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Suitability and use of two molecular markers to track race-specific resistance *striga gesnerioides* in cowpea (*Vigna unguiculata* (L.) Walp.)

L. O. Omoigui[1]*, M. F. Ishiyaku[2], B. S. Gowda[3], A.Y. Kamara[4] and M. P. Timko[3]

[1]Department of Plant Breeding and Seed Science, College of Agronomy, University of Agriculture, Makurdi, Nigeria.
[2]Department of Plant Science, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Biology, University of Virginia, Charlottesville, VA 22904, USA.
[4]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

The obligate root parasitic weed *Striga gesnerioides* poses a severe constraint to cowpea productivity in the dry savannahs of West and Central Africa, where cowpea is a major crop. At least seven races of *S. gesnerioides* have been identified within the cowpea-growing regions of West and Central Africa, based on host differential response and genetic diversity analysis. Molecular markers linked to resistance to different races of *S. gesneriodes* have been identified. It was desirable to demonstrate the applicability and efficiency for use in marker-assisted selection (MAS) to fast-track the development of cowpea for resistance to *S. gesnerioides.* The objective of the study was to determine the suitability of two molecular markers in tracking race-specific *S. gesnerioides* resistance in cowpea (SG3), the predominant race found in Nigeria. F_2 mapping populations and recombinant inbred lines (RILs) derived from the cross involving IT97K-499-35 and a susceptible local landrace (Borno Brown), and another resistant parent B301 with the same susceptible land race (Borno Brown) were assayed using two linked markers. Genetic analysis showed that resistance to *S. gesnerioides* in cowpea is qualitatively inherited with single dominant gene action. Two SCAR markers, 61RM2 and C42-2B were validated in the same F_2 populations and subsequent recombinant inbred lines (RILs). The two markers were able to discriminate between resistance and susceptibility and the genotypic score was quite similar to the phenotypic score with the markers score showing greater efficiency in selection than phenotypic score. The 61RM2 had two bands in resistant cultivars and amplified a ~450 bp fragment with marker efficiency of 98% while C42-2B amplified a single ~250 bp fragment with marker efficiency of 96% in resistant cultivars and absent in susceptible cultivars. The genetic distance between 61RM2 and phenotypic score was 3.5 cM while that of C42-2B and phenotypic score was 8.5 cM. The two marker data set were significantly correlated with the phenotypic data (r=0.95). Based on the tight linkage with the resistant locus, 61RM2 was found to be a utility marker to initiate MAS in cowpea breeding for resistance to *S. gesnerioides.*

Key words: Cowpea, *Striga*, molecular marker, genetic distance, race-specific, obligate parasitic weed, *Vigna unguiculata.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important grain legumes grown in tropical and subtropical regions of the world, primarily in sub-Saharan Africa (Singh, 2005; Timko et al., 2007; Ehlers and Hall, 1997).

The majority of cowpea is grown by poor farmers in West and Central Africa, where its grain is highly valued for food, and the fodder as source of animal feed (Langyintuo et al., 2003). Cowpea is a food legume of

significant economic importance worldwide with high protein and mineral content. It plays a critical role in the lives of millions of people in Africa and other parts of the developing world where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops high in carbohydrate (Lambot, 2002). In addition, cowpea fixes nitrogen symbiotically with root rhizobacteria and helps to restore soil fertility (Carsky et al., 2002; Sanginga et al., 2003).

Cowpea production is constrained by a wide range of biotic and abiotic factors. Among the major biotic constraints are the obligate root-parasitic weeds *Striga gesnerioides* and *Alectra vogelii* of the Orobanceae family. *S. gesnerioides*, in particular, causes extensive damage to cowpea in the Sudano-Sahelian belt of West and Central Africa (Parker, 2009) where its damaging effects are compounded by drought (Obilana, 1987). Successful parasite establishment creates a strong sink for nutrients to the detriment of the host, leading to drastic growth reduction (Keyes et al., 2001; Joe et al., 2006). Yield losses range from 83 to 100% in severely infested fields (Emechebe et al., 1997; Omoigui et al., 2011). Farmers with crop fields severely infested with *Striga* often resort to abandoning their fields, contributing to an already severe non availability of farm lands. In northeast Nigeria, where cowpea is the most important legume crop, Dugje et al. (2006) reported that more than 97% of cowpea fields in the dry savannas were infested with *S. gesnerioides,* leading to serious crop losses. Therefore, the rapid spread of this parasitic weed to new regions would constitute a severe threat to cowpea production in those areas, and the virulence of the different races of *S. gesnerioides* further compounds the problem. The damage to host is already done before the *S. gesnerioides* shoots emerge from the soil. Control of *S. gesnerioides* is difficult to achieve due to the number of seeds of the parasite and their viability in the soil for over 20 years (Ouedraogo et al., 2012). The *Striga* seed germinate in response to the specific stimulants exuded by the host's roots (Worshmam, 1987). Several methods are available for the control of *Striga* in cowpea. However, the use of resistant cultivars is considered the most practical, sustainable and effective method to control the parasite. The lack of broad or horizontal resistance, however, is one of the biggest problems when trying to develop resistant cultivars across different races. Cowpea cultivar with complete resistance to *Striga* stimulates germination and permit attachment of *Striga* radicles to their root but the haustorium development is inhibited. This mechanism involves the plant recognizing parasite virulence effectors, usually through intracellular resistance proteins (R-proteins), causing effector-triggered immunity (ETI). ETI corresponds to what is classically referred to as gene-for gene, vertical or race-specific resistance (Flor, 1955; Dodds and Rathyen, 2010). Resistance to *Striga* generally follows a qualitative mode of inheritance where resistant and susceptible reactions are clearly differentiated

