RESPONSES OF RECOMBINANT INBRED LINES OF COWPEA [(*VIGNA UNGUICULATA* (L.) WALP] TO *STRIGA GESNERIOIDES* INFESTATION IN GHANA

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Abstract:

Cowpea [*Vigna unguiculata* (L.) Walp.] production in West Africa is constraint by *Striga gesnerioides* parasitism associated with 83-100% yield loses which warrants development of resistance varieties against the parasite. An exotic resistant genotype, IT97K-499-35 developed by International Institute of Tropical Agriculture (IITA) was crossed with SARC-LO2, a local susceptible genotype. SSR-1 and C42-2B markers previously mapped in the region of *S. gesnerioides* resistance loci were used to amplify genomic DNA of advance recombinant inbred lines (RILs) of this cross. The responses of the RILS to *Striga* infection in pot culture and a field trial conformed to segregation ratio of resistance to susceptible genotypes of 1:1 among F_7 progenies suggesting monogenic dominant inheritance of the resistance. The markers SSR-1 and C42-2B mapped in the region of the resistance locus, but presented as dominant markers, with amplification only in resistant genotypes. The selective efficiency of SSR-1 (92.6%) was better than that of C42-2B (85.7%). In field trials, growth and morphology of susceptible genotypes were adversely affected by *S. gesnerioides* which resulted in significant ($P \leq 0.05$) reduction in seed yield compared to resistant genotypes. The resistant RILs identified in the current work would be further evaluated in multi-location trials prior to their release to farmers for cultivation.

Key Words: Cowpea, Marker-assisted selection, *Striga*, Recombinant inbred lines

Introduction

Cowpea [*Vigna unguiculata* (L.)Walp] is one of the most economically important indigenous African grain legumes with enriched protein as source of food for both human and animal nourishment and a major crop in regional trade within West and Central Africa (Langyintuo *et al*., 2003). The relatively high protein content of cowpea makes it an essential supplement to the diet of many Africans (Bressani, 1985) who consume high carbohydrate but low in protein cereals, root and tuber crops (Omoigui, 2007). Besides, cowpea is also a valuable commodity that provides income for farmers and fixes atmospheric nitrogen to restore soil fertility for succeeding cereal crops growing in rotation with it. West and Central Africa produce 69% of the world production (Langyintuo *et al*., 2003). However, a major biological constraint to increase production in smallholder farms is the infection by the parasitic weed, *Striga gesnerioides* (Willd) Vatke (Ehlers and Hall, 1997). Cowpea yield losses associated with *S. gesnerioides* range from 83 to 100% (Cardwell and Lane, 1995; Emechebe *et al*., 1991)

The extent of damage to cowpea by *S. gesnerioides* infection is related to the close parasitic association between the host and the parasitic weed. Seed germination in *S. gesnerioides* occurs in response to specific stimulants exuded by host roots in the soil (Muller *et al*., 1992). The extremity of the radical modified into haustorium (Okonkwo and Nwoke, 1978), that attaches and penetrates the vascular tissues and establish vascular connections (Ba, 1983) to derive water, minerals and organic compounds from the cowpea for the development of the parasite (Graves *et al*. 1992). Indeed, no single method is adequate to control the parasite, however, host plant resistance appears to have the potential to effectively and economically control the parasite since it is affordable to resource-poor farmers (Omoigui *et al*., 2007) as well as being environmentally friendly. Breeding for resistance to *S. gesnerioides* has led to development of some resistant cowpea cultivars (Parker and Polniaszek, 1990; Aggarwal, 1991).

Development of cowpea host plant resistance to *S. gesnerioides* requires the application of phenotypic and genotypic diagnostic protocols to screen a population segregating for resistance to the parasite. Indeed molecular markers for identification and selection of Striga-resistant genotypes have been developed for most of the races of the parasite prevalent in West Africa. However, the differential virulence of races of *S. gesnerioides* on cowpea genotypes (Lane *et al*., 1994; Singh, 2002) has serious implication to breeding and selection procedures. Hence, the need to use race specific markers to complement conventional breeding methods to identify cowpea resistant genotypes. Until now, 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 (Lane *et al*., 1996). These races are designated as SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) and SG6 (Senegal). According to Botanga and Timko (2005), race formation in cowpea-*Striga* association is largely a result of host-driving selection, because the parasite is autogamous with floral features that make occurrence of out-crossing very low. Identification of race-specific responses in cowpea is relevant for the development of target resistant genotypes.

