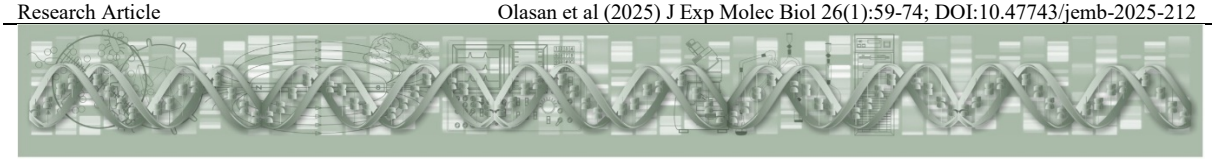

ANALYSIS OF GENETIC POLYMORPHISM FOR SCAB RESISTANT COWPEA VARIETIES USING SSR AND SNP MARKERS

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Abstract

Cowpea scab disease, caused by *Sphaceloma* sp., significantly reduces yield. This study screened nine cowpea varieties for resistance to scab using both phenotypic and molecular markers (SSR and SNP). The varieties were artificially inoculated, and disease incidence and severity were recorded. Genetic analysis with SSR markers revealed polymorphisms between resistant and susceptible varieties. Results showed significant genetic diversity among cowpea varieties, with IT99K-573-1-1 and TVx-3236 being resistant, while FUAMPEA-4 and UAM-09-130-20-4 were susceptible. SSR markers CP 29/30, CLM 0348, and CP 67/68 were the most informative in tracking resistance.

Keywords: Cowpea, Scab Resistance, SSR Markers, SNP Markers, Genetic Polymorphism, Disease Resistance

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an indigenous leafy vegetable and a grain legume widely grown in the semiarid areas of sub-Saharan Africa (Da Silva et al. 2019). The importance of cowpea in this region stems from its drought tolerance and ability to grow under water stress conditions (Carvalho et al. 2017), and its crucial role in ensuring food security and supporting the livelihoods of millions of smallholder farmers who depend on it for their economic and nutritional well-being (Bolarinwa 2022). The nutritional value of cowpea stems from its high protein content (25%) (Ogbonnaya et al. 2003, Mekonnen et al. 2022), which plays a considerable role in balancing the predominantly carbohydrate-based nutrition of the rural population in the West Africa subregion (Krasova-Wade et al. 2006, Singh et al. 2022). Cowpea is also an integral part of a sustainable agriculture and land use system (Ogbonnaya et al. 2003) and an essential component of traditional intercropping systems (Singh 2002). Integration of cowpea in cropping systems promotes buildup of soil organic matter and carbon and nitrogen fixation and ultimately improves soil fertility physical characteristics such as the water infiltration and retention capacity (Sánchez-Navarro et al. 2019a,b).

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Cowpea is grown on about 14, 911, 307 million hectares worldwide, with an annual grain production of about 8, 986, 191.25 million tons (FAO 2021). Nigeria produced about 3, 628, 612.65 million tons of cowpea, making her the world largest producer; followed by Niger (2, 661, 882.93 million tons), Burkina Faso (705, 768.3 tons) and Kenya (250, 260 tons) (FAO 2021). In Nigeria, cowpea is predominately grown in the drier northern parts of the country; however, advances in crop development have opened opportunities for its production in the wetter agroecologies (Nwofia et al. 2006), with the north-central guinea savanna zone contributing 29% production in 2020 (NAERLS 2020).

Although the West African sub-region accounts for over 95% of world cowpea production (Samireddypalle et al. 2017), its production has largely been due to increase in land mass rather than productivity per unit area. Studies have shown that the yield from farmers' fields is very low (500 kg/ha) compared to that obtained in the USA (2000 kg/ha) and in Australia (2200 kg/ha) (Quin 1997). The low yield is attributed to the effect of several biotic and abiotic factors (Omoigui et al. 2007). However, biotic stresses like cowpea scab disease, caused by *Sphaceloma* sp. (Emechebe 2014), threaten cowpea production. The disease affects all above-ground parts of the cowpea plant, including leaves, stems, pod and severe infections can lead to significant yield losses (Afutu et al. 2016). Molecular markers have emerged as effective and reliable tools for the genetic analysis of plant traits such as disease resistance (Sharma and Sharma 2020). Genetic analysis offers a powerful approach for characterizing scab resistant and susceptible varieties, towards the development of resistant varieties using molecular tools (Meuwissen et al. 2016). Identifying resistant varieties through phenotypic and molecular approaches such as the use Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNP) marker systems is crucial for breeding programs aimed at improving resistance and ensuring sustainable cowpea production.

Materials and Methods

Field Screening of cowpea for Reaction to Scab Infection

Plant Materials, Experimental Design and Location

Nine cowpea varieties (FUAMPEA-4, Gujarat Cowpea-3, Gujarat Cowpea-5, Gujarat Cowpea-6, IT99K-573-1-1, Pant Lobia-4, Pant Lobia-1, TVx-3236, and UAM-09-130-20-4) were used. They were obtained from the cowpea breeding program of the Molecular Biology Laboratory, Joseph Sarwuan Tarka University Makurdi, from the Germplasm of the Stress Tolerant Orphan Legume (STOL) project comprising Germplasm from Nigeria and India.

The field experiment was conducted at the Teaching and Research Farm of the Joseph Sarwuan Tarka University of Agriculture, Makurdi, following a randomized complete block design with three replications.The field was laid out in plots according to the experimental design. Each plot consisted of a single row, 4 m long, and seeds were sown at an intra-row spacing of 25 cm, resulting into 16 hills and 32 plants per plot.

Cultural Practices

At sowing, Pendimethalin, a pre-emergence herbicide was applied to subdue weeds until crop establishment. The herbicides were applied at a dilution of 150 mls per 20 litre knapsack sprayer.

Nitrogen, Phosphorus and Potassium were applied in the form of NPK 15:15:15 fertilizer at the rate of 100kg/ha. This is the equivalent of 0.075kg (75g) per plot, applied by side placement as a single dose at one week (7 days) after planting (7 DAP). Weeding was done manually and it was carried out for all plots at 3 weeks (21 days) after planting and subsequently as needed to keep the field free from weeds till maturity. Cypermethrine + Dimethoate was sprayed at the rate of 50 g a.i/ ha to control insect pests.