Recently, there are increasing interests in studies aiming at the molecular characterization of the plant-parasitic weed interaction and its resistance through expression analysis of genes, proteins and metabolites involved in these processes (Dos Santos et al., 2003; Castillejo et al., 2004). The availability of molecular markers tightly linked to *S. gesnerioides* resistance genes opens up the possibility of applying Marker-Assisted Selection (MAS) to cowpea breeding. To-date, limited information is available on large scale implementation of marker assisted selection (MAS) in cowpea breeding programs. Heritable sources of resistance in cowpea to both *S. gesnerioides* and *A. vogelii* have been reported (Timko and Singh, 2008). However, most of these resistant lines have poor agronomic characteristics and therefore, their direct use is limited. These germplasm are being used as donor parent to introgress resistant gene(s) into local adapted cowpea cultivars, but the delivery of improved varieties to the farmers is slow. Among the limitations to successful development of improved *Striga*-resistant cowpea is the fact that *S. gesnerioides* is variable in its parasitic abilities, showing both host and cultivars-specific selectivity. At least seven distinct races of *S. gesnerioides* (designated SG1 through SG7) have been identified throughout West Africa (Lane et al., 1997a, b; Botanga and Timko, 2006). Most cowpea plants are susceptible to *Striga* parasitism, although some local landraces have been identified that show resistance to one or more of the known races (Timko et al., 2007), with resistance being conferred by a single dominant gene (Aggarwal et al., 1984; Atopkle et al., 1995). Vos et al. (1995) and others have been able to map several of the race-specific resistance genes to two linkage groups on the cowpea genome via the application of amplified fragment length polymorphism (AFLP) markers (Ouedraogo et al., 2001, 2002; Boukar et al., 2004). The *S. gesnerioides* race SG1 and SG3 resistance genes Rsg2-1, Rsg1-1 and Rsg4-3, present in the resistant cowpea lines B301, IT82D-849 and TVu14676, respectively, were mapped to LG1. Whereas, the *S. gesnerioides* race SG1 resistance genes Rsg3-1 and Rsg2-1 present in Suvita-2 (Gorom local) and IT81D-994, respectively, were mapped to LG6 (Ouedraogo et al., 2001, 2002). One of the *Striga* resistance genes, RSG3-B301 has been cloned and shown to be effective only to SG3 race (Li and Timko, 2009).

Over the years, significant progress has been made by national and international centres toward developing

*Corresponding author. E-mail: luckyomoigui@gmail.com.

Striga-resistant lines in different breeding programs, but the constraint of pyramiding these resistant genes still lingers. To alleviate these constraints and for other reasons (for example, speeding breeding efforts, possibility of identifying other races of the parasite's development), MAS has been proposed as an alternative solution for pyramiding resistance genes (Haley et al., 1994; Ouedraogo et al., 2001). Several molecular marker technologies have been exploited for MAS. Amplified fragment length polymorphism (Vos et al., 1995), combined with bulked segregant analysis (BSA) (Michelmore et al., 1991), have been used to discover markers closely associated with economically important traits in many crop species including cowpea.

Studies conducted by Ouedraogo et al. (2001) using these techniques (AFLP and BSA) identified three markers tightly linked to the resistance gene Rsg2, effective against S. gesnerioides race 1 from Burkina Faso, and present in IT82D-849; and six AFLP markers associated with the resistance gene Rsg4, effective against S. gesnerioides race 3 from Nigeria, and present in TVu 14676. Two of the markers, E-AAC/M-CAA300 and E-ACA/M-CAT150, were linked to Rsg2 and Rsg4, respectively. One of the AFLP markers has been converted into a SCAR marker, 61R and an improved SCAR 61RM2 (Ouedraogo et al., 2012). Both were also reported to be linked to race 3 resistances. These two markers were dominant markers with wider applications. However, Boukar et al. (2004) also reported a SCAR marker for race 3 resistance that is co-dominant in nature.

Even though a single gene controlling resistance has been identified in the parasite, the transfer of the gene and genes pyramiding through marker assisted backcrossing (MAB) is the most effective and efficient way to develop stable and durable Striga resistant cultivars. The identification of markers for major gene in one segregating population does not mean that the same marker work well for similar genes in other segregating populations. Such findings represent an important advance in the genetic analysis of the character. The recent development of tightly linked markers in cowpea for resistance to Striga provides the opportunity to initiate molecular study to this important trait. As a contribution to the development and implementation of MAS approaches, our study was to investigate the efficiency of one SCAR marker and another gene specific marker that are tightly linked to the S. gesnerioides SG3 and SG5 resistance, respectively, in discriminating between resistance and susceptible individuals in genetic populations produced from a cross between improved and local cultivar.

MATERIALS AND METHODS

Plant materials, development of advanced populations, and phenotypic screening for Striga resistance

Seeds of the cowpea genotypes used in this study were obtained from the International Institute of Tropical Agriculture (IITA), Kano Station, Nigeria. The Striga resistant lines B301 and IT97K-499-35 have been previously described (Singh et al., 2006).

Development of genetic populations

The populations used for this study were developed from the cross Borno Brown x IT97K-499-35 and Borno Brown x B301. The resulting F_1 plants were allowed to self-pollinate yielding two F_2 populations. The backcross populations were also developed from the respective crosses. The F_2 seeds were planted in 13 cm diameter pots containing about 1 L unsterilized sieved sand and top soil (sandy loam) mixture (1:1 vol/vol) previously inoculated uniformly with about 2000 S. gesnerioides seeds as described (Singh and Emechebe, 1990; Atopkle et al., 1995). Two hundred F_2 and the corresponding F_1 and two parents were planted in plastic pot. At four to five weeks after planting (WAP), the pots were scored for day of first Striga emergence and scoring continued on a daily basis until termination of the experiment at 75 days after planting. Striga shoot count was done at 7, 8, 9 and 10 (WAP). After the last Striga shoot count at 10 weeks, the soil was washed off the plant roots after submerging each pot in a 20 L bucket of water for about 5 min. The roots of each plant that had entangled were gently separated from the other and carefully freed from any remaining soil. The cowpea plant root was examined closely for Striga attachment. Plants allowing attachment, haustoria development, and emergence of Striga were categorized as susceptible. Those without any attachment and free of infection were categorized as resistant.