Several race-specific resistance genes have been identified and located to linkage groups 1 and 6 (LG1 and LG6) of the current cowpea genetic map (Ouédraogo *et al*. 2001 and 2002). The genetics of cowpea *Striga*-resistance varies according to the biotype of the parasite; however, it is inherited mainly as a single nuclear dominant gene (Singh and Emebeche, 1990; Atokple *et al*., 1993; Lane *et al*., 1993; Moore *et al*., 1995; Toure *et al*, 1997; Carsky *et al*., 2003). A few reports, however, have pointed out that the resistance is conferred by two independent dominant genes (Dubé, 2000) or a recessive single gene (Toure *et al*., 1997). The implication of this variation in resistance is that reliable screening protocols are required to select recombinant inbred lines for the resistance or susceptibility to the parasite. Therefore, the focus of the current work was to screen recombinant inbred lines of the cross between Striga-resistant exotic line and locally adapted variety susceptible to the parasite to select resistant lines as basis for developing better adapted varieties.

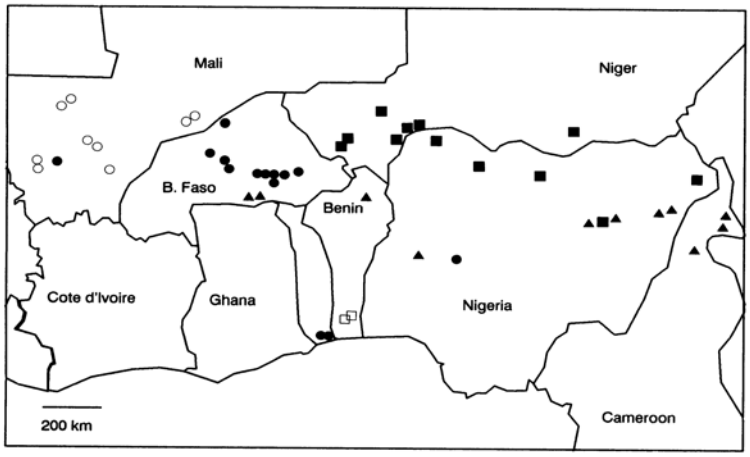


Fig. 1. Distribution of races of *Striga gesnerioides* in West Africa: closed circles, race 1; open circles, race 2; closed squares, race 3; open squares, race 4, and triangles, race 5.

Source: Lane *et al*. (1996)

Materials and Methods

Plant culture and DNA extraction

Cowpea seedlings of 98 recombinant inbred lines (F_7) derived from a cross of IT97K-499-35 (resistant parent) × SARC-LO2 (susceptible parent) were raised in plastic pots filled with sandy loam soil in the Botanical Garden of the School of Biological Sciences of the University of Cape Coast. Three cowpea seeds were sown in each plastic pot and maintained with regular watering at two-day intervals or when necessary. Three young leaves for each potted cowpea were harvested at 14 days after sowing (DAS) seeds, labeled and frozen in liquid nitrogen.

The frozen dried leaves were homogenized and total genomic DNA isolated from each sample using the plant DNAzol® ES (MRC Inc, Cincinnati, OH) as per manufacturer's protocol with slight modification. The homogenate was transferred into 2ml Eppendorf tubes containing 750µL DNAzol® ES reagent and after vigorous shaking, 750µL chloroform was added. The mixture was centrifuged at high speed (12,000rpm for 10minutes) and supernatant was transferred to a new microfuge tube. Absolute ethanol (750 µL) was added to precipitate the DNA and the DNA pellet recovered by centrifugation at low speed (6000 rpm for 5minutes). Precipitated DNA was rinsed gently with 70 % ethanol, air-dried and suspended in 200 µL TE buffer (pH 8.0) and stored in a fridge at -4 ^{o}C overnight. The suspended DNA was incubated at 65 ^{o}C for 30 minutes in a water bath. The bottom of centrifuge tube containing DNA was tapped gently and centrifuged at 6000 rpm for 5minutes. The DNA was quantified with a spectrophotometer (Thermo Scientific, Wilmington, DE) and intactness of DNA was checked by resolving 1µL genomic DNA in 0.8 % (w/v) agarose gel. A DNA working solution of100 ng/µL was prepared and stored in a freezer at -20 ^{o}C until use.