Disease Inoculation

Cowpea plants were inoculated with *Sphaceloma* sp. at 14 days post-planting, and disease incidence and severity were recorded at intervals. The Spore suspension for disease inoculation was prepared at the Crop and Environmental protection Laboratory as described by Afutu et al. (2016). The concentration of spores in the solution was determined using a hemocytometer, and adjusted to 10^5 spores/mL for the inoculation.

Cowpea plants were artificially inoculated with the scab disease by spraying the spore suspension of *Sphaceloma* sp. onto the plants at the flower initiation stage. The inoculum was applied to the plants' canopy with a hand-held sprayer until runoff at 14 DAP. After inoculation, water spray was applied to plants in the evening to maintain high humidity for disease development.

Data Collection and Statistical Analysis

Observations were made a plot by plot basis, on the incidence and severity of scab infection. Disease incidence and severity were assessed at 14 days post-inoculation (DPI) and then every 7 days after, up to 28 DPI.

According to Afutu et al. (2016), the symptoms of cowpea scab disease caused by *Sphaceloma* spp. include:

1. Small, circular, dark brown to black spots or lesions on the leaves, stems, and pods.
2. Lesions may merge to form larger, irregularly shaped spots.
3. Spots may have a reddish-brown border and a grayish center.
4. Severe infections can lead to defoliation, reduced pod formation, and lower yields.

Disease incidence was measured as the percentage of plants showing symptoms of scab infection (Gerstman, 2015) as shown below:

Incidence (%) = (Number of infected plants / Total number of plants) × 100

Disease severity was determined using a rating scale of 1 to 10, with 1 indicating minimal infection and 10 indicating severe infection (Gerstman 2015). The disease severity scale is as follows:

0: No symptoms
1: 1-10% infection
2: 11-20% infection
3: 21-30% infection
4: 31-40% infection
5: 41-50% infection
6: 51-60% infection
7: 61-70% infection
8: 71-80% infection
9: 81-90% infection
10: 91-100% infection (plant death)

Genetic Analysis

DNA Extraction

Using disease incidence and severity score, Scab resistant and scab susceptible cowpea varieties were identified. For the Genetic analysis, DNA was extracted from young, healthy trifoliate leaves of 7 days old plants of Scab resistant and scab susceptible cowpea varieties using the cetyltrimethylammonium bromide (CTAB) method (Xin and Chen 2012) at 7 DAP. The method was adapted with slight modification; leaf samples were collected from each cowpea variety in silica gel placed in small appropriately labelled ziploc bags for drying to a crispy state (suitable for grinding). The quality and quantity of the extracted DNA were assessed using agarose gel electrophoresis. Gels were prepared as described by (Sambrook and Russell 2001).

PCR Amplification.
Polymerase chain reaction (PCR) was performed using 13 Simple Sequence Repeat (SSR) markers to screen for polymorphism between the resistant and susceptible varieties. Each PCR reaction contained 20 ng of genomic DNA, 10 µL of PCR master mix, 1 µL of forward and reverse primers, and nuclease-free water to a final volume of 20 µL. Polymerase Chain Reaction (PCR) was carried out in a Biorad Thermal cycler under the following thermal cycler conditions for PCR reaction. 35 cycles of denaturation (95ºC) for 30 seconds, annealing (55ºC) for 30 seconds and extension (72ºC) depending on the product size.

Gel Electrophoresis
The amplified products were separated on a 2% agarose gel stained with ethidium bromide. The gels were run at 100 volts for 1 hour and visualized under ultraviolet light. Gel images were captured using a Digital camera. Band patterns were observed and amplification of single DNA band was scored as 1, amplification of multiple bands was scored as 2 and no band was scored as 0. This scoring generated the genotypic (molecular) data for genetic analysis.

Results and discussions

Table 1 shows that 9 cowpea varieties evaluated for their reaction to scab infection showed varied reactions in terms of disease incidence and severity measurement. The variety FUAMPEA-4 and UAM-09-130-20-4 showed the highest incidence at 71.67%, while IT99K-573-1-1 and TVx-3236 exhibited the lowest incidence at 1%. All the cowpea varieties recorded disease severity scores greater than 5, except for IT99K-573-1-1 and TVx-3236. The variety UAM-09-130-20-4 had the highest severity score of 6.7, followed by Pant Lobia-1 with a score of 6.2, while IT99K-573-1-1 and TVx-3236 showed the lowest severity score of 1.

Table 1. Mean Disease Incidence and Severity Response of 9 Cowpea varieties to Scab infection

Variety	Mean Incidence (%)	Mean Severity (1-10)
FUAMPEA-4	71.67	5.67
UAM-09-130-20-4	71.67	6.67
Gujarat Cowpea-3	53.33	5.33
Gujarat Cowpea-5	15.00	6.00
Gujarat Cowpea-6	13.67	5.50
Pant Lobia-4	11.67	5.67
Pant Lobia-1	6.67	6.17
IT99K-573-1-1	1	1
TVx-3236	1	1
Standard Error (SE)	1.41	0.15

This result indicates that FUAMPEA-4 and UAM-09-130-20-4 were susceptible to scab caused by *Sphaceloma* sp. while IT99K-573-1-1 and TVx-3236 were resistant. In similar studies, variations in susceptibility to cowpea scab caused by *Sphaceloma* sp. have been observed, with some varieties demonstrating higher levels of resistance. This aligns with the findings of Jorem et al. (2023), who reported that disease severity and incidence in cowpea vary depending on the genetic makeup of the varieties tested, environmental conditions, and the virulence of the pathogen.

Genetic resistance is a critical factor in managing scab disease. Research by Emechebe and Florini (1997) noted that certain cowpea genotypes express resistance to a range of pathogens,

including *Sphaceloma* sp., responsible for scab disease. Their work supports the idea that resistant varieties like IT99K-573-1-1 and TVx-3236 are crucial for integrated disease management strategies, as noted by Amayo et al. (2014). and are also essential for breeding programs aimed at improving cowpea resilience. They emphasized the role of breeding programs in developing scab-resistant cowpea varieties as a sustainable approach to disease control.

The variation in disease incidence in this study may be attributed to host-pathogen interactions, which play a significant role in determining the outcome of infection. A study by Jorem et al. (2023) explored how cowpea's genetic diversity influences its interaction with pathogens, leading to varying levels of disease resistance.

