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Research Article

Assessment of *Striga gesnerioides* (Willd.) Resistance and Genetic Characterization of Forty-Six Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes in Ghana

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The parasitic weed, *Striga gesnerioides*, imposes physiological stress on cowpea (*Vigna unguiculata* (L.) Walp.) resulting in significant yield loss in the regions of northern Ghana. This warranted identification of resistant cowpeas for sustainable production. The current work aim was to identify *Striga*-resistant cowpea genotypes and assess their genetic relatedness. Forty-six (46) cowpea genotypes were screened in pots for their reaction to *Striga* samples obtained from the upper east, upper west, and northern regions of Ghana and validated with C42-2B and 61R-M2 markers involving DNA amplification by PCR assay. Sixteen polymorphic SSR primer pairs were used to assess genetic relatedness among 46 cowpea genotypes. Data were analyzed with PowerMarker V. 3.25 and a dendrogram was generated with MEGA 4. On the whole, 65.2% of the cowpea genotypes had stable resistance to *S. gesnerioides* from the regions of northern Ghana and 34.8% were susceptible. The C42-2B marker resolved as a single DNA band of 280 bp with segregation efficiency of 80% and 61R-M2 marker as double DNA bands of 320 bp and 380 bp with segregation efficiency of 60% associated with *Striga* resistance. Sixteen (16) polymorphic SSR primers distinguished all 46 cowpea genotypes into three clusters. Gene diversity ranged from 0.04 to 0.49 with an average of 0.29. The average allele frequency is 0.78, with a mean genetic diversity of 0.29. Polymorphism information content (PIC) varied from 0.08 to 1.00 with an average of 0.55. Therefore, cowpeas with *Striga* resistance and other desirable traits can be evaluated and released as varieties for farmers to cultivate.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is the second most important legume in the regions of northern Ghana, after groundnuts in terms of area under cultivation, quantity produced, and annual consumption [1]. Ghana is the fifth highest producer of cowpea in Africa [2] with an average of 143,000 MT produced annually on about 156,000 ha of land. There has been a projection that the rate of cowpea production for the period between 2010 and 2020 would increase by 11.1% [3]. However, cowpea production in the major cultivation regions of northern Ghana is constrained by both abiotic and biotic factors [4]. One of the major biotic constraints to cowpea productivity among small-holder and resource-poor farmers in the regions of northern Ghana is the attack by parasitic weed, *Striga gesnerioides* (Willd.) Vatke [5].

S. gesnerioides is a devastating obligate root hemiparasite that primarily parasitizes dicotyledonous species, including cowpea and other legumes [6, 7]. Cowpea yield losses associated with *S. gesnerioides* infestations range from 83 to 100% [5, 8]. The degree of damage to cowpea caused by *S. gesnerioides* is as a result of the close parasitic association between the host cowpea and the parasitic weed. Seeds of *S. gesnerioides* in the soil germinate in response to specific stimulants produced by cowpea roots [9]. Haustoria developed by the parasites [10] are used to attach the roots and penetrate the vascular tissues to establish vascular connections with the host cowpea [11]. Water, minerals, and organic compounds (photosynthates) are drawn from the

cowpea for the development of the parasite [12]. Several control strategies have been developed including cultural practices, the use of chemical control, and breeding for resistance [13]. Control strategies based on the use of herbicides are too expensive for low-input farming systems, whilst cultural practices are inefficient [14]. However, according to [15], host plant resistance has the potential to efficiently control the parasitic weed, which can be accessible to resource-poor farmers as well as environmentally friendly [5].

Seven races of *S. gesnerioides* have been identified based on host differential response and genetic diversity analysis within the cowpea growing regions of West Africa [16]. The races of *Striga* are designated as SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon), and SG6 (Senegal). However, the race of *S. gesnerioides* across the three regions of northern Ghana has not been confirmed. It is also unclear whether the *S. gesnerioides* in the northern Ghana is one of the known races in West Africa or a novel biotype. According to [17], race formation in cowpea-*Striga* association is largely a result of host-driving selection. Identification of race-specific responses in cowpea is relevant for the development of target-resistant genotypes.

Asare et al. [18] showed that only limited sources of resistance exist against *S. gesnerioides* in Ghanaian cowpea germplasm, and this underscores the need for more extensive analysis of breeding lines and exploitation of exotic genetic resources for cowpea improvement in Ghana. Some recombinant inbred lines (RILs) of cowpea, local cowpea accessions and exotic cowpea genotypes from the International Institute of Tropical Agriculture (IITA), exist in the Department of Molecular Biology and Biotechnology of the University of Cape Coast. However, these cowpea genotypes lack comprehensive assessment of their reactions to *Striga* infestation and genetic analysis which are necessary to facilitate selection and evaluation towards the release of adaptable cowpeas as varieties. The objective of the current study was to assess the reactions of 46 cowpea genotypes to *S. gesnerioides* infestation and to determine resistant genotypes.

