# AFLP and AFLP-Derived SCAR Markers Associated with *Striga gesnerioides* Resistance in Cowpea

O. Boukar, L. Kong, B. B. Singh, L. Murdock, and H. W. Ohm*

## ABSTRACT

**Cowpea, *Vigna unguiculata* (L.) Walp., is an important grain legume grown in tropical and subtropical regions, primarily Africa. The parasitic weed *Striga gesnerioides* (Willd.) Vatke is one of the most important constraints to cowpea production. Host plant resistance is the only practical control method. Five virulence genotypes (races) of *S. gesnerioides* have been identified in different regions of Africa. Several host resistance genes have also been identified that are effective against specific races of *S. gesnerioides*. The rapid spread of this parasitic weed creates an urgent need for cowpea varieties with multiple resistance genes. A recently identified cowpea breeding line, IT93K-693-2, has resistance to all known races. The objective of this research was to develop DNA markers that are useful for marker-assisted selection (MAS) in breeding cowpea for resistance to *S. gesnerioides*. An $F_2$ population developed from the cross between IT93K-693-2 and the susceptible cultivar IAR1696 was characterized for resistance against race 3 of *S. gesnerioides* for genetic analysis and molecular mapping. IT93K-693-2 was found to have a single dominant gene for resistance. Four amplified fragment length polymorphism (AFLP) markers, designated E-ACT/M-CTC$_{115}$, E-ACT/M-CAC$_{115}$, E-ACA/M-CAG$_{108}$ and E-AAG/E-CTA$_{190}$, were identified and mapped 3.2, 4.8, 13.5 and 23.0 cM, respectively, from *Rsg1*, a gene in IT93K-693-2 that gives resistance to race 3 (or Nigerian strain) of *S. gesnerioides*. The first two markers were validated in a second $F_2$ population developed from crossing the same resistant parent with 'Kamboinse local', a different susceptible cultivar. The AFLP fragment from marker combination E-ACT/M-CAC, which is linked in coupling with *Rsg1* was cloned, sequenced, and converted into a sequence characterized amplified region (SCAR) marker named SEACTMCAC83/85, which is codominant and useful in breeding programs.**

Cowpea is an important leguminous crop in various regions of the world, including tropical and subtropical areas of Asia, Africa, and Latin America, as well as parts of southern Europe and the USA (Singh et al., 1997). The high protein content represents a major advantage in the use of cowpea in nutritional products, for infant and children's food, and to compensate for the large proportion of carbohydrates often ingested in African diets (Lambot, 2002).

Cowpea production is limited by several abiotic and biotic factors, including parasitic weeds, among which *Alectra vogelii* Benth. and *S. gesnerioides* are the most important. *Alectra vogelii* is restricted to Africa while *S. gesnerioides* is found in Africa, and in parts of Asia and the USA (Musselman et al., 1991; Parker and Riches, 1993). In West Africa, *S. gesnerioides* is an increasingly serious problem. When there is drought, its impact becomes even more significant (Obilana, 1987). Yield losses due to *S. gesnerioides* range from 15 to 100%. Aggarwal and Ouédraogo (1989) have recorded 30% yield losses while farmers in northern Nigeria experienced 100% losses because of *Striga* (Emechebe et al., 1991). Furthermore, its rapid spread to new regions constitutes a severe threat to cowpea production (Agbobli, 1991).

Control measures including cultural practices, chemical control, biological control, and host plant resistance have been reviewed (Dubé and Alain, 2001; Boukar, 2002). No single method seems to provide a complete control of this parasite, although host plant resistance appears to be the most effective and economical approach. Significant progress toward developing *Striga*-resistant lines has been reported in different breeding programs and by the International Institute for Tropical Agriculture (IITA), but important constraints linger.

To alleviate these constraints and for other reasons (e.g., speeding breeding efforts, possibility of identifying other strains of the parasites, reduction of environmental factors on the parasite's development), MAS has been proposed as an alternative solution for pyramiding resistance genes (Haley et al., 1994; Ouédraogo 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), has been used to discover markers closely associated with economically important traits in many crop species including cowpea. Recent studies conducted by Ouédraogo 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-CAA$_{300}$ and E-ACA/M-CAT$_{150}$, were linked to both *Rsg2* and *Rsg4*, respectively.