Progeny testing

The resistant F_2 plants were further classified into homozygotes and heterozygotes by a progeny testing. For this analysis, 16 individuals from each of 30 randomly selected F_3 families were rescreened for Striga resistance.

DNA extraction

Young leaves from two weeks old plants were collected from clearly labeled plants. A total of 100 F_2 plants from each cross were sampled. Genomic DNA was isolated from each plant on FTA paper matrix and processed as previously described by Omoigui et al. (2012).

PCR amplification of genomic DNA

To amplify regions of genomic DNA, 25 µl of PCR mixture was added to a tube containing a processed 1.2 mm FTA disc in 0.2 ml PCR tube. 25 µl PCR ready mix (Sigma-Aldrich) had18 µl of sterilized water, 2.5 µl of dNTPs mix, 2.5 µl 10 x PCR buffer, 0.05 µl of Taq DNA polymerase and 1 µl each of the forward and reverse primers (synthesized by Integrated DNA Technologies, Coralville, IA). Each of the DNA samples was amplified using two different primer pairs, 61RM2 (*M2F: 5'*-gatttgtttggtttccttaag-3'; M2R: 5'-ggttgatcttggaggcatttt-3') and C42-2B (C42-2BF: 5'-cagttccctaatggacaacc-3'; C42-2BR: 5'-caagctcatcatcatctcgatg-3'). 61RM2 primer is a modification of the 61R marker linked to S. gesnerioides (Ouedraogo et al 2012); C42-2B is a primer developed by Gowda, BS and Timko MP (Manuscript in Preparation).

Amplification was performed in a heated lid thermal cycler programmed at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57.5°C for 30 s, and

extension at 72°C for 1 min followed by final extension of 10 min at 72°C to ensure completion of the final amplification products. For C42-2B marker, a similar procedure was followed but the annealing temperature used was 67.5°C. The F_3 families were successfully characterized for resistance to *S. gesnerioides* using the BIONER AccuPower PCR premix.

Gel electrophoresis of PCR products

After performing the PCR, 20 µl of each of the PCR products were electrophoresed on a 2% agarose gel pre-stained with ethidium bromide as previously described (Omoigui et al., 2012). Representative gels were photo-documented and the presence and absence of the polymorphic bands associated with 61RM2 and C42-2B were scored, and these data, along with phenotypic segregation, were used for linkage analysis.

Genetic analysis

Goodness-of-fit of observed to expected segregation ratios was tested by chi-square.

Marker analysis

Linkage analysis between either of the markers 61RM2/C42-2B to the phenotypic score was performed using the computer-aided program Joint MAPMAKER/EXP version 3.0 (Lander et al., 1987) and the Kosambi's mapping function (Kosambi, 1943) for correcting the recombination values to cM distances.

The genetic distance matrices test of the markers to each other was computed using NTSYSpc (Exeter Software, Setauket, New York, USA) as follows:

$$D = \frac{1 - N_{xy}}{(N_x + N_y - N_{xy})}$$

Where, D_{xy} = the genetic distance between marker "x" and marker "y"; N_{xy} = the number of bands shared by marker "x" and marker "y"; N_x = the number of bands in marker "x"; N_y = the number of bands in marker "y".

RESULTS

Phenotypic data

Emergence of *Striga* on susceptible plants began at 29 days after planting (DAP). At about 70 DAP, several *Striga* plants had emerged in those pots containing susceptible plants and the differences in level of susceptibility was quite clear. *Striga* emergence was delayed up to 75 days in some pots involving the cross, Borno Brown x IT97K-499-35. Symptoms of infection such as leaf chlorosis, stunted growth, and partial defoliation were quite visible even before the parasite emerged above soil level. Some plants developed symptoms, but *Striga* did not emerge from the soil. Some of the highly susceptible plants that had emerged *Striga* at 29 days after planting died before reaching the reproductive phase. Classification of individual plants into

resistant and susceptible groups on the basis of emerged *Striga* was complicated because some pots had un-emerged *Striga* but the plant showed *Striga* symptom. Thus, the segregation ratios were ascertained only after plant roots were carefully washed off soil and the parasite's attachment had been observed before classifying cowpea plants as resistant or susceptible. The differences between plants were clear and easily noticed so that a resistant plant would be completely free of attached *Striga* while a heavily *Striga* attached plants were termed susceptible.

Striga infestation on plants derived from cross Borno Brown x IT97K-499-35 showed that the number of attached *Striga*, including those which emerged, ranged from 1-6 *Striga* on susceptible individual plant. Resistance to *Striga* in the resistant parents 'B301 and IT97K-499-35' was characterized by a lack of attachment of the parasite to the roots. This result confirmed that both cultivars exhibited vertical resistance as they were completely resistant to *Striga*. A total of 60 plants of each of Borno Brown were screened in the study, all of which were heavily attacked by *Striga*. All the 16 F_1 hybrid plants derived from the cross had neither emerged *Striga* nor haustoria's attachment. All the plants were as resistant as their resistant parent (IT97K-499-35) indicating the complete dominance of resistance over susceptibility.