2. Materials and Methods

A total of forty-six (46) cowpea genotypes were obtained from the Department of Molecular Biology and Biotechnology, University of Cape Coast. Twenty-eight (28) of the cowpea genotypes were recombinant inbred lines developed from a cross between IT97K-499-35 (resistant parent) and Apagbaala and SARC-LO2 (susceptible parents); fourteen (14) were cowpea genotypes from International Institute of Tropical Agriculture (IITA), Nigeria, the three parental genotypes (IT97K-499-35, Apagbaala, and SARC-LO2) as well as the local landrace, GH3684.

2.1. Pot Screening. The pot culture screening method used by [5, 17] was employed to assess the reaction of the cowpea genotypes to *S. gesnerioides* infestation. Each cowpea genotype was screened against *Striga* from Manga (upper east region), Lawra (upper west region), and Walewale (northern region). The test was conducted at the Manga Research Station of the Savannah Agricultural Research Institute (SARI) of the Council for Scientific and Industrial Research (CSIR) in Bawku. The potted soil medium was inoculated with *Striga* seeds, and four seeds of each cowpea genotype were sown per pot and replicated three times. The seedlings were thinned out at two weeks (14 days) after sowing and three plants were maintained per pot. The soil was kept moist by watering regularly every two days or when necessary. Hand picking of weeds was done when necessary. The days to emergence of *Striga* and the number of *Striga* per pot were recorded after 6 weeks. The plant-soil mass was removed from each pot and gently agitated to loosen the soil mass. The roots were washed thoroughly free of soil and examined using hand lens for the presence of necrotic hypersensitive lesions, attachment of *S. gesnerioides*, and tubercles. Cowpea plants that favoured attachment and emergence of *S. gesnerioides* were classified as susceptible and those that were intact and free from infestation, thus without any *Striga* attachment, were categorized as resistant.

2.2. DNA Analysis

2.2.1. DNA Extraction. The genomic DNA from each plant was fixed on FTA plant card (Bioneer) and processed as described by [19, 20], with slight modifications. Each isolated leaf from cowpea seedlings was cleaned with 70% ethanol and then placed over the marked circle with the underside of the leaf facing down on top of the FTA matrix card. The leaf was overlaid with parafilm, and a small porcelain pestle was used to apply moderate pounding over each sample circle area to burst the cell walls of the plant tissue. The back of the FTA card matrix was checked to observe the plant tissue extract drawn through the matrix. The FTA card was air-dried at room temperature for about one hour thirty minutes after the plant tissue extract transfer was completed. The FTA matrix card was placed on FTA sample mat and 2.0 mm Harris micro-punch tool was used to isolate several discs from the center of the dried sample area into 1.5 ml microfuge tube. Alcohol was used to wipe the punch tip after picking samples from a particular cowpea genotype before being reused to pick samples from another cowpea genotype. The leaf discs in each tube were washed with 70% ethanol for 5 minutes and repeated until the disc turned white. About 200 μl of FTA purification reagent was added to each tube, capped, inverted twice, and incubated for 5 minutes at room temperature. The FTA reagent was pipetted up and down twice to ensure that the disc remained in the tube. A pipette was used to remove and discard the FTA purification reagent. This was repeated for two FTA reagent washes. The discs were allowed to completely air dry for a minimum of one hour at room temperature and stored at 4°C until being ready for PCR amplification.

2.2.2. Primer Screening. A total of 100 SSR primers were screened for polymorphism involving two cowpea parental genotypes, IT97K-499-35 and SARC-LO2, to ensure optimal

performance. Optimal PCR amplification was achieved within the range of 55 to 60°C annealing temperatures. Sixteen SSR polymorphic primer pairs (Table 1) were selected and used for genetic analysis of the cowpeas.

2.3. Polymerase Chain Reaction (PCR) Analysis. AccuPower Taq PCR Premix containing Taq DNA Polymerase, dNTPs, reaction buffer, tracking dye, and patented stabilizer ordered from Bioneer was used in this work. 10 μl of the complete PCR amplification mixture including 1 μl of primer pair was added directly to the PCR tube containing the dried FTA disc. Each of the 16 primer pairs (Table 1) was used to amplify genomic DNA of the 46 cowpea genotypes. PCR amplification was carried out in Bio-Rad T100™ thermal cycler (Applied Biosystems). PCR conditions involved denaturing at 94°C for 3 minutes, annealing at 55–60°C (Table 1) for each primer pair for 30 seconds, and extension at 72°C for 30 seconds. This cycle was repeated 35 times, with a final extension at 72°C for 10 minutes, and PCR products were stored at 4°C.

