dr Alpha Kamara assisted in reviewing the manuscript. Professor Ebenezer Ekefan assisted in disease culture and inoculation. Professor Michael Timko provided the markers used for the study and provided guidance for the work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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