Segregation in the F_2 population yielded 231 resistant plants (no *Striga* emergence or attachment) and 69 susceptible plants with either fully emerged *Striga* or with Tubercle attachment or both (Table1). The observed segregation ratio of resistant versus susceptible in this cross fits closely to a 3:1 (resistant: susceptible) expected ratio ($\chi^2 = 0.65$; p = 0.05), thus, indicating the inheritance of resistance by a single dominant gene conferring resistance to *S. gesnerioides.*

The segregation for *Striga* in the cross derived from Borno Brown × B301 followed the same pattern conformed with the 3:1 R:S segregation ratio. The 20 plants of the susceptible parental line (Borno Brown) were severely infested with *Striga;* most of the plants died before reaching reproductive stage due to severe attack by the parasite. All the 16 F_1 hybrid plants derived from the cross had neither emerged *Striga* nor haustorial attachment. All these plants were as resistant as their resistant parent (B301) indicating the complete dominance of resistance over susceptibility. Segregation in the F_2 yielded 217 resistant plants (no *Striga* mergence or attachment) and 83 susceptible plants with either fully emerged *Striga* or with haustoria attachment or both. The number of *Striga* count including those with haustoria's attachment and emerged *Striga* on susceptible plants ranged from 1 to 10. The observed segregation ratio of resistant versus susceptible plants in this cross fits closely to a 3:1 (resistant: susceptible) expected ratio ($\chi^2 = 1.137$; p = 0.05), thus, indicating the inheritance of resistance by a single dominant gene.

Table 1. Segregation analysis of Striga resistance in a cross between Borno Brown x IT97K-499-35

Population	Generation	Total no. of plants	No. of plants		Genetic ratio	X-value	Pr>ChisSq
			Resistant	Susceptible			
Borno Brown	Parent 1	20	1	19			
IT97K-499-35	Parent 2	20	20	0			
Borno Brown x IT97K-499-45	F1	16	16	0			
Borno Brown x IT97K-499-45	F2	300	231	69	3:1	0.65	0.64

The F_3 progeny testing of 30 families randomly selected from seeds of F_2 resistant plants showed that 10 families bred true for *Striga* resistance while 20 families segregated for resistance and susceptibility. The genetic analyses conformed to the 1: 2 non-segregating: segregating families (p= 0.999) (Tables 2 and 3). This further confirmed the inheritance of resistance by a single dominant gene for the cross. The 20 families segregating for *Striga* yielded 140 resistant plants and 53 susceptible plants which fits closely to a 3:1 ratio (χ^2 = 0.623; p = 0.42). This analysis further confirmed that single dominant gene confers resistance to *S. gesnerioides* in IT97K-499-35 and B301.

Marker analysis

The two primers used were very informative as they showed polymorphism between the parents and were subsequently used to screen the individual F_2 plants of the two segregating populations. The two markers (61RM2 and C42-2B) analysis, conducted on the F_2 populations derived from the crosses Borno Brown x IT97K-499-35 and Borno Brown x B301, showed that the bands were clearly readable for the different markers used. The result of the analysis of marker segregation in the cross derived from Borno Brown x IT97K-499-35 is presented in Figure 1.

The electrophoresis of the PCR product generated polymorphic bands that were highly reproducible and scoreable. Of the 100 F_2 individual plants screened with 61RM2, amplification of product was detected in both susceptible and resistant lines. In the resistant lines, two bands appeared consistently, which is a characteristic of 61RM2 dimorphic marker (Figure 1). The 61RM2 marker amplified a 450 bp fragment similar to that reported in mapping population for *Striga* race 3. 61RM2 which has a characteristic dimorphic banding pattern with one band similar in both the resistant and susceptible genotypes and a lower fragment that was polymorphic, being present only in resistant genotypes but absent in susceptible genotypes. The inability of the marker to differentiate between homozygotes and heterozygotes resistance indicates the dominant nature of the marker (Figure 1).

On the other hand, the primer C42-2B has a monomor-

phic banding pattern and identifies resistant lines with a definite single band while susceptible line had no band (Figure 2). In the screening of the parental cultivars with 61MR2 marker, all the 12 plants of the susceptible parent (Borno brown) showed a single band while those of IT97K-499-35 showed double bands similar to that observed in the segregating F_2 resistant lines for 61RM2. As it was observed with the phenotypic score, all the F_1 plant DNA samples were also resistant using the markers. Scoring of the F_2 segregation shows that of the 100 plant DNA analyzed, 81 of them showed double bands while 19 plants showed single band which closely fit the expected genetic ratio of 3:1. The phenotypic score data did not significantly deviate from the expectation of random segregation when compared with the marker score 61RM2 tagging SG3 resistance gene.

Percent co-segregation of the marker between susceptible and resistant relative to the phenotypic data was found to be 98% with less than 2% recombinants. The genetic distance of the marker using Joint MAPMARKER was found to be 3.4 cM from the resistance gene indicating a tight linkage. However, the genetic distance from the phenotype score was found to be 5.9 cM (Figure 3). The markers were also used to validate an F_2 population derived from the resistant parent B301 crossed to a susceptible parent Borno Brown, and similar band patterns to that of the first population was observed. The electrophoresis of the PCR product generated polymorphic bands that were reproducible and scorable. A double band was present in resistant lines while a single band was showed by the susceptible line for 61RM2.

In the resistant lines, two bands appeared consistently, which is a characteristic of a dimorphic marker. The presence of a double band indicates resistance while presence of a single band indicates susceptibility. All the susceptible plants showed the 500 bp amplification product.

The resistant plants showed 450 bp band in addition to 500-bp band (homozygous or heterozygous). Linkage analysis performed by Mapmarker confirmed the association of these markers with the resistant gene. Their flanking status was maintained and their distance from the resistance gene were 3.4 and 8.7 cM for the markers 61RM2 and C42-2B, respectively, which were similar to that obtained from the first population (Figure 4).