The severity of scab infection has direct implications for cowpea yield, as observed in this study. High disease severity can reduce plant vigor and yield. A study by Mbong et al. (2012) highlighted the negative impact of scab disease on yield performance in susceptible varieties, stressing the importance of early detection and the use of resistant cultivars.

Plates 1, 2 and 3 show the agarose gel images of 13 screened Simple Sequence Repeats (SSR) Marker employed in the DNA amplification for polymorphism between scab resistant cowpea varieties (**IT99K-573-1-1 and TVx-3236**) and susceptible cowpea varieties (**UAM-09-130-20-4 and FUAMPEA-4**). The primers showed varying degrees of genetic polymorphism depending on the DNA of the cowpea varieties amplified and the SSR primer used. All primers produced visible bands except in CP15/16, CLM1190 and CLM1182. DNAs of scab resistant and susceptible varieties were well resolved in primers CLM059, CP85/86, CP29/30, CLM0348 and CP 67/68.

Table 2 presents the banding pattern of 13 SSR primers employed to identify polymorphism between scab resistant and susceptible varieties of cowpea. Presence of bands were indicated by 1 or 2 to represent single or double bands respectively while absence of band was indicated as zero (0). Double bands were produced by CP 29/30, CLM 0348 and CP 67/68 in IT99K-573-1-1(scab resistant variety). Double bands were produced by CLM 057, CP 85/86, CP 29/30, CLM 0348 and CP 67/68 in FUAMPEA-4 (scab susceptible variety). Other banding patterns occurred singly where present. Nine (9) primers produced bands in the four varieties studied representing 69.2% of the primers.

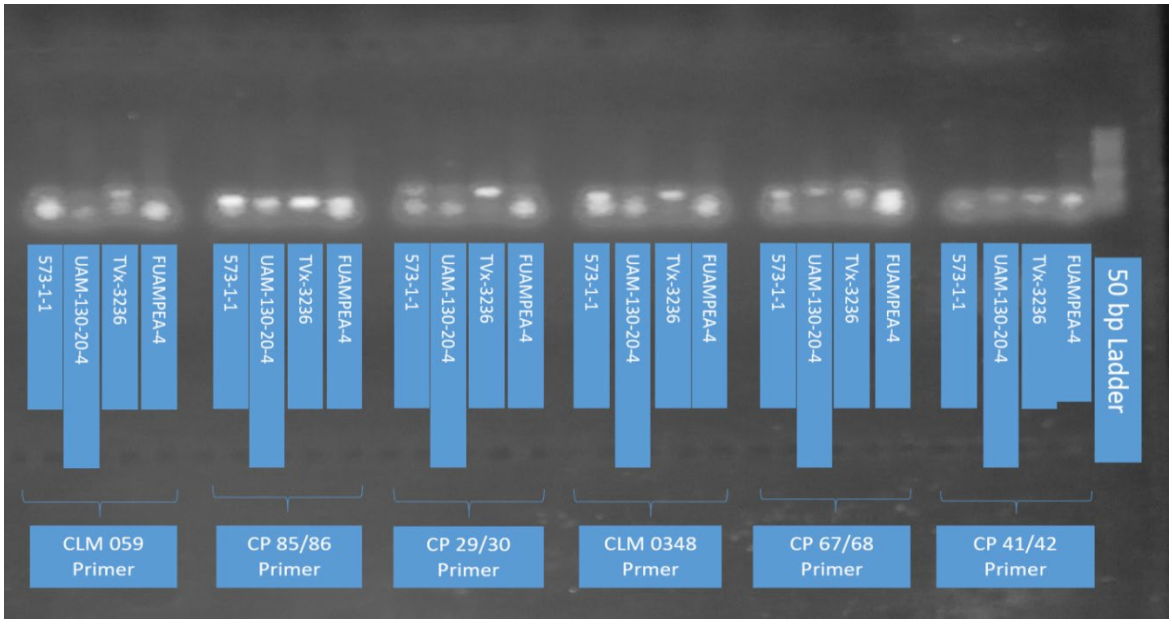

Plate 1. Agarose gel image showing screening of SSR markers (SET 21) for polymorphism between Scab resistant cowpea varieties (IT99K-573-1-1 and TVx-3236) and susceptible cowpea varieties (UAM-09-130-20-4 and FUAMPEA-4)

Each group of four represents screening with a single marker as labelled. Lane 1 and 3 in each group is DNA from resistant parents IT99K-573-1-1 and TVx-3236 respectively, while Lane 2 and 4 is DNA from susceptible parents UAM-09-130-20-4 and FUAMPEA-4 respectively.

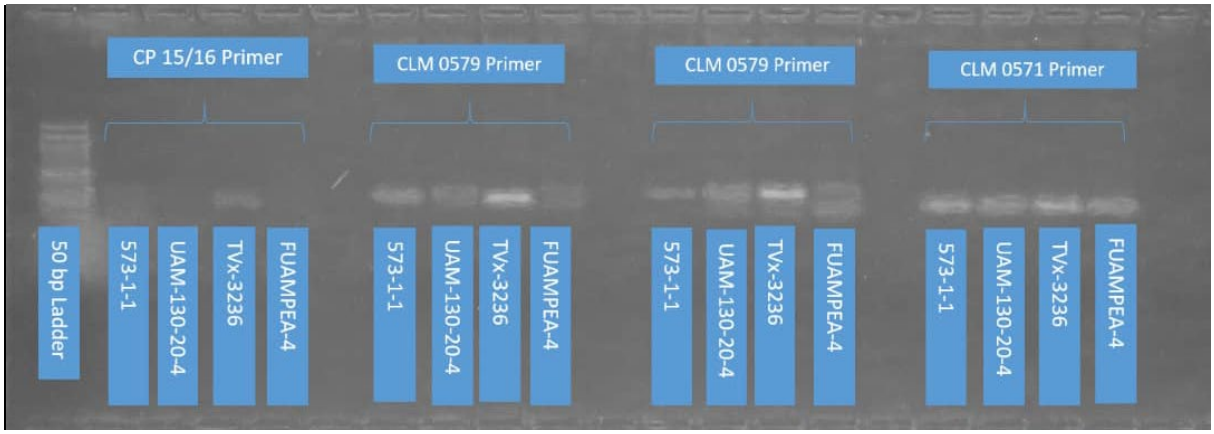

Plate 2. Agarose gel image showing screening of SSR markers (SET 2) for polymorphism between Scab resistant cowpea varieties (IT99K-573-1-1 and TVx-3236) and susceptible cowpea varieties (UAM-09-130-20-4 and FUAMPEA-4)
Each group of four represents screening with a single marker as labelled. Lane 1 and 3 in each group is DNA from resistant parents IT99K-573-1-1 and TVx-3236 respectively, while Lane 2 and 4 is DNA from susceptible parents UAM-09-130-20-4 and FUAMPEA-4 respectively.