2.3.1. Gel Electrophoresis. The 2% agarose gel was casted in a tray (27.5 cm × 24.5 cm) with 15-well-forming comb inserted to create wells. A 40 ml agarose gel was prepared by dissolving 0.8 g of the agarose in 40 ml of ×1 TBE buffer in a microwave. The mixture was stained with 3.0 μl ethidium bromide. The mixture was then poured into the tank and distributed across the whole surface and allowed to solidify. The whole assembly was transferred into electrophoretic tank after the comb was removed; the assembly was submerged in ×1 TBE buffer. The PCR products were loaded into the wells. The lid of the electrophoresis tank was then fixed. The PCR products were resolved for 1 hr. at 120 mA and 90 V and visualized on a UV transilluminator (M-15; UVP, Upland, CA, USA). The DNA bands in the gel were photodocumented with a digital camera (Sony SELP1650, Thailand). The size of DNA bands in base pairs was determined using the 100 bp DNA standard ladder (N0551S, Bioneer).

2.4. Data Analysis. Morphological and physiological data collected from the pot experiment were subjected to the Analysis of Variance (ANOVA) using General Statistics (Genstat) analytical software (version 12.1.0.3338). Varietal means were compared using the Least Significant Difference (LSD) at 5% level of probability. The scoring and analysis of molecular data followed the format used by [21], with slight modifications. All genotypes were scored for the presence and absence of DNA bands. Only clear and repeatable polymorphic DNA bands were scored as "+" for presence of band associated with *Striga*-resistant and "−" for absence of band associated with *Striga*-susceptible. The 100 bp standard ladder was used to determine the size of polymorphic DNA bands across the cowpea genome. Data matrix was created and used to calculate the genetic distance and similarity using PowerMarker V. 3.25. The related genetic parameters including the number of polymorphic bands, alleles per locus, genetic diversity, and polymorphism information content (PIC) are generated through the similarity matrixes for cluster analysis using PowerMarker V. 3.25. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) on the similarity indices was performed to identify genetic variation patterns among cowpea genotypes, and the resulting dendrogram generated was observed in MEGA 7 software.

3. Results

3.1. Phenotypic Screening. The 46 cowpea genotypes expressed resistance or susceptible responses across the spectrum of *S. gesnerioides* from Walewale (northern region), Manga (upper east region), and Lawra (upper west region). In all, 65.2% (30) of the cowpea genotypes were resistant to *S. gesnerioides* and 34.8% (16) were susceptible (Table 2). The cowpea genotypes that expressed complete resistance to the "witchweed" in Ghana were not associated with the emergence of *S. gesnerioides* (Figure 1(b)), no attachment of *S. gesnerioides* to the roots, or necrotic hypersensitive lesions on the roots. Susceptible cowpea genotypes were characterized by an average of 15 emerged *S. gesnerioides* seedlings per pot (Figure 1(a)) and attachment of tubercles to the roots. The seedlings of germinated *Striga* emerged on the surface of the soil after 31 days (Table 3) of sowing. The *Striga*-infested cowpea plants expressed varied symptoms including stunted growth, leaf necrosis, chlorosis, senescence, defoliation and reduced size of young leaves, low flowering, and pod formation.

3.2. Molecular Screening. The amplicons of C42-2B marker across the genome of 46 cowpea genotypes appeared as single DNA bands of 280 bp, associated with resistant cowpea genotypes but that of 61R-M2 marker was expressed as double DNA bands of 320 bp and 380 bp associated with resistant cowpea genotypes. Susceptible cowpea genotypes showed only single DNA band of 380 bp for 61R-M2 or no band at all (Table 3).

3.3. SSR Polymorphism. The 16 informative SSR primers distinguished all the 46 (100%) genotypes of the cowpea including those from same parents and those with similar seed size and seed coat colour. The sizes of polymorphic amplicons (DNA bands) ranged from 120 bp for SSR-6315 to 750 bp for SSR-6375. The number of alleles detected per primer pair varied from a minimum of 2 to a maximum of 22 with an average of 5.25. The allele frequencies yielded by the 16 SSR primers ranged from 0.57 to 0.98 with an average of 0.78. Gene diversity also ranged from 0.04 to 0.49 with an average of 0.29. The PIC varied from 0.08 to 1.00 with an average of 0.55, from a total of 8.76 (Table 4). Fifty percent of the primers had PIC of 0.5 or above. There was significant ($P \leq 0.05$) correlation between the allele frequency and the gene diversity. There was, however, no significant ($P \geq 0.05$) correlation between the allele frequency and the PIC (P-value = 0.322; $r = 0.265$) and between the gene diversity and PIC (P-value = 0.246; $r = -0.308$).

Table 1: SSR primers used, their sequences, and annealing temperatures.