To improve the efficiency of molecular marker screening, AFLP markers and other molecular markers, such as randomly amplified polymorphic DNA (RAPD), can be converted into SCAR markers (Paran and Michelmore, 1993). In several species such as carrot (*Daucus carota* L.) (Bradeen and Simon, 1998), mustard

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**Abbreviations:** AFLP, amplified fragment length polymorphism; BSA, bulked segregant analysis; CTAB, hexadecyltrimethylammonium bromide; EDTA, ethylenediamine tetra-acetic acid; IITA, International Institute of Tropical Agriculture; MAS, marker-assisted selection; PCR, polymerase chain reaction; SCAR, sequence characterized amplified region; TBE, Tris-borate.

[*Brassica juncea* (L.) Czern.] (Negi et al., 2000), soybean [*Glycine max* (L.) Merr.] (Meksem et al., 2001), apple (*Malus domestica* Borkh.) (Xu et al., 2001), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (Shan et al., 1999), and rice (*Oryza sativa* L.) (Jia et al., 2001), the amplified AFLP band was cloned and sequenced and then used to produce extended primers. The use of a pair of specific primers to amplify genomic DNA allows the development of SCAR markers. These markers are useful to plant breeding and genome analysis because of their simplicity, low cost, and efficiency. Depending on crop species, SCAR markers generally generate a single polymorphic band that is more reproducible across labs, is easier to score, and applicable to use with low-quality DNA obtained through rapid DNA extraction procedures than polymorphic bands generated by AFLP or RAPD markers (Kelly and Miklas, 1998). The codominant feature of certain SCARs has a direct application for MAS in plant breeding. As a contribution to the development and implementation of MAS approaches, the objectives of this study were to map *Rsg1* and develop an AFLP-derived SCAR marker to facilitate MAS for this gene.

## MATERIALS AND METHODS

### Plant Material and Phenotypic Data

The plant material used in this study was obtained from IITA, Kano Station, Nigeria. Resistant parent line IT93K-693-2, used in this study, is an inbred selection from a three-way cross: (IT88D-867-11 × IT90K-76) × IT89KD-374. Of these three parents, IT90K-76 is an inbred selection from a backcross: (B301 × IT90K-2246-4) × IT90K-2246-4. Among these, IT88D-867-11 has resistance to the strain of *S. gesnerioides* originating in the Benin Republic, but is susceptible to the strain that originated in Nigeria and that was used in tests in this study. B301 has the resistance gene *Rsg1* (Singh and Emechebe 1990; Atokple et al., 1995), which is effective against four strains of *S. gesnerioides* originating in Burkina Faso, Mali, Niger/Nigeria, and Cameroon, respectively (Lane et al., 1997). The cowpea varieties IT90K-2246-4 and IT89KD-374 are susceptible to all five strains of *S. gesnerioides* but have good agronomic attributes. The IITA breeding line IT93K-693-2 has resistance to all of the five known strains of *S. gesnerioides* (Singh, 2002) and thus, has *Rsg1* from B301 and the resistance of IT88D-867-11. IT93K-693-2 was crossed to a susceptible line, IAR1696, selected from a local land race at the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria, and an $F_2$ population (population 1) was developed. A second population (population 2) was developed from a cross of the same resistant parent, IT93K-693-2, by the susceptible parent, Kamboinse local (a susceptible cowpea line different from IAR1696).