Bulked Segregant Analysis

The Bulked Segregant Analysis (BSA) following Michelmore *et al.*, (1991) and Boukar *et al.* (2004) was employed to assess the segregation patterns and select more informative molecular markers for genomic analysis of the RILs. Equivalent amounts of genomic DNA from 10 resistant and 10 susceptible F_7 plants respectively from the population derived for the cross between IT97K-499-35 and SARC-LO2 were pooled to form resistant and susceptible bulks. Both bulks were used along with the parents to and screened with three sequenced characterized amplified region (SCAR) primers 61R, 61RM2 and C42-2B and one microsatellite primer, SSR-1. The ethidium bromide-stained gels were visualized on a UV transilluminator (M-15 UVP Upland, CA 91786 USA) and photographed using a digital camera. The highly polymorphic informative primers were selected and further used to analyze the population to determine the association of the markers with cowpea resistance or susceptibility to *S. gesnerioides*.

Polymerase chain reaction (PCR) analysis

Each PCR reaction mixture contained 8 µL Bioneer AccuPower® TLA PCR PreMix desolved in Molecular Grade Distilled water (MGDw), 0.5 µL of forward primer, 0.5 µL reverse primer and 1 µL genomic DNA of sample were added to make up 10 µL total volume. The PCR amplifications were performed in an Eppendorf Mastercycler (Techne TC-512) comprising an initial denaturation at 94 $^{\circ}$C for 1 min followed by 35 cycles of denaturation for 5 min, annealing at 55 $^{\circ}$C or 60 $^{\circ}$C for 1 min, extension at 72 $^{\circ}$C for 1 min and end with final extension at same temperature for 5 min. The PCR products were resolved for 1 h at 120 V on 2% (w/v) Agarose gel in 1 × TAE buffer using a horizontal gel electrophoresis apparatus (Model V16.2 or V16; Gibco BRL, Gaithersberg, MD, USA). The gels were stained with ethidium bromide and visualized on a UV transilluminator (M-15 UVP Upland, CA 91786 USA) and photo-documented with a digital camera. The size of DNA bands in base pairs was determined using the 1 kb DNA standard ladder (Invitrogen, Carlsbad, CA, USA).

Pot culture screening of cowpea against *Striga-gesnerioides* infection

The pot culture screening method used by Botanga and Timko (2005) was employed to assess the response of recombinant inbred lines of cowpea derived from IT97K-499-35 (resistant) × SARC-LO2 (susceptible) with a local check GH3684 (resistant) to *Striga gesnerioides* infection in the glasshouse of SARI, Tamale-Nyankpala in 2009. Each pot (17 × 11cm) filled with garden was inoculated with about 1000 seeds of *Striga gesnerioides* from the Upper East Region of Ghana. Four seeds of cowpea were sown per pot in 3 replications. The seedlings were thinned out and two were maintained per pot at 2 weeks after germination. The soil was kept moist by watering regularly every two days or when necessary. Destructive sampling was carried out at 8 weeks. The plant-soil mass

was removed from each pot, immersed into a bucket of water, and gently agitated to loosen the soil mass. The roots were washed thoroughly free of soil and examined using hand lens for presence of necrotic hypersensitive lesions, attachment of *Striga gesnerioides* and tubercles. Plants that favoured attachment, healthy development and emergence of *Striga gesnerioides* were classified as susceptible and those that appeared free from infection, without any attachment were categorized as candidate resistant genotypes.

Response of cowpea breeding lines to *S. gesnerioides* infection in field trial

Nighty-eight (98) recombinant inbred lines (RILs) selected by pot screening and molecular markers for resistance and susceptibility to the parasite were composed into a field trial. The trial was conducted under rain-fed sandy soil conditions from 3rd August- 31st October 2011 at the Savannah Agriculture Research Institute (SARI), Manga Station in Bawku located within latitude 11° 11′ 0, longitude 10° 40′ N and at an altitude of 249 m above sea level in a *Striga* seed-infested field (hotspot). with a local cowpea line GH3684 with resistance to Striga was included as a check. A 20 m × 40 m plot of land was ploughed, harrowed and ridges were constructed. The field was divided into two blocks at 1.5 m apart.