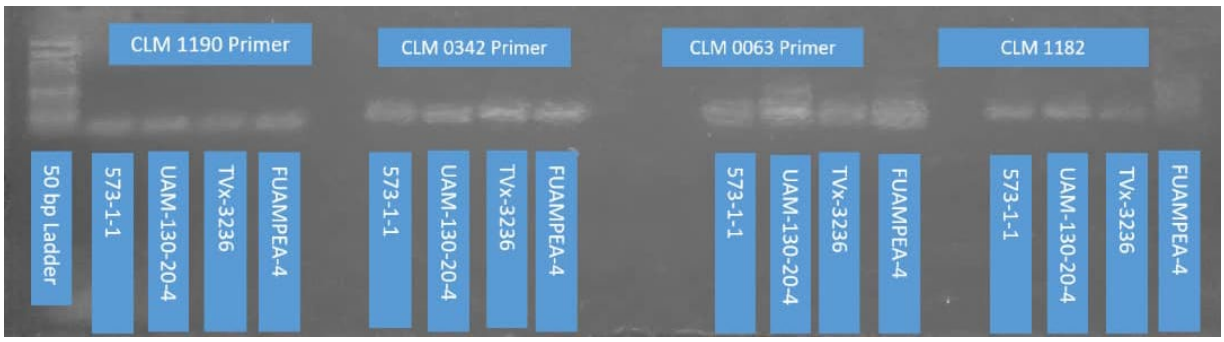

Plate 3. Agarose gel image showing screening of SSR markers (SET 3) for polymorphism between Scab resistant cowpea varieties (IT99K-573-1-1 and TVx-3236) and susceptible cowpea varieties (UAM-09-130-20-4 and FUAMPEA-4)
Each group of four represents screening with a single marker as labelled. Lane 1 and 3 in each group is DNA from resistant parents IT99K-573-1-1 and TVx-3236 respectively, while Lane 2 and 4 is DNA from susceptible parents UAM-09-130-20-4 and FUAMPEA-4 respectively.

Band analysis in IT99K-573-1-1 variety (resistant type) showed 13 bands representing 100% of the total primers employed in the DNA amplification. There were 3 double bands (23.1%) and 9 single bands (69.2%) as shown in Figure 1. UAM-09-130-20-4 (susceptible variety) had 10 bands (76.9%) and all were single bands as shown in Figure 2. In TVx-3236 variety (resistant type), there were 13 (100%) bands grouped into 1 double band (7.7%) and 12 single bands (92.3%) as shown in Figure 3. In FUAMPEA-4 (susceptible variety), there were 11 (84.6%) bands grouped into 5 double bands (38.5%) and 6 single bands (46.2%) as shown in Figure 4.

Table 2. Band Pattern of SSR Primers to Identify Polymorphism Between Crab Resistant and Susceptible Varieties of Cowpea

Primer	Forward / Reverse sequence	IT99K-573-1-1	UAM09-130-20-4	TVx-3236	FUAMPEA-4
CP-15/16	GTAGGGAGTTGGCCACGATA CAACCGATGTAAAAAGTGGACA	0	0	1	0
CLM 0579	CCTAAGCTTTTCTCCAACTCCA CAAGAAGGAGGCGAAGACTG	1	0	1	2
CLM 0571	GATTTGTTTGGTTTCCTTAAG GGTTGATCTTGGAGGCATTTT	1	1	1	1
CLM 1190	GTCAAAGCAATGGACTAA TGAATTTGATACACACACTACT	1	1	1	1
CLM 059	AAACTGACACTTGAACACGA CTCATGCAGAGTTCAAGATC	1	0	2	1
CP 85/86	GATCACCTCCCACACCTCAG TAGCAGTTTCCCACCAGCTT	1	1	1	2
CP 29/30	AATGACCCACAAAGCAAAGT TTGGCCCAAAATATCACACA	2	1	1	2
CLM 0348	GCTTTGCATGTGGATTTCCT GGGGAGAATGAAACTAAAGTAATGTT	2	1	1	2
CP 67/68	GATGCTGGTGCTTGTATGGA TAATTTCTACGCAAGGGAGAGAG	2	1	1	2
CP 41/42	ACCTGCATTGCCTCATATCC GCTGATTCGGCTTGTTCTTC	1	1	1	1
CLM 0342	GATCCAACATTTCCTGTGTCTC GGAGCACCCGACAAGCCCCT	1	1	1	1
CLM 0063	ACTTCGCACACAGATCCAAC AATTGCCGGCTTTCCCATTG	1	1	1	1
CLM 1182	TTCAGACAGCATAGCTCCCA GGCCGTATCAAGGATGAACA	1	1	1	0

The markers CLM059, CP85/86, CP29/30, CLM0348, and CP67/68 were able to distinguish between the resistant and susceptible varieties clearly, showing good resolution on the agarose gel. SSR markers are highly effective for revealing genetic diversity due to their co-dominant nature and ability to detect even small differences in the DNA of different genotypes. The presence of polymorphism in this study screened markers indicates genetic variability between resistant and susceptible cowpea varieties. This is consistent with findings by Diouf and Hilu (2005), who demonstrated that SSR markers are reliable for detecting genetic differences among cowpea genotypes, especially in relation to disease resistance traits.

These markers showing genetic polymorphisms not only reveal diversity but are also essential for identifying loci associated with scab resistance. SSR markers linked to resistance traits in cowpea have been well documented in other studies, such as the work of Omo-Ikerodah et al. (2008) who used AFLP and SSR cowpea linkage map to identify QTLs for resistance to flower bud thrips. Similarly, Gioi et al. (2012) used SSR markers to identify and validate a QTL for cowpea yellow mosaic virus (CYMV) resistance in cowpea. According to Asare et al. (2010) SSR markers can effectively differentiate between resistant and susceptible genotypes, making them valuable for marker-assisted selection (MAS) in breeding programs.