Population 2 was used to validate the linked molecular markers obtained from population 1. The $F_2$ populations derived from both crosses were screened for resistance to the Nigerian strain of *S. gesnerioides* (prevailing in Nigeria). Thus, the populations were characterized for resistance conditioned by *Rsg1* from parent line B301 and not for resistance from IT88D-867-11. Plants were grown with the pot-culture technique (Singh and Emechebe, 1990, Atokple et al., 1995). Two $F_2$ seeds were planted in each plastic pot, 13-cm diam. and 13 cm deep containing about 1 L of unsterilized sieved sand and top soil (sandy loam) mixture (1:1 vol/vol) previously inoculated uniformly with about 800 *S. gesnerioides* seeds. About 10 wk after planting, 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 were gently separated, carefully freed from any remaining soil and examined for *Striga* attachment. Plants allowing attachment, healthy development, and emergence of *Striga* were classified as susceptible. Those without any attachment and free of infection were categorized as resistant.

### DNA Extraction

Five weeks after planting, one young fresh leaflet from each plant was collected in an Eppendorf tube and put on ice. The samples were taken to IITA headquarters, Ibadan, Nigeria, where they were lyophilized. Dry leaf samples were transferred to Purdue University, where they were stored at −80°C before their use.

Leaf tissues from individual plants were ground to a fine powder under liquid nitrogen before DNA isolation. Total genomic DNA was extracted by the CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof et al., 1984) following the procedure of Hoisington et al. (1994) with minor modifications. Two-percent CTAB extraction buffer [100 m*M* Tris-HCl buffer pH 8.0, 2% (w/v) CTAB, 100 m*M* $Na_2EDTA$ (ethylenediamine tetra-acetic acid), and 1.4 *M* NaCl] was used instead of the 1.67% CTAB outlined by Hoisington et al. (1994). The DNA concentration in each sample was measured with the Hoefer DyNA Quant 200 Fluorometer (Hoefer Pharmacia Biotech Inc., Buckinghamshire, UK). A total of 62 and 35 $F_2$ individual plants from the crosses IT93K-693-2 × IAR1696 and IT93K-693-2 × Kamboinse local, respectively, were screened against *S. gesnerioides*. The first population was used to identify markers linked to the targeted striga resistance gene, and the second population was used to validate linkage between the striga resistance and the SCAR marker.

### Bulked Segregant Analysis

For BSA (Michelmore et al., 1991), equivalent amounts of genomic DNA from 10 resistant $F_2$ plants and 10 susceptible $F_2$ plants from the population derived from the cross between IT93K-693-2 and IAR1696 were pooled to form resistant and susceptible bulks. Both bulks were used along with the parents to identify markers showing polymorphisms between the four samples. These polymorphic markers were further used to analyze individual $F_2$ plants to determine linkages between AFLP markers and the *S. gesnerioides* resistance gene.

### AFLP Analysis

Amplified fragment length polymorphism analysis was performed according to the procedure described by Vos et al. (1995), with a commercially available kit (AFLP® Analysis System I, Invitrogen, Life Technologies, Carlsbad, CA) and following the manufacturer's instructions with minor modifications. Approximately 500 ng DNA of each sample was digested with *Eco*RI/*Mse*I restriction enzyme solution. After the ligation of the digested DNA, the reaction mixture was diluted five-fold rather than 10-fold with TE buffer containing 10 m*M* Tris-HCl pH 8.0, 0.1 m*M* EDTA. The number of cycles was increased to 25 cycles for preamplification reaction and 30 cycles for the selective amplification rather than 20 and 23 cycles, respectively. Primer labeling was performed by phosphorylating the 5′ end of *Eco*R I primers with [$\gamma$-$^{33}$P]ATP (adenosine 5′-triphosphate disodium salt) and T4 kinase in selective amplification. The preamplification product was also diluted 1:20 rather than 1:50. The amplification products were separated on 6% (w/v) polyacrylamide gels containing 29:1 acrylamide: Bis-acrylamide (Fisher Scientific, Chicago, IL), 7.5 *M* urea, and 1 × TBE (1,1,2,2-tetrabromoethane) buffer used with 0.4-mm spacers and a sharktooth comb. The gels were preelectrophoresed for about 20 min with 1 × TBE. Electrophoresis was performed at constant temperature and wattage (45–50°C, 100 W) for about 2.5 h. The gels were transferred onto 3-MW gel blot paper (Midwest Scientific, Valley Park, MO) and dried at 80°C for 2 h on a gel-drier (Bio-Rad, Hercules, CA). The dried gels were exposed to x-ray film (X-OMAT AR, Eastman KODAK Corp., Rochester, NY) for 2 to 3 d at −80°C without an intensifying screen. The bands were visualized with a transluminator (Fisher Scientific, Chicago, IL).