Name	Sequence	Annealing temperature (°C)
SSR-6265	CAG AAG AGG TGA AAA TTG AAC GCA TGT TGC TTT GAC AAT GGT	57
SSR-6299	GGC GCA GAA AGA CAG GTT AC CTG CAG CAC CTA ACT CAC CA	60
SSR-6315	CGC AGT GAA AAG GAA AAG GA ATC AGC GTC CAA TCC AAA AA	55
SSR-6929	GCC CAT GTA ATG CTG TAT AGT GGC GTT AGA ACT ACT CCA GTT	57
SSR-6965	GCA TTC AGC TAC GAT GTG TTC GGC ACT TTG TAA AAG ACA GGC	59
SSR-6291	TCA TGA GTT TCC ACA CAC CAA CCT TCG TAT GTA TAT GTG GCT ACT G	60
SSR-6375	GCT CGG ATA TGG TCC TGA AA TCA GTG TCA GCA CCA TAC CC	60
SSR-6777	CGA AGC ATG TGG ACA CGT AC CAT TGA ACA AAC ATC GCT GAA GC	60
SSR-6273	CCC CCA GAA CAA ATA GAA ACT C TGA ATT TGA AGA AGA GAT AAT TG	60
SSR-6248	GGG TGC TTT GCT CAC ATC TT TCC ATG TGT TTA TGA CGC AAA	58
SSR-6171	ATT CGA TCC AAC CCA ATG AC AGC GAA GGC ATG TTC GTA AG	58
SSR-6235	TTT TCC CTC CAC CTG TTT GA GAA GCA TTG ACC AAG CAA CA	56
SSR-6243	GTA GGG AGT TGG CCA CGA TA CAA CCG ATG TAA AAA GTG GAC A	60
SSR-6260	AAA GTT TTA ATA TTA CCA ACA ACA A CAA CCA GGC AAA TGG AAA TC	56
SSR-6240	TTC AAT GTG GGA GGA TGA GA GGT TCC GGA TTC AAT TTT CC	56
C42-2B	CAG TTC CCT AAT GGA CAA CC CAA GCT CAT CATCAT CTC GAT G	58
61RM2	GAT TTG TTT GGT TTC CTT AAG GGT TGA TCT TGG AGG CAT TTT	57

3.4. Cluster Analysis. Sixteen (16) polymorphic primers differentiated the 46 cowpea genotypes into three major clusters, A, B, and C, at 0.15 similarity coefficient (Figure 2). Cluster A was the largest comprising 26 cowpea genotypes, out of which 15 were inbred lines from the University of Cape Coast, 8 cowpea genotypes from IITA, and 3 local genotypes (GH3684, Apagbaala, and SARC-LO2). Sixteen of the cowpea genotypes in cluster A are *Striga* resistant and 10 are *Striga* susceptible.

Cluster B had 17 cowpea genotypes, out of which, 12 are *Striga* resistant and 5 are *Striga* susceptible. Cluster C was made up of only two cowpea genotypes (UCC-122 and UCC-24) which are both *Striga* resistant. SARC-LO2 and Apagbaala, as well as UCC-497 and UCC-514, were the most genetically similar with a genetic distance of 0.05 cM. The longest genetic distance (0.50 cM) existed between UCC-11 and UCC-24, UCC-11 and UCC-523, and UCC-32 and UCC-473.

The 30 *Striga*-resistant genotypes were spread throughout the spectrum of the three clusters. Generally, they were diverse, with only a few of them closely related. UCC-241 and UCC-428, IT10K-499-35 and UCC-478, and IT08K-150-12 and IT10K-817-3 were the most closely related with a genetic distance of 0.07 cM. UCC-11 and UCC-24 and UCC-32 and UCC-473, which are all *Striga*-resistant genotypes, showed the highest diversity among the 30 *Striga*-resistant genotypes with a genetic distance of 0.50 cM. Ten (10) out of the fifteen *Striga*-susceptible cowpea genotypes were clustered together (Cluster A). They were, however, not closely related, with the exception of Apagbaala and SARC-LO2 and IT10K-125-107 and UCC-white with genetic distances of 0.05 cM and 0.08 cM, respectively.

4. Discussion

4.1. Phenotypic Analysis. The overall resistance or susceptibility expressed by the 46 cowpea genotypes to the *S. gesnerioides* from Walewale (northern region), Manga (upper east region), and Lawra (upper west region) gave implication that probably the *S. gesnerioides* samples may be of the same race or the cowpea genotypes may have multiple resistance to the parasitic weed in Ghana. Indeed, [18] observed similar responses to *S. gesnerioides* from Bawku among recombinant inbred lines (RILs) of cowpea. This suggests that the Ghanaian biotypes of *S. gesnerioides* may have similar virulence [5]. Besides, the *Striga*-resistant cowpeas may possess the *Striga*-race-specific resistant gene

Table 2: The responses of cowpea genotypes to *S. gesnerioides* infestation.