Three seeds of each cowpea breeding line were randomly sowed at 40 cm within row and 60 cm between rows. The cowpea seedlings were thinned to one per stand at 14 days after germination, allowing 12 cowpea stands per breeding line in each row arranged in a randomized complete block design. The local cowpea accession, GH3684 included in the test trial was also used as border plant for the entire set-up. Cultural practices, including hand weeding, were carried out at 3 and 6 weeks after sowing seeds, and insecticide (Dimiprid® 200 SL at 35ml/15L knapsack capacity) sprayings were carried on 4 and 7 weeks old plants. The number of *S. gesnerioides* emerged per plot was recorded for each cowpea breeding line. The effects of the parasitic stress on growth and morphology of cowpeas caused by *S. gesnerioides* were observed from 6 to 8 weeks of the growth period. The weight of hundred seeds was determined. The data were subjected to analysis of variance (ANOVA) using Minitab 16.2.2 statistical software. The differences between mean values were assessed using the least significant difference (LSD) at 5% level of significance.

Results

The pot test on F_7 RILs of advanced cowpea progenies derived from a cross of IT97K-499-35 (resistant parent) × SARC-LO2 (susceptible parent) expressed a segregation ratio of 1R:1S (χ^2 = 0.003; P = 0.995). This ratio conforms well to those of SSR-1 and C42-2B molecular markers in the cowpea genome and further confirmed by the response of the cowpea genotypes to *S. gesnerioides* infestation on the field (Plates 1 and 2; Table 1). The susceptible genotypes of cowpea had *Striga* seedlings attached to the roots after 45 days of germination or germinated *Striga* seedlings emerged on the surface of the soil. These cowpea plants expressed varied symptoms with age of culture due to *Striga*-parasitic stress including stunted growth, leaf necrosis, chlorosis, senescence, defoliation and reduced size of young leaves. The susceptible cowpea plants also had reduced flowering and pod formation as well as poor rooting and nodulation. Contrary, the resistant recombinant inbred lines (RILs) of cowpea had normal growth and development without *Striga* attachment or emergence comparable to the resistant parental genotype IT97K-499-35 and the local genotype cowpea accession, GH3684 used as a check.

The details of *Striga* emergence and degree of parasite infection for the individual RILs in the field trial are presented in Table 1. There were significant ($P \leq 0.05$) differences in the rate of emergence of *S. gesnerioides* among the genotypes of the F_7 RILs of cowpea. The emergence of *S. gesnerioides* ranged from 1 – 13.0 plants per plot (Table 1). The RILs that were not associated with *Striga* emergence and had the SSR-1 or C42-2B maker were observed as resistant genotypes. However, the cowpea RILs that were associated with *S. gesnerioides* emergence on the field or in the pot, and devoid of the SSR-1 and C42-2B resistance marker in the genome were susceptible genotypes. On the whole, the cowpea RILs differed in their growth responses with corresponding 100 seed yield under *Striga* infestation in the field.

The mean 100-seed dry weight for susceptible RILs was significantly ($P \leq 0.05$) lower (15.2g) than that (16.6g) for the corresponding resistant RILs. The influence of gene segregation, coupled with the *Striga* infection on 100-seed dry weight apparently followed the regular distribution pattern for a continuous variation (Fig. 2). The 100 seed weight ranged from 3.2g in the *Striga*-susceptible progeny UC96-05 to 23.0g in the *Striga*-resistant progeny UC96-223. On uninfected plots, the

susceptible parent (SARC-LO2) had large seeds with higher 100-seed weight (26.8g) (data not shown) compared to seeds from infected plots (19.8g). The 100-seed weight of the resistant parent (IT97K-499-35) was greater (16.2g) compared to the resistant local genotype GH3684 with a 100-seed weight of 13.3g.

Bulk segregant analysis in addition to the pot and field screening data revealed that C42-2B and SSR-1 markers were more informative in distinguishing resistant from susceptible bulks. SSR-1 and C42-2B markers produced single bands of 150bp and 180bp PCR products, respectively, with amplification only in resistant genotypes (Plate 1 and 2). The marker segregation efficiency of SSR-1 was better (92.6 %) than that of C42-2B (85.7%) in identifying resistant cowpea genotypes among the RILs.

Twelve RILs (UC96-36, UC96-46, UC96-50, UC96-85, UC96-171, UC96-177, UC96-191, UC96-264, UC96-290, UC96-333, UC96-357, and UC96-357) which had the C42-2B marker for resistance were found to be susceptible. Similarly, thirteen others (UC96-07, UC96-10, UC96-38, UC96-47, UC96-48, UC96-64, UC96-113, UC96-189, UC96-206, UC96-209, UC96-229, UC96-243 and UC96-274) with both SSR-1 and C42-2B markers were also susceptible in field trials indicating crossing-over between the marker locus and the gene locus for resistance.