### Marker Segregation and Linkage Analysis

Data were analyzed by the chi-square ($\chi^2$) test to ascertain the goodness of fit between the expected ratio for a single dominant gene, and the segregation of the phenotypic data. Linkage analysis between the AFLP markers and the *S. gesnerioides* resistance loci was performed with the software package MAPMAKER/EXP version 3.0 (Lander et al., 1987). Map units were computed by applying the Kosambi function (Kosambi, 1944). The LOD score of 5 and the maximum distance of 25 cM were used in the determination of linkages.

### Cloning and Sequencing of the Target AFLP Band

The gel slice containing the DNA fragment was excised from the dried AFLP polyacrylamide gel with a sharp-edged clean razor blade, and then eluted with 30 μL of 1 × TE (10 m*M* Tris-HCl, pH 8.0 and 1mM EDTA pH 8.0) for overnight at 4°C. From this, 1.0 μL of supernatant was used as template for polymerase chain reaction (PCR) amplification with the same primer combination and PCR conditions as that of the selective amplification with the only difference of not labeling the *Eco*RI primer (E-ACT).

The amplified products were electrophoresed at 70 V in a 1.2% low-melting-point agarose gel. The critical fragment was excised from the gel and purified with QIAquick gel-extraction Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instructions. An aliquot of 3.0 μL of the purified DNA was ligated into a pGEM-T easy vector (Promega, Madison, WI) according to the procedures described by the manufacturer with minor modifications. The host strain DH5α was used as competent cells for transformation. The recombinant plasmids were plated on selective Luria-Bertani media containing ampicillin {(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0)heptane-2-carboxylic acid} and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; BioVectra, Charlottetown, PE, Canada).

QIAprep Miniprep Kit (Qiagen, Inc., Valencia, CA) was used for plasmid DNA extraction. To check the presence of the target insert, PCR amplification was performed with 25 μL total volume of 1 × PCR buffer, 1.5 m*M* $MgCl_2$, 200 m*M* dNTP, 0.4 μ*M* T7, 0.4 μ*M* SP6, 0.2 μL of *Taq*, 10 ng of template DNA. The amplification profile consisted of one cycle at 94°C for 2 min, followed by 36 cycles of 45 s at 94°C, 1 min at 62°C and 1.5 min at 72°C, with a final extension of 7 min at 72°C.

All 10 samples showed a single amplification product in a 1.2% agarose gel immersed in 0.5 × TBE buffer (90 m*M* Tris-Borate, 1 m*M* EDTA, pH 8.0). The ten corresponding purified plasmid DNAs were sent to the DNA Sequencing Laboratory at the Genomic Center (Purdue University) for sequencing.

### Designing SCAR Primers

The following oligonucleotide primers, designed on the basis of the identical sequence of plasmid DNA, led to polymorphisms between the parents and the bulks.

Primer-reverse:

5′-ACAGACACAGTTGTAGTTTATCAGC-3′ (25-mer) and

Primer-forward:

5′-CTATACTTTTGCTCCTTGTGTGGC-3′ (24-mer).

The 5′ end of the reverse and forward primers contained two selective bases (AC and CT) of *Mse*I and *Eco*RI primers, respectively. These primers were synthesized by Integrated DNA Technologies (Coralville, IA) and were used to screen the parents, both resistant and susceptible bulks and individual $F_2$ plants. The optimal PCR amplification was conducted with 25 μL of reactions containing 25 ng of template DNA; 10 m*M* Tris HCl, pH 8.3; 50 m*M* KCl; 1.5 m*M* $MgCl_2$; 200 μ*M* of each dNTP; 0.3 μ*M* of each primer; and 1 unit of *Taq* DNA polymerase. After an initial heat denaturation step at 94°C for 2 min, DNA fragment amplification was performed for 43 cycles, comprising 45 s at 94°C, 1 min at 62°C, and 1.5 min at 72°C. Final extension was for 7 min at 72°C. To separate the amplified products, 3.5% Metaphor agarose gels (BMA, Rockland, ME) stained with ethidium bromide were used and the products were visualized by illumination with ultraviolet light.