Table 2. Marker segregation analysis of F_2 and F_3 progeny testing of the population derived from the cross Borno Brown × IT97K-499-35.

| Population | Generation | Total number of plants | Number of plants | | Genetic ratio | χ^2-value | Pr>ChiSq |
			Resistant	Susceptible			
Borno Brown	Parent 1	20	0	20			
IT97K-499-35	Parent 2	20	20	0			
Borno Brown x IT97K-499-35	F_1	16	16	0			
Borno Brown x IT97K-499-35	F_2	100	82	18	3:1	2.61	0.10
Borno Brown x IT97K-499-35	F_3	30 families	10 homozygote	20 heterozygote	1:2	0.00	0.999
Non-segregating family =10		88	87	1	1:0		
Segregating family=19 =20		193	140	53	3:1	0.623	0.42

Table 3. Marker segregation analysis of F_2 and F_3 progeny testing of the population derived from the cross Borno Brown × B301.

| Population | Generation | Total number of plants | Number of plants | | Genetic ratio | χ^2-value | Pr>Chi Sq |
			Resistant	Susceptible			
Borno Brown	Parent 1	20	1	19			
B301	Parent 2	20	20	0			
Borno Brown x B301	F_1	16	16	0			
Borno Brown x B301	F_2	100	78	22	3:1	0.48	0.49
Borno Brown x B301	F_3	30 families	11 homozygote	19 heterozygote	1:2	0.1503	0.69
Non-segregating family =11		108	107	1	1:0		
Segregating family=19		183	134	49	3:1	0.307	0.57

Progeny testing with DNA marker

The identification of heterozygous F_2 plants was done through their F_3 progeny testing by growing 30 families obtained from F_2 plants carrying the dominant allele for *Striga*. Of the 30 families analyzed, 10 families breed true for *Striga* resistance while 20 families segregated for *Striga*. The proportions of the non-segregating to segregating families fit the theoretical 1:2 ratio as confirmed by chi-square test, with probability values of p=0.34.

Relative efficiency of 61RM2 and C42-2B in detecting SG3 race

These two primers generated polymorphisms that were linked to SG3 resistance in coupled phase. They only segregated with the dominant allele. Both markers are dominant markers. Comparison of the similarity values obtained with the two DNA markers used in the genetic populations gave highly significant correlations with the phenotypic score (Figure 3). This very strong correlation between the phenotype and the markers suggests

that a locus with a major influence on *Striga* was closely linked to 61RM2 on the linkage group. Thus, these markers, especially the 61RM2, can be used in screening segregating populations for *Striga* resistance instead of the phenotypic screening that is laborious and environmentally influenced. C42-2B similarity values (Table 4) correlated with those obtained using phenotype in the different population but these correlations should be interpreted carefully due to the low number of amplification products obtained with C42-2B and the discrepancies observed in the F_2
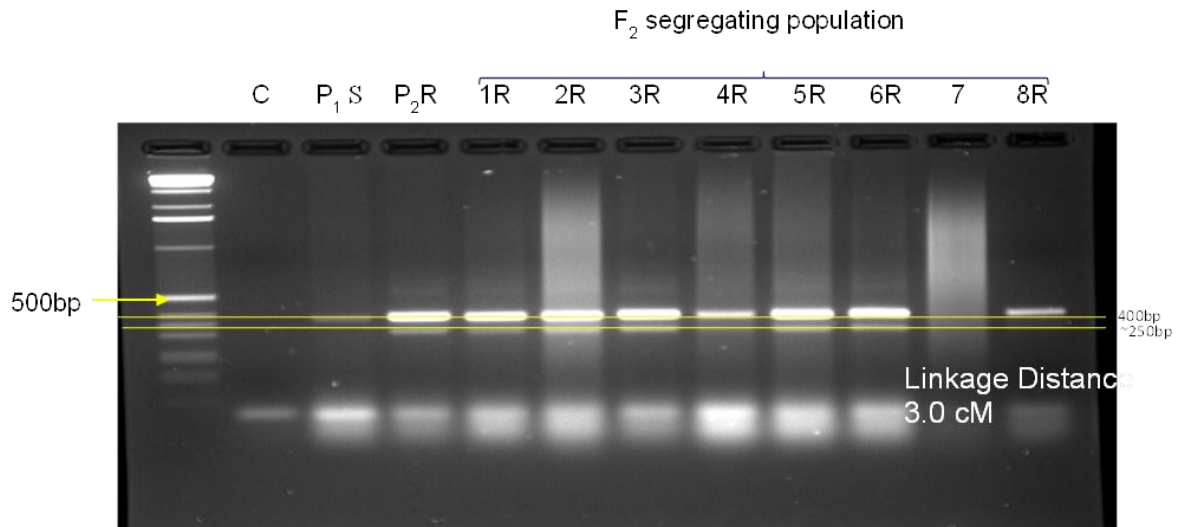
F$_2$ segregating population

Figure 1. A 2% agarose gel electrophoretic analysis of PCR amplified product using 61RM2 marker for F$_2$ progenies derived from Borno brown x IT99K-499-35. All F$_2$ lines are resistant except line 7 which is smeared. R and S indicate Resistant and Susceptible respectively. P$_2$, IT97K-499-35; P$_1$, Borno brown; C, Control without genomic DNA template; L, 100 bp ladder.
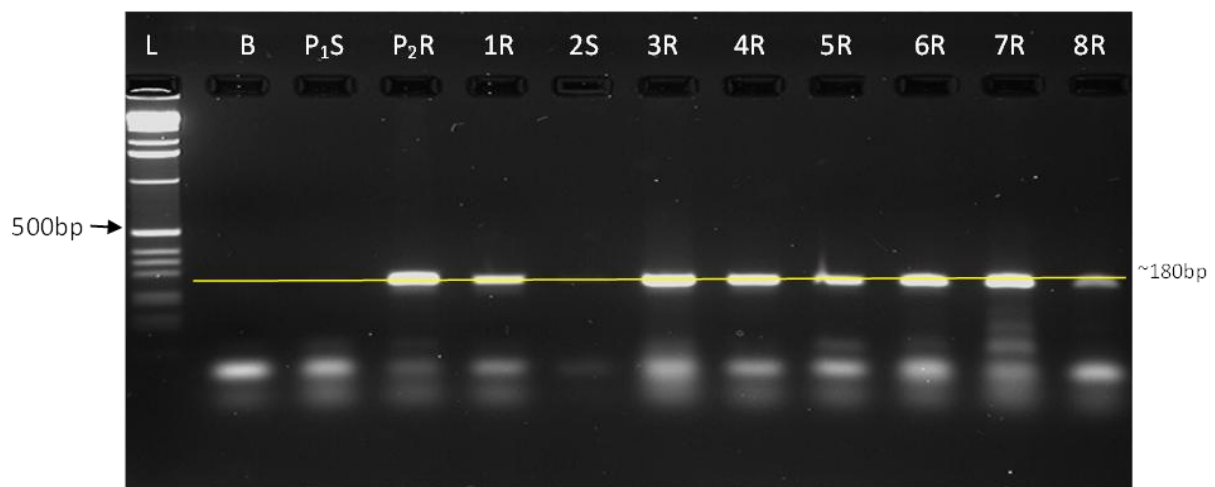