Genotype	Phenotype			Genotype	
	Manga	Lawra	Walewale	C42-2B	61R-M2
UCC-11	R	R	R	+	−
UCC-24	R	R	R	+	+
UCC-32	R	R	R	+	+
UCC-56	S	S	S	+	+
UCC-122	R	R	R	+	+
UCC-153	R	R	R	+	+
UCC-221	R	R	R	+	+
UCC-226	R	R	R	+	−
UCC-241	R	R	R	+	+
UCC-328	R	R	R	+	−
UCC-366	S	S	S	+	−
UCC-445	R	R	R	+	−
UCC-460	R	R	R	+	+
UCC-466	R	R	R	+	+
UCC-471	R	R	R	+	+
UCC-478	R	R	R	+	+
UCC-484	R	R	R	−	−
UCC-489	S	S	S	+	+
UCC-490	S	S	S	−	+
UCC-497	S	S	S	−	+
UCC-513	R	R	R	+	+
UCC-514	S	S	S	−	+
UCC-523	S	S	S	−	+
UCC-428	R	R	R	+	+
UCC-473	R	R	R	−	−
UCC-377	R	R	R	+	−
GH3684	R	R	R	+	−
UCC-White	S	S	S	−	−
UCC-Early	S	S	S	−	−
IT97K-499-35	R	R	R	+	+
SARC-LO2	S	S	S	−	−
Apagbaala	S	S	S	−	−
IT08K-193-14	R	R	R	−	−
IT10K-815-5	S	S	S	+	−
IT10K-125-107	R	R	R	−	+
IT10K-832-3	R	R	R	+	+
IT10K-456	R	R	R	+	−
IT10K-298-9	S	S	S	−	−
IT07K-297-13	S	S	S	+	+
IT08K-150-12	R	R	R	+	−
IT08K-126-19	R	R	R	+	+
IT08K-193-15	R	R	R	−	+
IT11K-61-82	S	S	S	−	+
IT11K-321-2	S	S	S	+	+
IT10K-817-3	R	R	R	+	−
IT10K-819-4	R	R	R	+	+

R: resistant, S: susceptible, +: presence of marker, and −: absence of marker.

to combat the Ghanaian isolate of the parasite. In addition, the cowpeas genotypes from IITA and the local landrace GH3684 may have similar *Striga*-resistant trait as IT97K-499-35. The *Striga*-resistant cowpeas in the current study showed robust and healthy growth forms compared to the *Striga*-susceptible cowpeas (Figure 1). The enhanced vigorous growth of *Striga*-resistant cowpea genotypes may be due to adequate biomass accumulation [22] that could be translated into grain yield contrary to the *Striga*-susceptible cowpeas.

4.2. Molecular Analysis. The resistance status of the cowpea genotypes to *S. gesnerioides* was validated with C42-2B and 61R-M2 markers. The results are consistent with those of Asare et al. [18], who used C42-2B to screen some breeding lines of cowpea. The marker segregation efficiency of 83.3% for C42-2B was better than that of 60% for 61R-M2 in identifying *Striga*-resistant cowpea genotypes. However, 92.6% discriminatory efficiency of SSR-1 marker was reported among cowpea breeding population [18].

The cowpea genotypes UCC-11, UCC-24′, UCC-56, UCC-122, UCC-221, UCC-226, UCC-241, and UCC-328 had stable resistance to *S. gesnerioides*. The stability of resistance to *S. gesnerioides* demonstrated by the cowpeas suggests that the *Striga*-resistant gene might be fixed in the F_9 recombinant inbred lines.

The current study observed double DNA bands of 380 bp and 320 bp for the 61R-M2 marker associated with *S. gesnerioides*-resistant cowpea genotypes. This was consistent with the similar result by Omoigui et al. [23], who also observed the double DNA band characteristic of 61R-M2 marker in *Striga*-resistant cowpea genome of F_1 and F_2 populations from a cross between Borno Brown × IT97K-499-35 and Borno Brown × B301. Four of the cowpea genotypes (UCC-56, UCC-489, IT07K-297-13, and IT11K-321-2) had the C42-2B and 61R-M2 markers but were susceptible against *S. gesnerioides* in the pot analysis. However, three cowpea genotypes (UCC-473, UCC-484, and IT08K-193-14) were resistant to the sampled *S. gesnerioides* but did not amplify for any of the markers (Table 2). Unlike SSR-1 primer where the marker is within the *Striga* race 3 (SG3) gene of B301 (1 cM), the C42-2B and 61R-M2 primers have their markers close to the resistant gene, with genetic distances of 8.5 cM and 3.5 cM, respectively [23]. The *Striga*-resistant gene can be separated from the marker during crossover. The marker could, therefore, be present, but the gene controlling the resistance to *S. gesnerioides* may be absent. This could account for the reason why some of the cowpea genotypes (UCC-56, UCC-489, IT07K-297-13, and IT11K-321-2) had the marker of both primers present but susceptible phenotypically.