Table 1. Reaction of cowpea RILs derived from a cross of IT97K-499-35 × SARC-LO2 to *S. gesnerioides* infection

		Phenotypic reaction			Genotypic reaction	
Genotypes	Pot Test	Field Trial	100-seed weight/g	Striga Emergence/ Plot	SSR-1 Marker 150bp	C42-2B Marker 180bp
IT97K-499-35	R	R	16.0	0	+	+
SARC-LO2	S	S	19.8	13	-	-
UC96-02	S	S	12.2	7	+	-
UC96-03	R	R	16.4	0	+	+
UC96-05	S	S	3.2	3	-	+
UC96-07	S	S	15.8	2	+	-
UC96-08	S	S	18.1	6	-	+
UC96-10	R	S	13.4	2	+	-
UC96-11	R	R	18.8	0	NA	NA
UC96-12	S	S	13	11	-	-
UC96-17	S	S	16.5	1	-	-
UC96-19	S	S	16.1	5	-	-
UC96-20	R	R	17.6	0	+	-
UC96-23	R	R	16.9	0	-	-
UC96-24	R	R	20.1	0	+	+
UC96-25	R	NA	-	0	+	-
UC96-30	R	NA	-	0	-	+
UC96-32	R	R	19.9	0	+	+
UC96-33	R	R	19.0	0	+	+
UC96-36	S	S	16.0	1	-	+
UC96-37	S	S	12.1	1	-	+
UC96-38	R	S	14.2	10	+	+
UC96-39	S	S	-	-	-	-
UC96-44	S	R	16.0	0	-	-
UC96-46	S	S	17.3	4	-	+
UC96-47	S	S	21.2	3	+	+
UC96-48	R	S	16.1	2	+	+
UC96-50	S	S	13.2	12	-	+
UC96-52	S	S	16.6	1	-	-
UC96-56	R	R	18.9	0	+	+
UC96-60	R	R	16.9	0	-	+

R: Resistant, S: Susceptible, +: Presence of marker, -: absence of marker or product, NA: Not applicable

Table 1. Continued

	Phenotypic reaction				Genotypic reaction	
Genotypes	Pot Test	Field Trial	100-seed weight/g	Striga Emergence/ Plot	SSR-1 Marker 150bp	C42-2B Marker 180 bp
UC96-64	S	S	20.9	3	+	+
UC96-73	S	S	-	2	-	-
UC96-76	S	S	20.2	1	-	-
UC96-77	R	R	17.8	0	+	+
UC96-80	R	R	15.1	0	+	+
UC96-85	S	S	14.7	4	-	+
UC96-86	R	R	14.6	0	+	+
UC96-99	S	S	14.6	1	-	-
UC96-113	R	S	16.5	2	+	+
UC96-122	R	R	16.7	0	+	+
UC96-128	R	R	14.1	0	+	+
UC96-139	R	R	15.6	0	+	+
UC96-141	R	R	16.5	0	+	+
UC96-144	R	R	-	0	+	+
UC96-145	S	S	10.9	3	-	-
UC96-148	R	R	16.3	0	+	+
UC96-151	R	R	16.6	0	+	+
UC96-153	R	R	17.7	0	+	+
UC96-154	R	R	21.4	0	+	+
UC96-168	R	R	16.9	0	-	+
UC96-171	S	S	18.4	4	-	+
UC96-173	R	R	16.2	0	+	+
UC96-174	S	S	-	-	-	-
UC96-177	S	S	18.4	2	-	+
UC96-178	R	R	16.5	0	-	+
UC96-186	R	R	-	0	-	-
UC96-189	S	S	-	2	+	+
UC96-191	R	S	16.8	4	-	+
UC96-194	R	R	16.0	0	+	+
UC96-198	R	R	15.9	0	+	+
UC96-199	R	R	16.6	0	+	+
UC96-200	R	R	16.5	0	+	-

R: Resistant, S: Susceptible, +: Presence of marker, -: absence of marker or product