## RESULTS

### Phenotypic Data

At about 6 wk after planting, *S. gesnerioides* emerged in pots with susceptible cowpea plants. These cowpea plants showed leaf chlorosis, reduced growth and vigor, and partial defoliation. Some plants developed symptoms, but *Striga* did not emerge from the soil. However, since each pot had two plants, the roots were washed and attachment of *S. gesnerioides* to plants was verified before classifying cowpea plants as resistant or susceptible.

$F_2$ populations 1 and 2 segregated 43 resistant:19 susceptible and 28 resistant:7 susceptible, respectively. These segregations fit a 3:1 ratio ($\chi^2 = 1.05$, $P = 0.30$ and $\chi^2 = 0.47$, $P = 0.49$, respectively) indicating that resistance to the Nigerian strain of *S. gesnerioides* in IT93K-693-2 is monogenic and dominant. The gene providing resistance to the Nigerian strain of *S. gesnerioides* in these populations is *Rsg1*, which is from B301.

### Marker Analysis

The AFLP analysis conducted on the population derived from the cross of IT93K-693-2 × IAR1696 showed that there were about 65-120 clearly readable bands for each *Eco*RI and *Mse*I primer combination. From the 64 AFLP primer combinations used, 20 showed polymorphism between the parents and the bulks, and were used to screen individual $F_2$ plants. Of these 20, four primer combinations produced bands that were linked to *Rsg1*. Primer combination *Eco*RI-ACT/*Mse*I-CTC showed an approximately 115-bp amplification product present in the susceptible parent IAR1696 and the susceptible bulk but absent in the resistant parent and

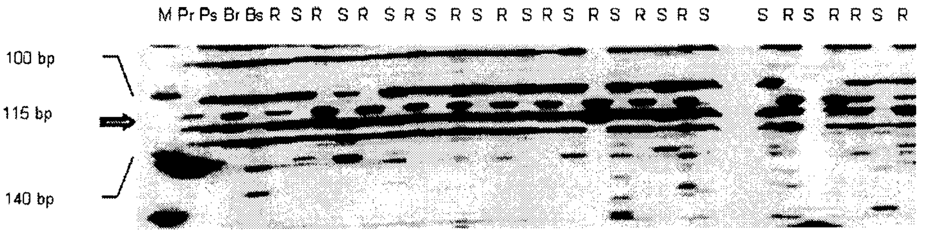

Fig. 1. Amplified fragment length polymorphism pattern on polyacrylamide gels around the polymorphic products (arrow) obtained by primer combinations E-ACT/M-CAC. M, Pr, Ps, Br, Bs, R, and S represent DNA ladder, resistant parent, susceptible parent, resistant bulk, susceptible bulk, and resistant and susceptible individual $F_2$ plants, respectively.

resistant bulk. This marker linked in repulsion phase to the resistance allele was designated E-ACT/M-$CTC_{115}$ following AFLP marker nomenclature described by Ouédraogo et al. (2001). The three other primer combinations *Eco*RI-AAG/*Mse*I-CTA, *Eco*RI-ACT/*Mse*I-CAC (Fig. 1), and *Eco*RI-ACA/*Mse*I-CAG revealed polymorphic fragments of 190, 115, and 108 bp, respectively. These primer combinations generated polymorphisms that were linked in coupling phase to the resistance allele with the marker present only in the resistant phenotype. They were designated E-AAG/M-$CTA_{190}$, E-ACT/M-$CAC_{115}$, and E-ACA/M-$CAG_{108}$, respectively.