4.3. Genetic Analysis. The genetic diversity and phylogenetic relationships of cowpea genotypes from Ghana have been evaluated using SSR markers [24, 25]. In this study, all the 16 SSR primer combinations used gave amplification products with 100% polymorphism. The 16 selected microsatellites (SSR) markers differentiated the cowpea genotypes and clustered them differently. There was increasing similarity among the cowpea genotypes from IITA and the inbred lines from UCC. SARC-LO2 and Apagbaala, as well as UCC-497 and UCC-514, were the most genetically similar with a genetic distance of 0.0476. The longest genetic distance (0.500) existed between UCC-11 and UCC-24; UCC-11 and UCC-532; and UCC-32 and UCC-473. UCC-11, UCC-24, and UCC-32 are progenies from a cross between IT97K-499-35 × SARC-LO2, while UCC-473 and UCC-532 are progenies from a cross between IT97K-499-35 × Apagbaala. Most of the cowpea genotypes are also populations from the cross between IT97K-

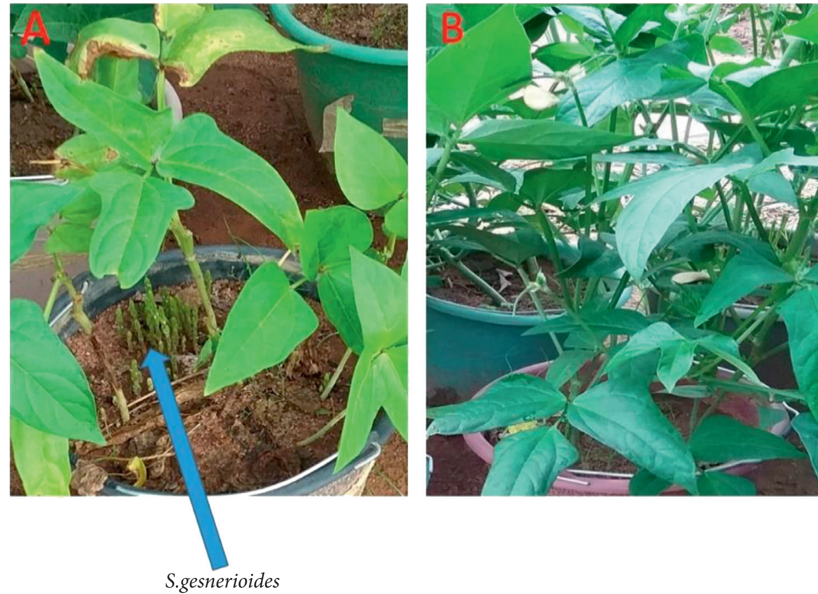

Figure 1: Response of cowpea plants to *S. gesnerioides* infestation at 5 weeks after sowing seeds in *Striga*-infested soil. (a) *Striga*-susceptible cowpea plants under stress by *Striga* seedlings parasitism. (b) *Striga*-resistant cowpea plants showing vigorous vegetative growth without *Striga* emergence.

Table 3: *Striga* count per pot and days to emergence of *Striga*.

Genotypes	Number of *Striga* seedlings per pot			Days to *Striga* emergence		
	M	W	L	M	W	L
I0T1K-298-9	10.67	7.00	12.00	39.67	34.33	34.30
IT07K-297-13	9.67	7.67	13.67	35.00	33.33	33.00
UCC-489	16.67	13.33	11.67	37.00	35.33	33.60
IT11K-321-2	30.33	20.33	20.33	36.00	35.00	33.30
APAGBAALA	12.00	23.00	15.67	32.00	33.33	33.00
UCC-514	15.33	12.67	3.33	32.00	33.67	31.67
UCC-490	20.00	17.33	7.33	32.00	33.33	31.33
UCC-56	17.33	11.33	14.33	31.00	33.33	34.33
UCC-497	25.33	21.00	1.33	34.00	33.67	33.00
UCC-White	27.33	23.33	16.00	34.00	33.33	33.00
IT11K-61-82	24.00	17.67	21.33	33.00	34.67	32.67
UCC-366	17.67	13.33	23.67	34.33	34.00	40.33
IT10K-815-5	10.67	8.33	5.67	49.33	33.33	33.67
SARC-LO2	17.33	20.67	9.67	34.00	33.67	33.67
UCC-Early	22.00	24.00	20.33	34.00	34.67	35.33

Table 4: Number of alleles, gene diversity, and polymorphism information content for 16 primers used.