Three markers CP15/16, CLM1190, and CLM1182 which did not produce visible bands, may suggest the absence of the specific loci they amplify in the cowpea varieties tested or inadequate primer annealing due to sequence mismatches. This kind of issues can also occur in PCR amplification when there are high levels of sequence variation or when primers are not well-suited to the varieties in question. As noted by Fatokun et al. (1993), such issues can arise when designing primers for highly polymorphic regions, especially in genetically diverse populations.

The successful resolution of DNA from resistant and susceptible varieties with the markers CLM059, CP85/86, CP29/30, CLM0348, and CP67/68 suggests that these primers are linked to regions of the cowpea genome associated with scab resistance. This agrees with Omoigui et al. (2019) who used SSR markers to identify genomic regions associated with *Cercospora* disease resistance in cowpea.

The identified polymorphisms between resistant and susceptible varieties using SSR markers can be leveraged in breeding programs aimed at enhancing scab resistance in cowpea. Similar studies have demonstrated the utility of SSR markers for developing improved varieties with resistance to fungal diseases. Boukar et al. (2016, 2019) emphasized the role of SSR markers in breeding strategies for cowpea, especially for traits like disease resistance, drought tolerance, and yield improvement.

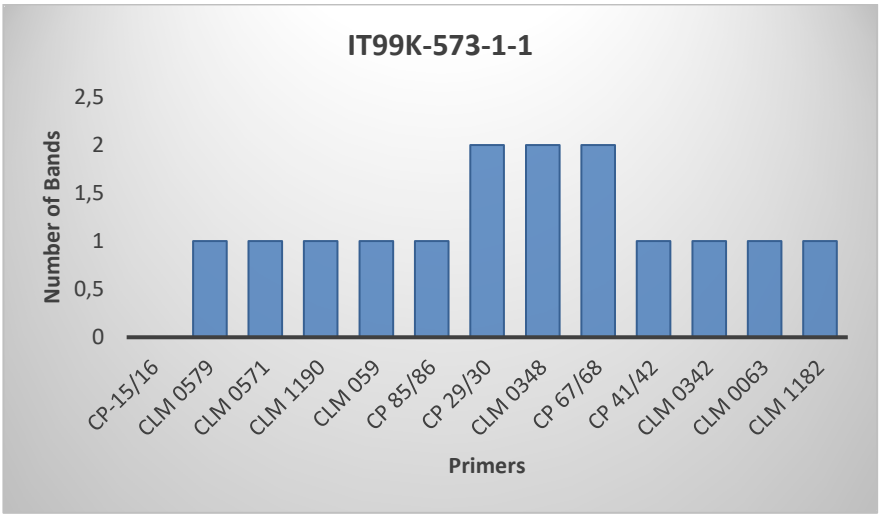

Figure 1. Band analysis in IT99K-573-1-1 Variety

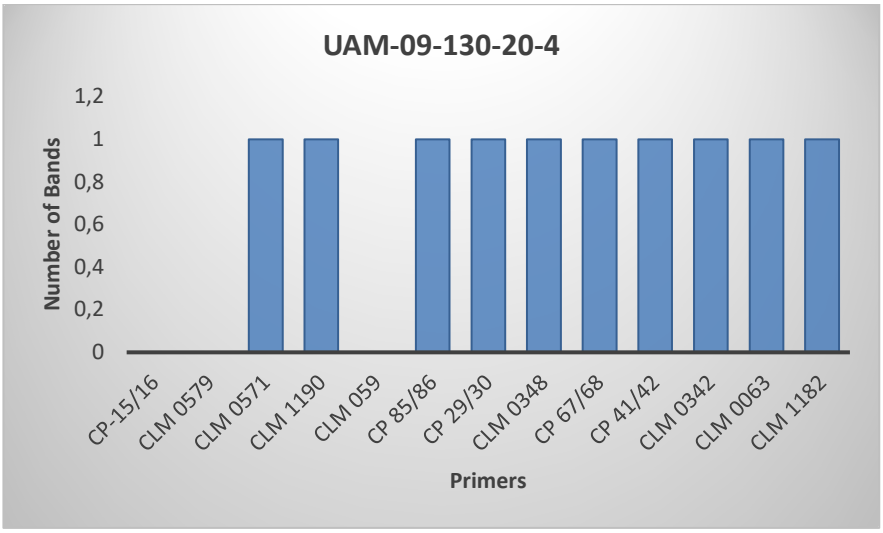

Figure 2. Band analysis in UAM-09-130-20-4 Variety

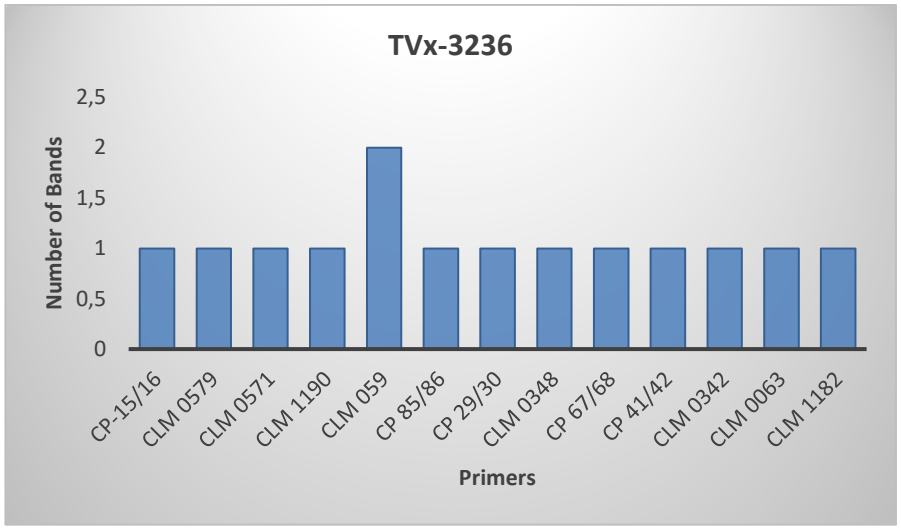

Figure 3. Band analysis in TVx-3236 Variety

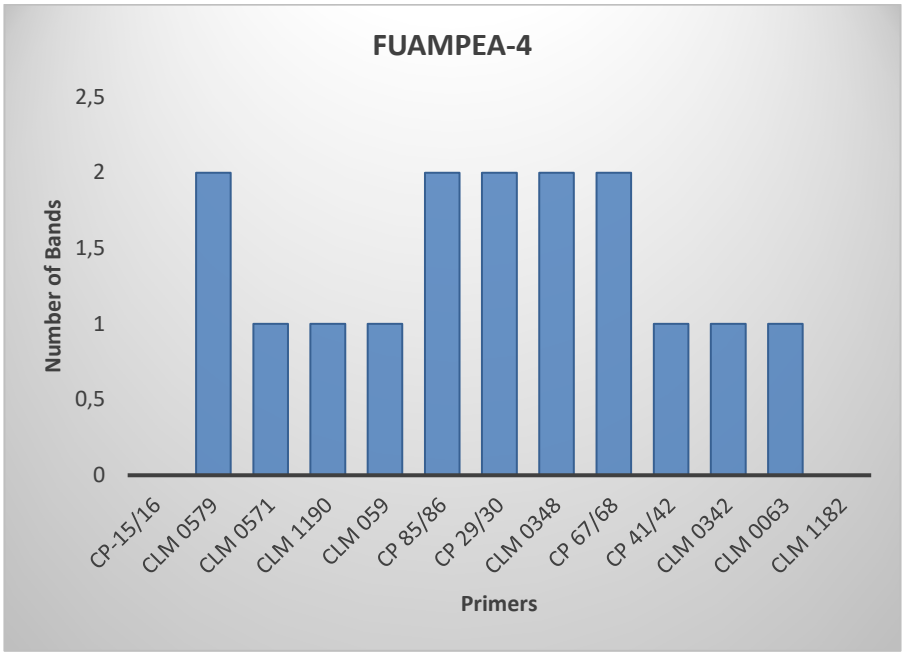

Figure 4. Band analysis in FUAMPEA-4 Variety

Primers were clustered on the basis of the DNA amplification results as shown in the dendrogram (Figure 5). Genetic distance ranged from 1.00 to 3.00 with similarity coefficient of 66.7 to 0.00 respectively. There were two clusters. Cluster 1 comprised a group of primers (CLM0571, CLM1190, CP41/42, CLM0342 and CLM0063) that produced single bands and appeared in all the four varieties of cowpea. The clustering of primers CLM0571, CLM1190, CP41/42, CLM0342, and CLM0063 in Cluster 1, which produced single bands across all four cowpea varieties, indicates that these primers are likely amplifying conserved regions in the cowpea genome. This suggests that these loci are not strongly associated with scab resistance or susceptibility, but rather represent general genetic similarity across