Figure 2. A 2% agarose gel electrophoretic analysis of PCR amplified product using C42-2B marker for the F$_2$ progenies derived from Borno brown x B301. All F$_2$ lines are resistant except line 2 which is susceptible as indicated by the absence of band. R and S indicate resistant and susceptible respectively. P$_2$, IT03K-338-1; P$_1$, Borno brown; C, Control without genomic DNA template, L, 100 bp ladder.

segregating population. For example, in some cases 61RM2 identified some lines as susceptible but phenotypic data and C42-2B marker analysis classified them as resistant. When the seeds of these lines were screened in the F$_3$ families, they were found to support many haustorial attachments. The linkage to the resistance gene in 61RM2 was found to be 3.4 cM while that of C42-2B was found to be 5.4 cM. 61RM2 showed consistent value of 99, 97, and 98%, respectively in

detecting SG3 resistance (Figure 3). On the other hand, C42-2B marker showed inconsistency in the different populations (98, 95, and 96%). Similarly 61RM2 had a relatively sharper product resolution and a tight linkage with the resistant gene locus compared with C42-2B. In this study, 61RM2 and C42-2B were found to be reliable markers for race 3 resistance. However, in IT97K-499-35, both markers are on the same side of the SG3 resistance gene, while in B301, the markers are (Figure 3) flanking
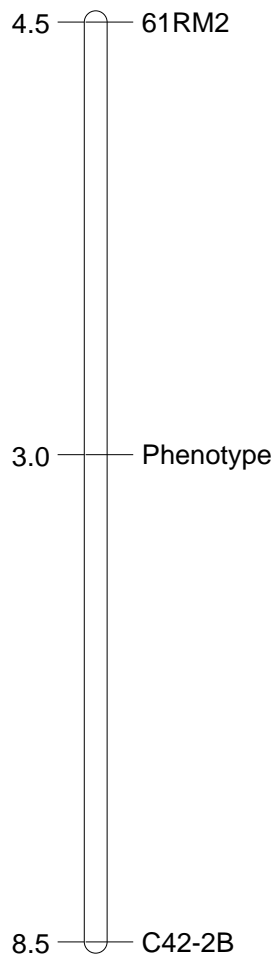
Figure 3. A genetic map generated using the Joint MAPMARKER from 100 F_2 lines showing the linkage relationships between markers for the cross Borno Brown x B301. Horizontal bars represent the map position of the markers and the names on the right side. Numbers on the left side indicate map distance between markers in centimorgans.

the SG3 resistance.

DISCUSSION

Reaction of parental lines to *Striga*

The results of the pot culture screening revealed that the cultivars B301 and IT97K-499-35 have resistance to the Prevalent race of *Striga* (SG3) from the northeast of Nigeria. Resistance to *Striga* of these lines was characterized by a lack of parasite emergence and attachment on the root. Also the F_1 hybrids resulting from crosses between these lines with the susceptible cultivar (Borno Brown) shows lack of parasite attachment which indicate the complete dominance of resistance over susceptible. These observations further confirm the

earlier report of Singh and Emechebe, (1991), Atokple et al. (1993) and Carsky et al. (2002). The local variety (Borno Brown) used in this study showed high levels of susceptibility to *Striga*, exemplified by several parasite attachments on the root with only one plant free of parasite attachment probably due to escapism.

Segregation analysis of the F_2 progenies of the two different genetic populations of susceptible x resistant cowpea crosses used in the present study showed that a single dominant gene conferred resistance to the *S. gesnerioides* collected from Maiduguri, Nigeria. The entire F_1 hybrids were resistant indicating a complete dominance of resistance over susceptibility. Segregation analysis fits expected genetic ratio of 3:1 confirming a monogenic dominant inheritance. This finding is consistent with the earlier work of Singh and Emechebe, (1990) and Atokple et al. (1993) who reported that a single dominant gene in cultivar B301 confers resistance to *S. gesnerioides* race 3 predominant in Nigeria.