Primers	Sample size	Number of alleles	Allele frequency	Gene diversity	PIC
SSR-6299	46.00	8.00	0.83	0.22	0.81
C42-2B	46.00	2.00	0.65	0.45	0.35
61RM2	46.00	4.00	0.59	0.48	0.73
SSR-6777	46.00	2.00	0.57	0.49	0.37
SSR-6375	46.00	22.00	0.79	0.30	0.23
SSR-6315	46.00	2.00	0.95	0.08	0.08
SSR-6569	46.00	2.00	0.70	0.42	0.33
SSR-6240	46.00	2.00	0.91	0.16	0.15
SSR-6243	46.00	4.00	0.75	0.37	0.82
SSR-6929	46.00	2.00	0.61	0.48	0.36
SSR-6273	46.00	2.00	0.65	0.45	0.35
SSR-6265	46.00	6.00	0.88	0.19	0.70
SSR-6248	46.00	4.00	0.77	0.27	0.86
SSR-6171	46.00	6.00	0.98	0.04	0.99
SSR-6235	46.00	4.00	0.98	0.04	1.00
SSR-6291	46.00	12.00	0.80	0.27	0.63
Mean	46.00	5.15	0.78	0.29	0.55

PIC = polymorphism information content.

499-35 × SARC-LO2 and IT97K-499-35 × Apagbaala and therefore may share similar genetic traits. The common donor, IT97K-499-35, could influence the genetic relationship among the cowpea inbred lines in particular.

The genetic diversity across the cowpea genome in the current study is low. This was in line with a similar report by Asare et al. [24], who also observed a lower level of genetic variability among Ghanaian cowpea accessions. Kuruma et al. [26] also found low level of genetic diversity among cowpea accessions in Kenya using molecular markers. The low genetic diversity among the cowpea genotypes could be as a result of common ancestral origin and self-pollination mechanism in cowpea. The number of alleles per locus of 2 to 22 with an average of 5.25 detected in the current study is in line with other recent reports. Li et al. [27] assessed the genetic similarities and relationships among 48 wild cowpea lines using SSR primers and detected between 4 and 13 alleles with an average of 7.5 alleles. Indeed, the authors in [28] observed that the number of alleles ranged from 1 to 9 per SSR primer combination in cowpea germplasm from Senegal. Sawadogo et al. [29] indicated that the 16 SSR primers used to assess the genetic diversity of cowpea cultivar in Burkina Faso generated a range of alleles between 5 and 12 fragments with an average of 8.2 bands per primer combination among cowpea genotypes. A combination of 25