varieties. As seen in studies by Timko et al. (2007), such primers often amplify housekeeping genes or other highly conserved sequences in cowpea. Divergent clustering pattern was observed in CP15/16 that produced a lone band in TVx-3236 resistant variety and in CLM059 that produced bands in all varieties except the UAM09-130-20-4 (susceptible variety). These observations suggest these primers target loci more specific to resistance traits. CP15/16 may be linked to a genetic locus

contributing to resistance in TVx-3236. According to Chen et al. (2004), certain SSR markers were specific to disease resistance genes. Cluster 2 comprised primers that produced 2 double and 2 single bands in the four varieties.

Figure 6 shows the dendogram of the four varieties of cowpea. Clustering pattern was divergent and independent of scab resistance or susceptibility status of the varieties. Coefficient of similarity ranged from 23.6 to 86.7 with genetic distance of 1.53 to 0.27 respectively. Results showed that IT99K-573-1-1 (scab resistant) and FUAMPEA-4 (scab susceptible) varieties shared closer genetic similarity than other varieties. The close genetic similarity between IT99K-573-1-1 (scab resistant) and FUAMPEA-4 (scab susceptible) indicates that despite their phenotypic difference in disease resistance, they may share similar genetic backgrounds. Similar results were reported by Price and Cishahayo (1986), where genetically similar cowpea varieties exhibited varying degrees of resistance to different pathogens, underscoring the complexity of disease resistance mechanisms. UAM09-130-20-4 (susceptible) was genetically divergent from IT99K-573-1-1 and FUAMPEA-4, suggesting it may possess unique genetic traits not shared with the other varieties. This could imply that UAM09-130-20-4 either lacks the resistance-associated alleles present in other varieties or carries susceptibility loci. This is consistent with the finding of Omo-Ikerodah et al. (2008), who identified divergent susceptible cowpea genotypes using SSR markers and linked them to specific susceptibility traits.

The most divergent and farthest in genetic distance among the four varieties was TVx-3236 (resistant type). This suggests that TVx-3236 may possess unique resistance mechanisms or genetic backgrounds. Its distinctiveness aligns with the divergent patterns seen in CP15/16, which was specific to this variety. This result echoes the finding of Asare et al. (2010), who reported that highly divergent cowpea varieties often carry unique alleles associated with specific disease resistance traits.