The results obtained from the F_3 progeny testing further revealed that inheritance of *Striga* resistance in each of the population derived from Borno Brown x B301 and Borno Brown x IT97K-499-35 is controlled by a single dominant gene. Single dominant gene inheritance of resistance to *S. gesnerioides* in B301 has been earlier reported using similar populations derived from a cross involving B301 and another susceptible cultivar (Lane and Bailey, 1992; Atokple et al., 1995), and a gene symbol *Rsg1* (resistance to *S. gesnerioides*) was proposed for this trait. The result of the pot culture techniques was also confirmed using two molecular markers (61RM2 and C42-2B) earlier reported to be linked to *Striga* race 1 and 3 (Oudraogo et al., 2012 and Gowda et al., unpublished). The highly significant correlation between the phenotypic scoring and the markers indicates that the results of the phenotypic marker and genotypic marker are the same. The slight differences observed are well within the range expected from sampling, as shown by a relatively small deviation of the samples. There was a clear segregation pattern in the F_2 populations and the F_3 families. The F_2 populations segregated in the ratio 3:1 (resistant 75% and 25% susceptible), which suggest the inheritance of a single dominant gene for resistance. This was further confirmed in the F_3 progeny testing by growing 30 families randomly selected from F_2 plants which were resistant to *Striga* and have large seed size for each cross. The proportion of segregating to non-segregating F_3 families obtained fits the theoretical 1:2 ratio as confirmed by chi-square test, with probability values of p=0.999 and p=0.69 for the cross derived from Borno Brown x IT97k-499-35 and Borno Brown x B301 crosses, respectively. This result shows that selection for resistance in F_2 population is effective. However, the genetic diversity for resistance established by this study is therefore particularly significant in that it promises a long lasting protection against *Striga*. If more virulent races of these parasites
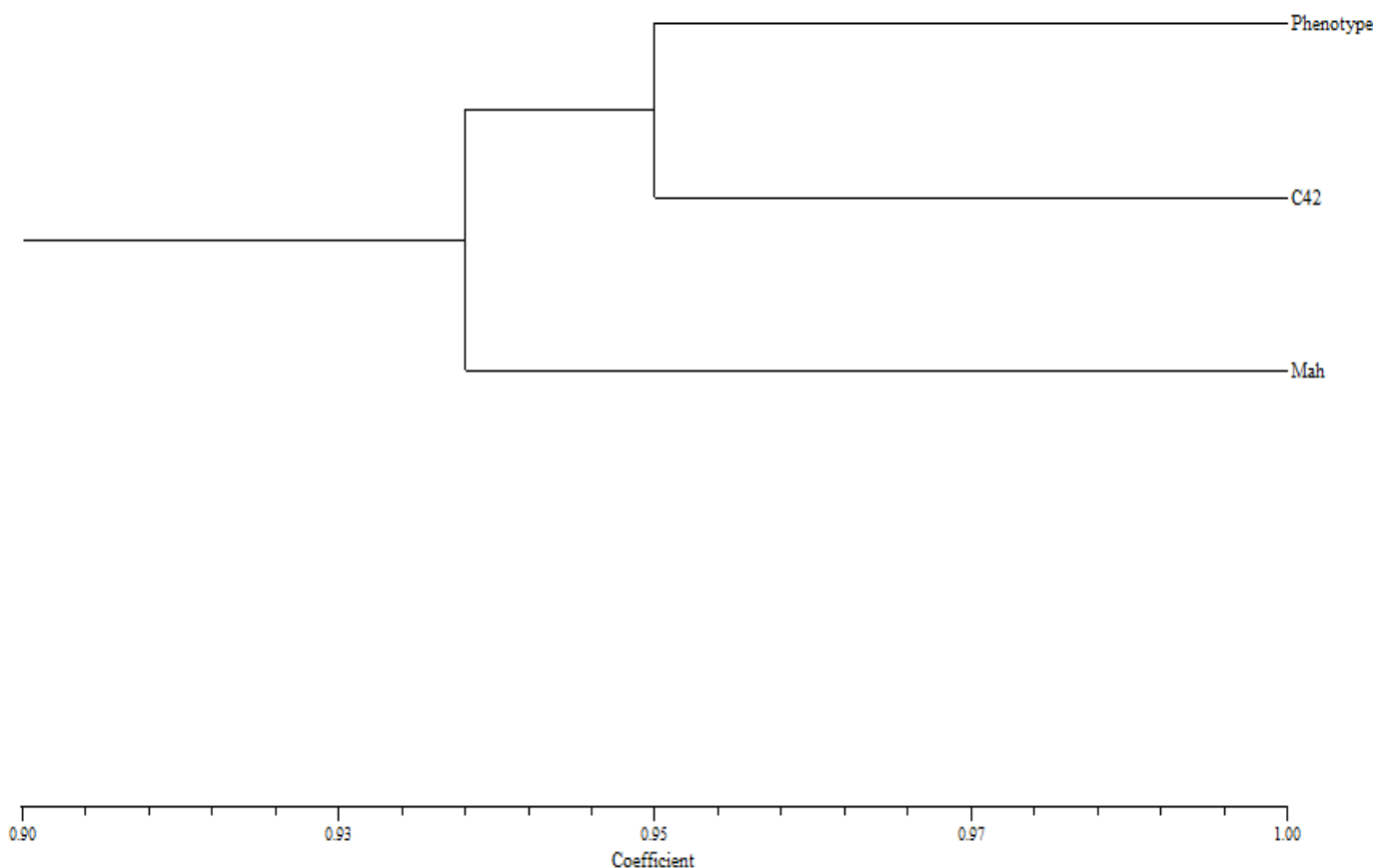
Figure 4. Dendogram for 100 lines derived from a NYST cluster analysis using Dice similarity coefficient based on three markers.

Table 4. Spearman rank correlation coefficient of marker similarity matrix

Population	DNA marker	Phenotype marker
Borno Brown x IT97K-499-35	61RM2	0.8346 (0.0001)
	C42B	0.8382 (0.0001)
Borno Brown x B301	61RM2	0.8207 (0.0001)
	C42B	0.91437 (0.0001)

Correlation values above 0.8 are considered to be good association (Rohlf, 1993). Values in parenthesis are level of significance expressed as probability

were to emerge, the strategy of sequential release of resistant varieties with varying backgrounds would have to be relied upon. The commercial use of varieties resistant to *Striga* is expected to offer both economical and practical method of control. Host plant resistance is used as a component in an integrated program of pest control in several crops. For *Striga*, the complete resistance available in cowpea however, should be used with the appropriate complementary agronomic practices of an integrated control package.

Marker screening

The two markers used to screen the F_2 segregating population showed polymorphisms with both the parents and the segregating populations. About 3% of the generated polymorphic fragments showed segregation distortion from the expected ratio of 3:1. Many authors have also reported segregation distortion of molecular markers in relation to a phenotype in F_2 mapping populations in other crops such as sunflower (Berry et al., 1995), rice (McCouch et al., 1998), lettuce (Landry et al., 1991), common bean (Paredes and Gepts, 1995). Based on linkage analysis using Joint Mapmaker, the two markers were identified to be associated with the gene conferring resistance to race 3 (Nigerian race) present in B301 and IT97K-499-35.