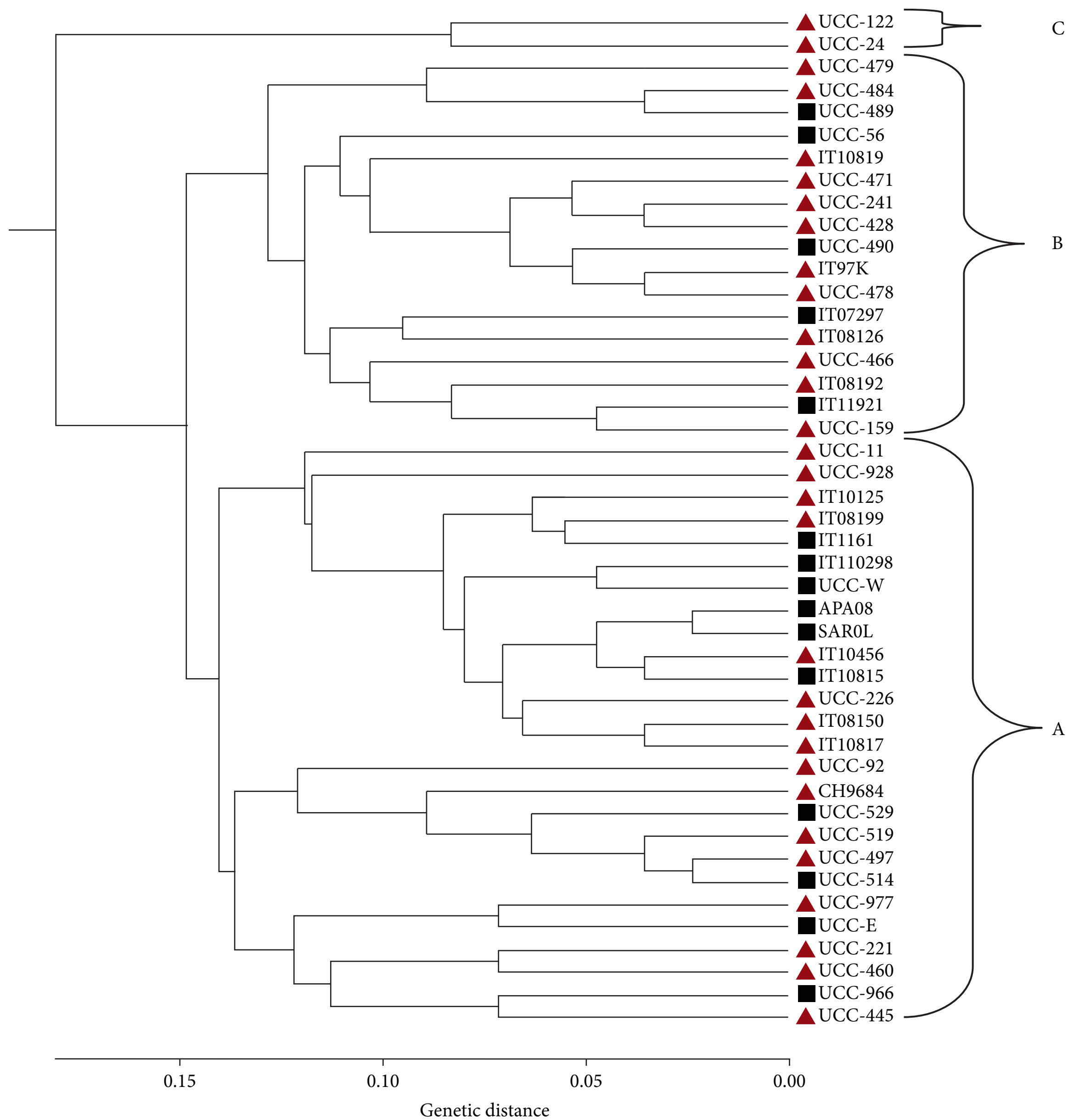

Figure 2: Phylogenetic relationship among 46 cowpea genotypes. A dendrogram was generated using sixteen informative SSR markers and sequential clustering algorithm (UPGMA) based on genetic similarity. UCC – E = UCC – Early; UCC – W = UCC – White; APAGB = Apagbaala; SARCL = SARC – LO2; IT97K = IT97K – 499-35; ▲ = *Striga*-resistant; and ■ = *Striga*-susceptible.

informative SSR primers were used to analyse Ghanaian cowpea germplasm and yielded 1 to 6 alleles per primer pair with a mean of 3.8 [24]. The assessment of 48 accessions of cultivated cowpea sampled from West Africa, Northeast Africa, Central Africa, and Southern Africa using 12 SSR markers revealed that the number of alleles per locus ranged from 2 to 5 with a total of 37 alleles generated for the primers [30].

The polymorphism information content (PIC) is a means of detecting alleles and distribution of their frequencies [31]. Data reported by [24] showed PIC ranging between 0.07 and 0.66 with a mean of 0.38. Sixteen SSR primers used to assess the genetic diversity and phylogenetic relationship among 252 cowpea genotypes in Sudan, yielding PIC between 0.33 and 0.83 with an average of 0.56 [31]. Genetic diversity studies among 32 cultivating cowpea, using 22 SSR primers, showed a PIC range from 0.25 to 0.63 with an average of 0.45 [21]. The mean PIC value (0.55) recorded in the current study is, therefore, in line with the results obtained from previous reports. The study in [30] produced a PIC valve ranging from 0.08 to 0.60 with a mean valve of 0.34 from a total of 4.47. Also, Larweh et al. [32] noted that polymorphism information content (PIC) values ranged from 0.32 to 0.36 with a mean of 0.34 in cowpea breeding lines. Ali et al. [33] reported that a mean PIC value ≥ 0.5 is highly informative, 0.25~0.50 reasonably informative, and <0.25 slightly informative, and loci (marker) with many alleles and a PIC value near 1 are most desirable.

This means that the primers used for the current study were highly informative. Gene diversity observed in this study was 0.29 on average ranging from 0.04 to 0.49. In Senegal, cowpea gene diversity varied from 0.08 to 0.42 with a mean of 0.28 [34], whereas in Ghana cowpea germplasm gene diversity ranged from 0.12 to 0.68 with an average of 0.44 [24]. The results of gene diversity reflect the proportion of polymorphic loci across the genome. Therefore, according to the result of the current study, the markers used were almost as polymorphic as those used by [24, 34].

5. Conclusion

The responses of 46 cowpea genotypes to *S. gesnerioides* samples from upper east (Manga), upper west (Lawra), and northern regions (Walewale) of Ghana were similar which implies that the *S. gesnerioides* in Ghana may be of the same biotype or the cowpea genotypes may have the same response to different races of *S. gesnerioides*. On the whole, 65.2% of the cowpea genotypes were resistant to *S. gesnerioides* associated with C42-2B and 61R-M2 markers and 34.8% were susceptible. The segregation efficiency of 80% of C42-2B marker (single DNA band of 280 bp) was better than that of 60% of 61R-M2 (double DNA bands of 320 bp and 380 bp) for identification of *Striga*-resistant cowpea genotypes. The 16 SSR primers were informative and could distinguish all the 46 cowpea genotypes into three major clusters in a dendrogram. The allele frequencies yielded by the SSR primers ranged from 0.57 to 0.98 with an average of 0.78. Gene diversity also ranged from 0.04 to 0.49 with an average of 0.29. The PIC varied from 0.08 to 1.00 with an average of 0.55 with a mean genetic diversity of 0.29.

Data Availability

The data used to support the findings of this study can be accessed from the corresponding author upon request.

Disclosure

The authors take full responsibility for any error.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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