All 62 $F_2$ individuals were analyzed and the Mapmaker *compare* command determined the most likely order within the linkage group comprising the resistance gene (*Rsg1*). The Mapmaker command *map* indicated the map distances between the resistance gene *Rsg1* and the associated AFLP markers (Fig. 2). The entire linkage group spanned a distance of 36.5 cM. Two flanking AFLP markers, E-ACT/M-$CTC_{115}$ and E-ACT/M-$CAC_{115}$, were estimated to be 3.2 cM and 4.8 cM from the resistance gene, respectively.

## Conversion of AFLP Marker E-ACT/M-$CAC_{115}$ into SCAR Marker

The 115-bp fragment obtained by AFLP primer combination E-ACT/M-$CAC_{115}$ was linked in coupling with the striga resistance gene, and it was cloned and sequenced to develop a rapid, inexpensive and reliable PCR-based marker. The successful cloning of the target fragment of 115 bp was confirmed by PCR-reaction with the eluted plasmid DNA as template. A single band was obtained on an agarose gel. Of the 10 colonies from a single transformation plate, seven inserts were found to be identical following a multiple sequence alignment produced by the software Clustal X (Jeanmougin et al., 1998). All the resulting sequences contained the *Eco*RI adaptor at one end and the *Mse*I adaptor at the other end. The oligonucleotide primers, designed on the basis of the identical sequence of plasmid DNA, led to polymorphisms between the parents and the bulks.

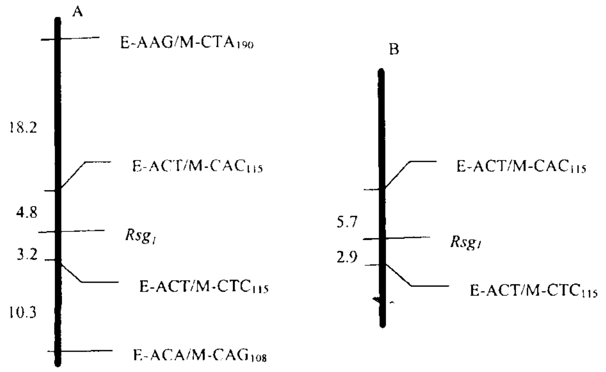

Fig. 2. Map showing amplified fragment length polymorphism markers associated with the *S. gesnerioides* resistance gene *Rsg1*. The map distances are displayed in centimorgans at the left side for (A) population IT93K-693-2 × IAR1696 and (B) IT93K-693-2 × 'Kamboinse local'.

These primers were used to screen the parents, both resistant and susceptible bulks and individual $F_2$ plants. An 85-bp DNA fragment was amplified from the susceptible parent, bulk and individual $F_2$ plants while an 83-bp fragment was amplified from the resistant parent, bulk and some individual $F_2$ plants. Other resistant $F_2$ plants showed both bands, representing heterozygous plants (Fig. 3). Thus, the AFLP marker was converted into a codominant SCAR marker designated as SEACTM-CAC83/85, meaning SCAR marker of size 83 to 85 bp derived from AFLP primer combination E-ACT/M-CAC.

## Validation of the Identified AFLP and the Converted SCAR Markers

The flanking markers were used to screen an $F_2$ population derived from the same resistant parent, IT93K-693-2, crossed to a susceptible line, Kamboinse local. Linkage analysis performed by Mapmaker confirmed the association of these markers with the resistance gene. Their flanking status was maintained and their distance from the resistance gene of 2.9 and 5.7 cM for markers E-ACT/M-$CTC_{115}$ and E-ACT/M-$CAC_{115}$, respectively, was similar to that obtained from the first population (Fig. 3).

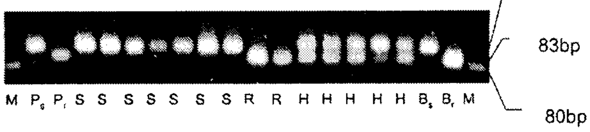

Fig. 3. Metaphor agarose gel (BMA, Rockland, ME) showing the results of polymerase chain reaction amplifications based on the use of the sequence characterized amplified region marker converted from E-ACT/M-$CAC_{115}$. M, $P_s$, $P_r$, $B_r$, $B_s$, R, H, and S represent 20-bp ladder, susceptible parent, resistant parent, resistant bulk, susceptible bulk, and resistant, heterozygous, and susceptible individuals, respectively, from the population IT93K-693-2 × IAR1696.