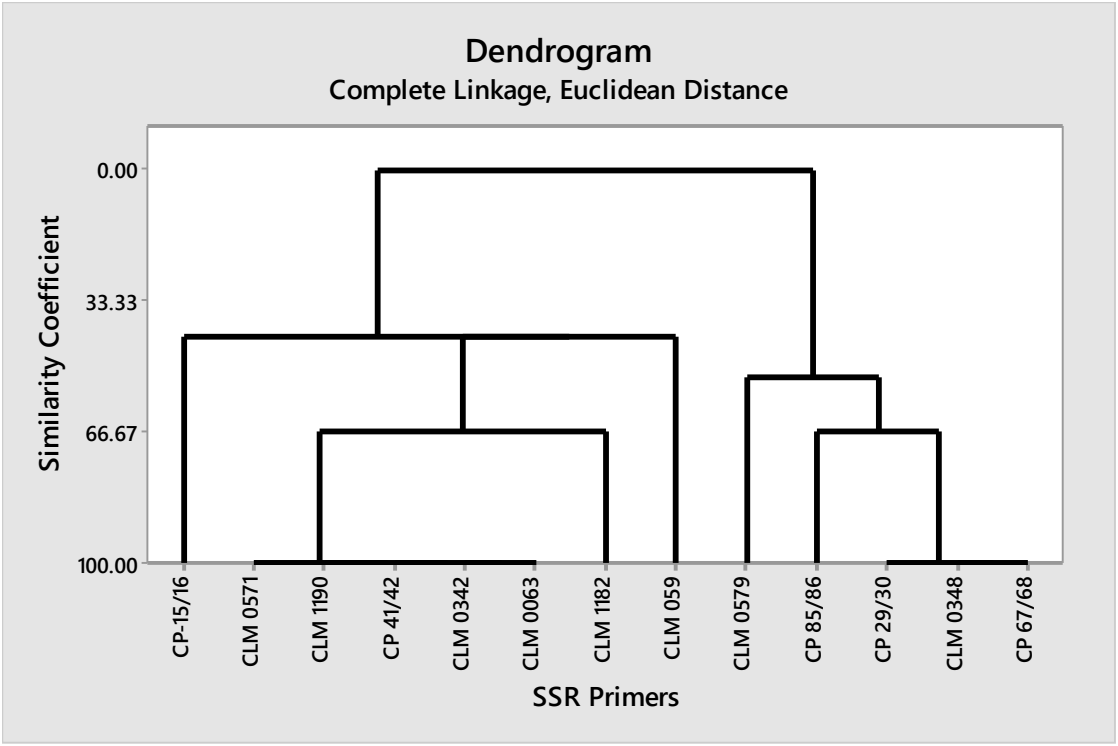

Figure 5. Dendrogram of SSR Primers

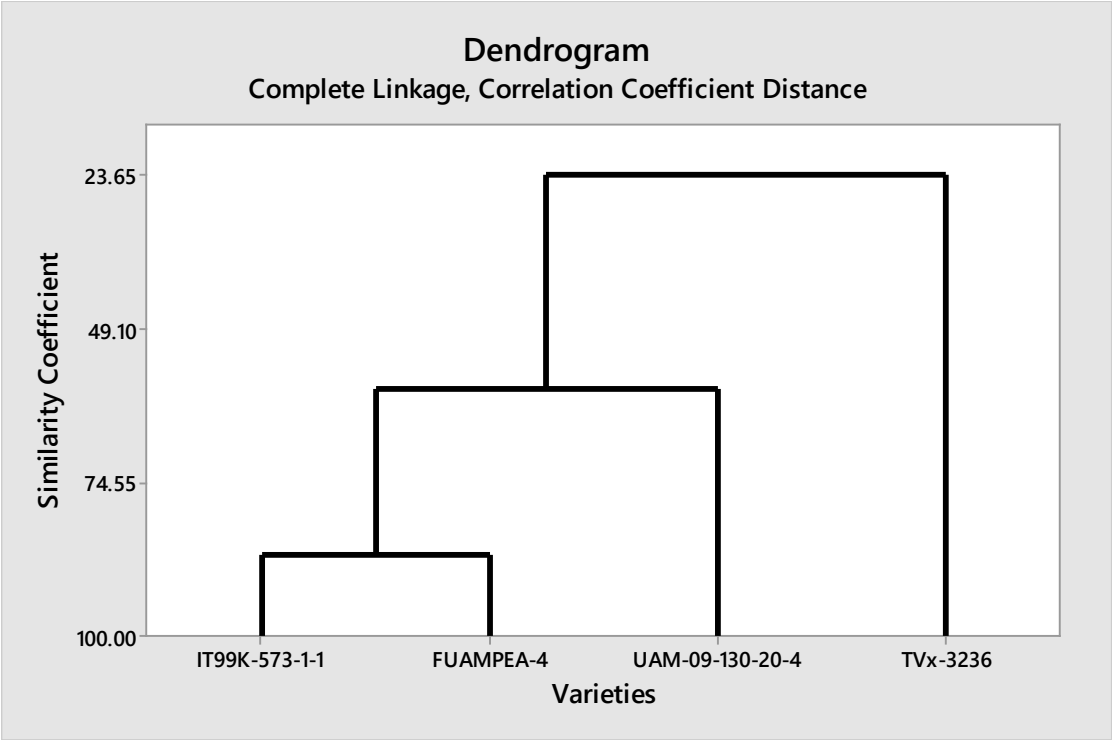

Figure 6. Dendrogram of Cowpea Varieties

Table 2 gives information on the total number of bands and relative polymorphic bands (RPB) of the SSR primers. The 13 SSR primers produced 55 bands. Number of bands per primer ranged from 1 in CP-15/16 to 8 bands. Primers CP 29/30, CLM 0348 and CP 67/68 produced 8 bands each with RPB of 7.6%. This group was followed by CP 85/86 primer that produced 5 bands with RPB of 6.3%. Varietal polymorphism was higher in the scab susceptible varieties than the resistant varieties in the following order: FUAMPEA-4 (27%), UAM09-130-20-4 (26%), IT99K-573-1-1 (24%) and TVx-3236 (23%).

Table 3 presents indices of polymorphism of SSR primers. Heterozygosity of primers (H) ranged from 0.26 in CLM 1182 to 0.62. Primers CLM 0579, CP 29/30, CLM 0348 and CP 67/68 had the highest H value (0.62) and Polymorphic Information Content (PIC) of 0.55. The highest Marker Index (MI) was found in CLM 0579 primer (MI =0.73). Effective Multiplex Ratio (EMR) of primers was highest in CP 29/30, CLM 0348 and CP 67/68 with value of 3.0 while Resolution Power (RP) was between 23.5 and 25.5 among the 13 primers.

Table 2. Frequency and Percentage of Polymorphism among SSR Primers

Primer	Total number of bands	Frequency of polymorphism	% Polymorphism
CP-15/16	1	0.01	1.3
CLM 0579	4	0.05	5.1
CLM 0571	4	0.05	5.1
CLM 1190	4	0.05	5.1
CLM 059	4	0.05	5.1
CP 85/86	5	0.06	6.3
CP 29/30	6	0.08	7.6
CLM 0348	6	0.08	7.6
CP 67/68	6	0.08	7.6