Table 1. Continued

Genotypes	Phenotypic reaction				Genotypic reaction	
	Pot Test	Field Trial	100-seed weight/g	Striga Emergence/ Plot	SSR-1 Marker 150bp	C42-2B Marker 180bp
UC96-204	R	R	17.9	0	+	-
UC96-206	R	S	14.1	2	+	+
UC96-209	R	S	16.0	3	+	+
UC96-211	S	S	14.0	5	-	-
UC96-212	R	R	13.1	0	+	+
UC96-216	S	S	7.3	4	-	-
UC96-221	R	R	16.9	0	+	+
UC96-222	R	R	16.4	0	+	+
UC96-223	R	R	15.8	0	+	+
UC96-226	R	R	23.0	0	+	+
UC96-227	R	R	16.4	0	+	+
UC96-229	R	S	16.0	1	+	+
UC96-231	R	R	16.6	0	+	+
UC96-236	R	R	17.1	0	+	+
UC96-239	R	R	18	0	+	+
UC96-241	R	R	21.3	0	+	+
UC96-242	R	R	13.9	0	+	+
UC96-243	R	S	13.7	1	+	+
UC96-244	R	R	-	0	+	+
UC96-247	R	R	17.5	0	+	+
UC96-253	R	R	15.3	0	+	+
UC96-264	S	S	10.0	3	-	+
UC96-270	R	R	-	0	+	+
UC96-274	R	S	-	2	+	+
UC96-275	R	R	18.1	0	+	+
UC96-276	S	S	13.9	2	NA	NA
UC96-288	R	R	15.8	0	+	+

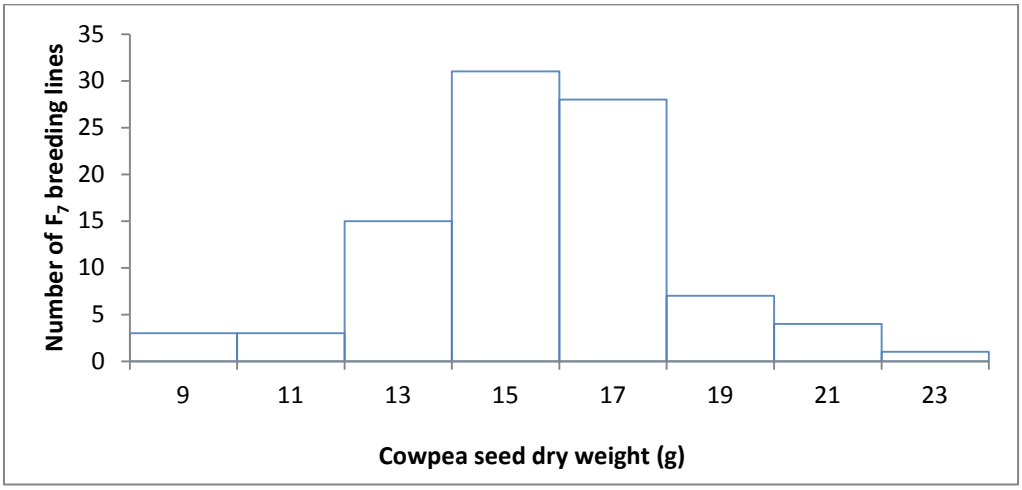
R: Resistant, S: Susceptible, +: Presence of marker, -: absence of marker or product, NA: Not applicable

Table 1. Continued

Genotypes	Phenotypic reaction				Genotypic reaction	
	Pot Test	Field Trial	100-seed weight/g	Striga Emergence/ Plot	SSR-1 Marker 150bp	C42-2B Marker 180bp
UC96-290	S	S	17.6	3	-	+
UC96-292	R	R	13.5	0	+	+
UC96-318	R	R	17.2	0	+	+
UC96-321	R	R	16.2	0	+	+
UC96-323	R	R	17.8	0	+	+
UC96-328	R	R	15.6	0	+	+
UC96-329	R	NA	-	-	+	+
UC96-333	R	S	14.6	1	-	+
UC96-346	R	R	18	0	+	+
UC96-352	R	NA	-	-	+	+
UC96-353	S	S	16.2	1	-	+
UC96-357	R	S	13.9	1	-	+
GH3684	R	R	13.2	0	+	+

R: Resistant, S: Susceptible, +: Presence of marker, -: absence of marker or product, NA: Not applicable

Fig.2. Variation in 100-seed dry weight of F_7 recombinant inbred lines of cowpea from a cross of IT97K-499-35 × SARC-LO2