Earlier study conducted by Ouèdraogo et al. (2001) using similar marker E61R found that E61R marker was linked to the resistance gene race 3 (Rsg3) present in B 301 from Nigeria. The 61RM2 and 61R markers were

reported to be effective in identifying resistance to Striga race 1 and 3 (Timko et al., 2007; Ouedraogo et al., 2012). If the genes conferring resistance to *S. gesnerioides* were clustered within the cowpea genome, the markers identified will be immediately useful in the analysis of other populations of cowpea segregating for other genes with race-specific resistance to *S. gesnerioides* (Ouèdraogo et al., 2001). The two molecular markers systems used in this study discriminated the segregating population for resistance and susceptibility to *Striga*. However, 61RM2 which had a relatively tight linkage with the resistance gene gave a better resolution among the segregating populations and was consistent in detecting resistance in the two genetic populations indicating the relative efficiency of the marker compared to C42-2B. The results reported here corroborate earlier findings of Boukar et al. (2004). In another study, both markers, E61R-M2 and C42-2B, have been shown to be effective in identifying resistance to *Striga* race 1 and 3 (Timko et al., 2007).

The resolution of a map and the ability to determine marker order are largely dependent on population size, the larger the mapping population the better. Markers linked at a distance less than 5 cM to the target gene can be used for effective indirect selection (Weber and Wrickle, 1994 cited by Brahm et al. (2000). The efficiency of MAS can be increased by employing markers flanking the gene of interest. This has been demonstrated for cowpea resistance gene in cowpea (Boukar et al., 2004), and for the common bean (Kelly and Miklas, 1998). The finding of flanking markers around the resistance gene is an important factor that can increase the efficiency of this indirect selection. When a marker used for selection is not tightly linked to the gene of interest, cross-over will occur between the marker and the gene of interest. This will lead to a high percentage of false-positive/negative selections in the screening process. Procunier et al. (1997) reported, however, that when flanking markers are used simultaneously, error due to cross-over will be reduced. Thus, the dominant nature of the SCAR markers used in the present study is an important factor for reliability in the linkage analysis. The reliability of the dominant markers has also dispelled the fear reported by other workers that the use of dominant markers in linkage analysis of an F_2 population can lead to errors (Beaumont et al., 1996).

The two markers used in the present study, have been identified to be linked to *S. gesnerioides* resistance and were reported to be effective in identifying resistance to race SG1, SG3 and SG5 (Timko and Gowda personal communication) suggesting that the *Striga* gene in the study could belong to linkage group 1. It is worth mentioning here that these two markers were efficient in characterizing the populations for resistance to *S. gesnerioides* although the efficiency of the two markers differs. From the results obtained, 61RM2 appeared to be very useful marker tool in characterizing populations for

resistance to *S. gesnerioides* race 3. The marker provides 2 reproducible bands and the process is fairly simple to carry out and easy to score.

Marker efficiency

The linkage analysis performed by Joint Mapmaker showed the association of these markers with the *Rsg*4-3. Their flanking status was maintained at a distance from the resistance gene of 2.5 and 4.5 cM for marker 61RM2 and C42-2B, respectively, which was similar to that reported for E61R by Ouedraogo et al. (2002, 2012). Markers linked at a distance less than 5 cM to the target gene, as those obtained in the present study, can be effectively used for indirect selection (Weber and Wrickle, 1994). In particular, the significant correlations of 61RM2 and C42-2B matrices with that of the phenotypic score commonly used in the classical genetic analysis indicate that these markers are very suitable for this kind of genetic study. Thus, these markers can be recommended for commercial use in screening genetic populations for *Striga* resistance.

61RM2 was the most tightly-linked marker compared to C42-2B. Percent co-segregation of 61RM2 between susceptible and resistant was found to be consistent with a high value 98. However, the advantages of these two markers indicate that both 61RM2 and C42-2B can be used in different genetic populations. Depending upon the parents used in the mapping population, the arrangement of markers and map distance between markers may vary as evidenced in the present study (Figures 3 and 4). The marker results reported herein are comparable with similar studies in *Striga* with reference to percentage polymorphism and number of amplified DNA fragments. The marker score was significantly correlated with the phenotypic score. This is in accordance with the findings of Sato and Takeda (1995), where correlation coefficients of 0.77 and 0.78 were obtained, thus, indicating that the results of the phenotypic marker and genotypic marker are the same, and either method can be used to screen populations for resistance to *Striga*. However, the efficiency of 61RM2 in screening genetic populations for SG3 resistance was quite high and reliable and appeared to be the most efficient marker compared to C42-2B which was specifically developed for SG5 resistance (Gowda personal communication). However, the advantage of genotypic marker over the conventional marker is the speed in detecting resistant and susceptible lines thus shortening the breeding period and avoids the laborious screening period for *Striga* resistance. It also eliminates the effect of genotype x environment interaction. The RIL derived from the crosses were evaluated in *Striga* hot spot field. Those lines that were selected based on marker result were completely free of *Striga* infestation on the field while some of the lines identified to be resistance based on

phenotypic pot screening were found to be susceptible when planted on heavily *Striga* infested field. This makes genetic marker a reliable stable tool for selection and for characterization of cowpea populations for resistance to *Striga*.

Conclusion

The results of this study shows that characterization of cowpea lines for *Striga* resistance using molecular markers linked to the trait is feasible and more reliable. The findings of this study have shown that the derived SCAR-Marker 61RM2 was found to be reliable in discriminating between resistant and susceptible individuals in a segregating population other than the population it was originally developed for, thus showing its wider application. The only weakness in the markers is that they are both dominant markers.

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Conflict of interests

The authors did not declare any conflict of interest.

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