CP 41/42	4	0.05	5.1
CLM 0342	4	0.05	5.1
CLM 0063	4	0.05	5.1
CLM 1182	3	0.04	3.8
	55		

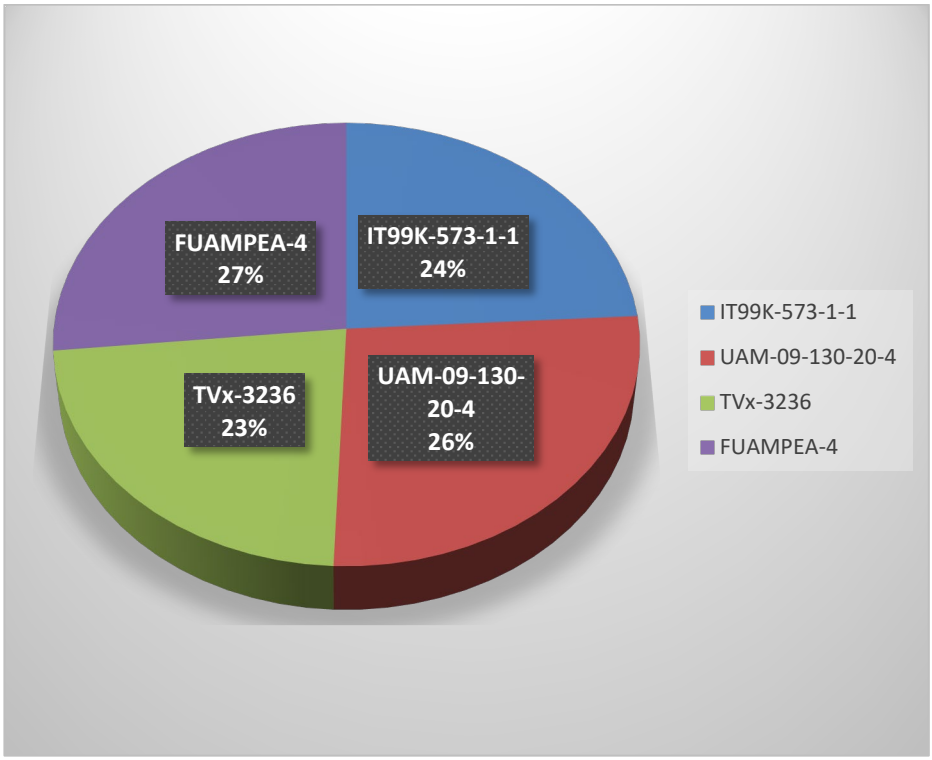

Figure 7. Percentage Polymorphism among Four Cowpea Varieties

Table 3. Polymorphic Indices of SSR Primers

Primer	H value	PIC	EMR	MI	RP
CP-15/16	0.50	0.38	0.50	0.19	25.5
CLM 0579	0.62	0.55	1.33	0.73	23.5
CLM 0571	0.32	0.27	0.5	0.13	25.5
CLM 1190	0.32	0.27	0.5	0.13	25.5
CLM 059	0.50	0.38	0.5	0.19	25.5
CP 85/86	0.54	0.47	1.33	0.62	23.5
CP 29/30	0.62	0.55	3.0	1.64	23.5
CLM 0348	0.62	0.55	3.0	1.64	23.5
CP 67/68	0.62	0.55	3.0	1.64	23.5
CP 41/42	0.32	0.27	0.5	0.13	25.5

CLM 0342	0.32	0.27	0.5	0.13	25.5
CLM 0063	0.32	0.27	0.5	0.13	25.5
CLM 1182	0.26	0.22	0.5	0.11	25.5

H= Heterozygosity of primers
PIC= Polymorphic Information Content of primers
EMR= Effective Multiplex Ratio of primers
M1= Marker Index
RP= Resolution Power

The indices of polymorphism in our study highlight the efficiency and informativeness of the SSR markers used to distinguish between scab-resistant and susceptible cowpea varieties. Primers with high heterozygosity, PIC, MI, EMR, and RP, such as CLM0579, CP29/30, CLM0348, and CP67/68, are particularly valuable for genetic studies aimed at improving resistance traits through marker-assisted selection (MAS). Studies like that of Li et al. (2011) have shown that SSR markers with high heterozygosity are more effective in identifying polymorphisms between different genotypes, making them useful for genetic mapping and breeding programs. PIC values above 0.5 are generally considered highly informative, as noted by Botstein et al. (1980), which suggests that these markers are reliable for studying genetic diversity. Higher MI values signify greater utility in distinguishing genotypes, as supported by studies such as Powell et al. (1996), which emphasize the importance of using markers with high MI for comprehensive genetic analysis. According to Varshney et al. (2007), high EMR values are advantageous in large-scale genotyping and breeding programs where high-throughput marker efficiency is required. According to Chesnokov and Artemyeva (2015) RP is a key indicator of a marker's discriminatory power. High RP values suggest a marker's strong ability to differentiate between closely related genotypes, making these markers particularly useful in genetic diversity studies.
Thus our findings show that the SSR markers used in this study are useful tools for detecting genetic diversity and potentially identifying loci linked to scab resistance. The high PIC and MI values further reinforce the utility of these markers for breeding programs, as they offer robust polymorphism and high discriminatory power. Additionally, the high RP values ensure that these markers can effectively resolve differences among cowpea genotypes, which is crucial for developing resistant varieties and improving overall crop resilience.

Conclusions

The study demonstrated significant genetic variability in cowpea scab resistance. Characterization with SSR Markers revealed genetic variability among the cowpea varieties screened expressed in the form of genetic polymorphism. SSR Markers CLM 0579, CP 29/30, CP 67/68 and CLM 0348 were the most informative markers in discriminating among the cowpea varieties at the molecular level. CLM 0571, CLM 1190, CLM 0342, CLM 0063 and CP 41/42 were monomorphic between cowpea varieties. CLM 0579, CP 29/30, CLM 0348 and CP 67/68 showed consistent polymorphism and band pattern between scab resistant and susceptible cowpea varieties indicating their suitability to track scab resistance in cowpea. SSR markers CP 29/30, CLM 0348, and CP 67/68 are useful for identifying resistant varieties, and IT99K-573-1-1 and TVx-3236 can serve as parental lines in breeding programs. In addition, the polymorphism observed in the SSR markers between resistant and susceptible cowpea varieties suggests that these markers could be linked to loci controlling scab resistance. These findings provide valuable insights for cowpea improvement programs aimed at enhancing scab

resistance. This insights will be crucial for breeding efforts aimed at improving cowpea resistance to scab and other diseases. Further studies could focus on mapping these markers to specific QTLs for marker-assisted selection, improving the efficiency of breeding programs targeting disease resistance.

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