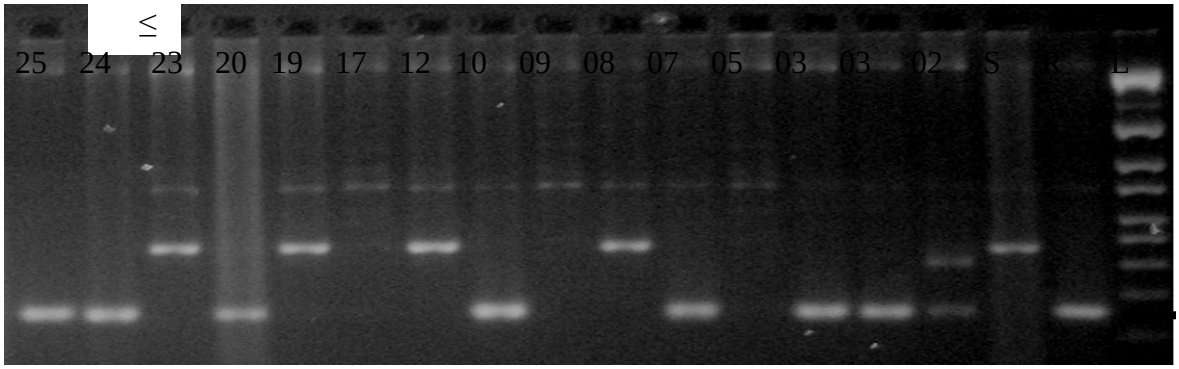
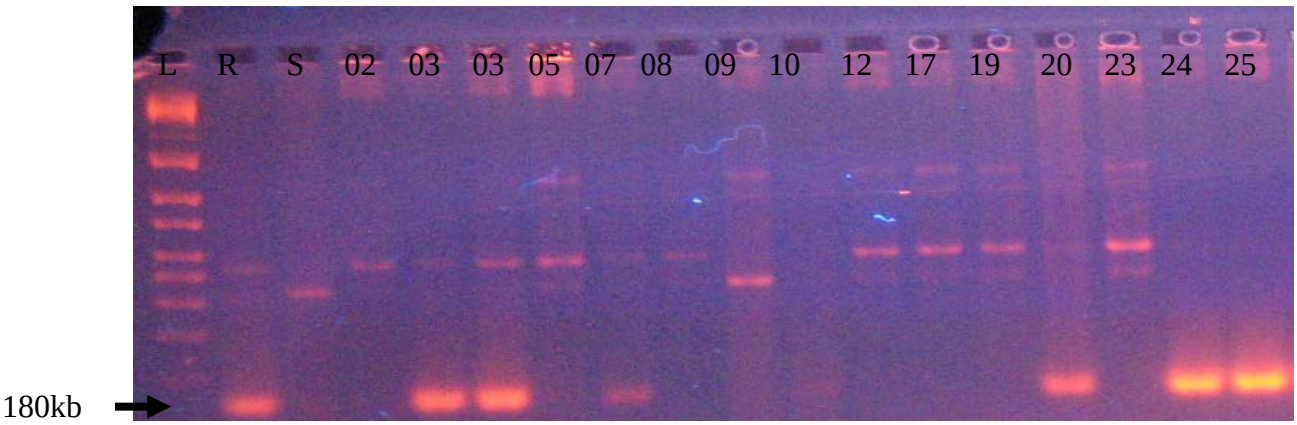


Plate 1: DNA bands from PCR amplification products of SSR-1 for some F_7 RILs of cowpea (derived from IT97K-499-35 × SARC-LO2) resolved in 2 % Agarose gel stained with ethidium bromide. The presence of 150bp band indicates resistant genotype and absence of this band indicate susceptible genotype. L represents the standard 1kb ladder.