The SCAR marker SEACTMCAC83/85 was also used to screen the $F_2$ population of IT93K-693-2 × Kamboinse local. Similar band patterns to that of the first population were obtained. The genotypes of plants based on the SCAR marker were the same as observed for the original AFLP marker. All the susceptible plants showed the 85-bp amplification product. The resistant plants showed either the 83-bp band (homozygous) or both the 85 and 83 bp (heterozygous), except that two resistant plants, which were assumed to be recombinant plants, revealed only the 85-bp band.

## DISCUSSION

*Striga gesnerioides* is a severe constraint to cowpea production in Africa, and at least five virulence genotypes have been identified. Gene pyramiding offers an effective strategy for the development of cultivars resistant to *S. gesnerioides* by pyramiding resistance from different genetic resistance sources. The results reported here and in the literature show that the AFLP technique is a reliable, stable, and rapid assay for use in molecular-marker screening (Jia et al., 2001). On the basis of linkage analysis, four primer combinations associated with the dominant resistance gene present in IT93K-693-2, were identified. The two flanking markers closely associated with the resistance gene were confirmed in the second population used in the present study.

Markers linked at a distance $< 5$ cM to the target gene, as those obtained in the present study, can be effectively used for indirect selection (Weber and Wrickle, 1994). The efficiency of MAS can be increased by employing markers flanking the gene of interest. This has been demonstrated for bacterial blight resistance genes in rice (Huang et al., 1997) and suggested, for example, for common bean (Kelly and Miklas, 1998) and wheat (Schachermayr et al., 1997).

In the present study, we were able to convert AFLP markers into easy, inexpensive, and reliable PCR-based markers like SCAR. Several studies in the literature revealed the inability to convert AFLP fragments with sizes $< 200$ bp (De Jong et al., 1997; Negi et al., 2000). According to Horn et al. (2003), the possibility of converting AFLP markers into sequence-specific markers is often restricted because of the very small size of the markers and the fact that most AFLP polymorphisms seem to originate in differences within the restriction sites. Bradeen and Simon (1998) pointed out that the AFLP fragment is too short for designing an appropriate PCR primer to amplify a polymorphic band, while Prins et al. (2001) reported that different AFLP fragments of the same size may migrate together in the gel, and a target polymorphic band may contain contaminating fragments from adjacent bands. Shan et al. (1999) found that the restoration of the original polymorphism remains difficult since the cloning procedure required for the AFLP conversion often contributes to the loss of the original polymorphism (Wei et al., 1999). Several procedures have been proposed to solve these problems. In the case of AFLP markers which are of 150 to 300 bp in size, Negi et al. (2000) pointed out that it is essential to isolate the flanking regions for the conversion to SCAR markers. These authors suggested the use of a PCR walking approach to isolate fragments adjacent to the AFLP markers. Other groups have reported the use of inverse-PCR to isolate the flanking regions for conversion to SCAR (Bradeen and Simon, 1998; De Jong et al., 1997). Recently, Brugmans et al. (2003) describe a general protocol for the conversion of AFLP markers into single-locus PCR assays and state that in principle, there is no minimal size of an AFLP marker as long as the internal sequence of the AFLP band is sufficiently long to allow the design of a highly specific PCR primer. The codominant nature of the SCAR obtained in the present study is an important factor for reliability in the linkage analysis. It has been reported that the use of dominant markers in linkage analysis with an $F_2$ population can lead to errors, as the amount of information created by each data point is decreased in situations where heterozygous genotypes are found (Beaumont et al., 1996). The conversion of the AFLP marker into a SCAR will facilitate the transfer of the *S. gesnerioides* resistance gene to desirable cowpea lines via MAS. Selection in segregating populations with this SCAR marker will be more efficient and less expensive than with AFLP markers.

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