Plates 2: DNA bands obtained from PCR amplification products of the SCAR primer C42-2B among some F_7 RILs derived from IT97K-499-35 × SARC-LO2 resolved in 2 % Agarose gel stained with ethidium bromide. Resistant lines have the 180bp band. L represents the standard 1kb ladder

Discussion

Significant effort has been made to identify natural sources of genetic resistance within cowpea and to select and breed for improved lines with resistance to *S. gesnerioides* (Singh and Emechebe, 1997; Singh *et al*., 2002). However, the use of most resistant varieties is limited due to concerns about the potential adaptability and small or medium seed size as found in variety IT97K-

499-35 (Omoigui, *et al*., 2007). IT97K-499-35 is a derivative from B301, local landrace from Botswana, which produces small seeds but is a multi-race resistant genotype to both *S. gesnerioides* and *Alectra vogelii* (Singh, 2002). Earlier inheritance studies indicated that the nature of resistance to *S. gesnerioides* races SG1, SG2, SG3 and SG4 in some cowpea genotypes is monogenic dominant (Singh and Emechebe, 1990; Atokple *et al*., 1993; Moore *et al*., 1995).The observed segregation ratio of 1R:1S in the present study is what is expected in a RIL population for a trait controlled by monogenic inheritance.. .

The SSR-1 and C42-2B primers distinguished between resistant and susceptible cowpea genotypes with different discriminating power. Indeed, the SSR-1 and C42-2B markers were found to co-segregate with *S. gesnerioides* race 3 or SG3 resistance gene (Li and Timko 2009; Omoigui, *et al*., 2009). Both primers identified resistant cowpeas by amplification of the band in only resistant genotypes. According to Omoigui *et al*. (2009) C42B-2B identified resistant lines with a single band while the susceptible lines had no band. In the current study, the 150bp SSR-1 marker was more efficient at 92.6% discriminating ability compared to that of C42-2B (85.7%). The implication is that SSR-1 might be closer to the *S. gesnerioides* race specific-resistant gene (SG3) than C42-2B as applied to the unknown race of *S. gesnerioides* in Ghana from the Upper East Region.

Crop yield losses due to stress imposed by *S. gesnerioides* can ranged from 83 to 100% (Aggarwal and Ouédraogo, 1989; Alonge *et al*., 2005; Cardwell and Lane, 1995; Emechebe *et al*., 1991) depending on the extent of damage and level of infestation. The observed stunted growth, leaf necrosis, chlorosis, senescence, defoliation, reduced size of young leaves, poor flowering and poor pod formation as well as poor rooting and nodulation from pot culture to field trial emphasized the devastating effects of *Striga* parasitism on the crop. The significantly ($P \leq 0.05$) low average 100-seed dry weight (15.2g) among susceptible genotypes compared to that of resistant genotypes (16.6g) could be due to the parasite-induced damages giving rise to yield loss. The decrease in seed weight might have resulted from reduced seed size and/or a direct effect of reduction in photosynthesis and translocation of photosynthates to sink due to *Striga* stress. Competition between parasite and host for solutes and water coupled with lower rate of photosynthesis in the leaves may retard root and shoot growth and, consequently, yield. The fact that dry seed weight of the progenies demonstrated a seemingly continuous variation, which closely conform to the normal distribution curve, suggest the existence of potential high yielding genotypes for cultivation in both *Striga* prone and non-*Striga* prone areas.

The current field trial involving F_7 progenies attracted low emergence of *S. gesnerioides* per plot (1-13) compared to data obtained for other studies (Carsky *et al*., 2003; Kamara *et al*., 2008). The resultant low parasite emergence might have been influenced by poor rainfall during the trial or low density of seeds present in the soil. However, the identification of susceptible and resistant RILs conformed to the selection procedure by Singh and Emebeche (1990) and confirmed with the presence or absence of distinct markers associated with *S. gesnerioides* resistance. The combined conventional and DNA marker technology used facilitated selection of 59.2% genotypes of the RILs with the same resistance traits as the parent IT97K-499-35 which compared well with the local resistant check (GH3684) in both pot and field assessments.

Conclusions

The segregation ratio of resistance to susceptible cowpeas of 1:1 observed among the F_6 progenies indicates that inheritance of resistance to the race of Striga in Ghana is monogenic. Resistant genotypes were identified with the presence of single distinct DNA bands of 150bp and 180bp for SSR-1 and C42-2B, respectively, which were absent in susceptible genotypes. SSR-1 was more efficient (92.6%) than C42-2B (85.7%) in discriminating between resistant and susceptible genotypes. The small number of crossover events between the markers and the gene indicates reliability of these markers in improving cowpeas for Striga resistance in Ghana. The stress imposed by *S. gesnerioides* infection resulted in significant ($P \leq 0.05$) yield reduction compared to resistant genotypes due to reduced vegetative and reproductive growth. The resistant RILs identified in the current study would have to be further evaluated for release to farmers to cultivate.

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