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Identification of AFLP markers linked to resistance of cowpea (Vigna unguiculata L.) to parasitism by Striga gesnerioides

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Abstract AFLP and bulked segregant analysis were used to identify molecular markers linked to resistance of cowpea [Vigna ungiculata (L.) Walp.] to parasitism by Striga gesnerioides (Willd.) Vatke. Segregation analysis of F_2 progeny from a cross of Tvx3236, a Striga-susceptible line, with IT82D-849, a resistant cultivar, showed that resistance to S. gesnerioides race 1 from Burkina Faso was controlled by a single dominant gene, designated Rsg2–1. Three AFLP markers were identified that are tightly linked to Rsg2–1: E-AAC/M-CAA_{300} (2.6 cM), E-ACT/M-CAA_{524} (0.9 cM), and E-ACA/M-$CAT_{140/150}$ (0.9 cM), which appears to be codominant. Segregation analysis of a different F_2 population resulting from a cross of the Striga-susceptible line IT84S-2246–4 with Tvu 14676, a S. gesnerioides race 3 resistant line, showed that resistance to S. gesnerioides race 3 was also controlled by a single dominant gene, designated Rsg4–3. Six AFLP markers linked to Rsg4–3 were identified: E-ACA/M-CAG_{120} (10.1 cM), E-AGC/M-CAT_{80} (4.1 cM), E-ACA/M-CAT_{150} (2.7 cM), E-AGC/M-CAT_{150} (3.6 cM), E-AAC/M-CAA_{300} (3.6 cM), and E-AGC/M-CAT_{70} (5.1 cM). Segregation analysis of the E-AAC/M-CAA_{300} and E-ACA/M-CAG_{120} markers in recombinant inbred lines derived from IT84S-2049×524B determined that both are located within linkage group 1 of the cowpea genetic map. The identification of AFLP markers linked to Striga resistance provides a stepping stone for a marker-assisted selection program and the eventual cloning and characterization of the gene(s) encoding resistance to this noxious parasitic weed.

Keywords Cowpea · AFLP markers · Bulked segregant analysis · Striga resistance · Parasitic plants

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Introduction

Cowpea [Vigna unguiculata (L.) Walp.] is a major food legume grown in the semi-arid regions of West and Central Africa, South America, and India, and for many people in these regions it is an important dietary protein source (Aggarwal 1991). Like other food crops, cowpea is attacked by a variety of pathogens, among which the parasitic flowering plant Striga gesnerioides (Willd.) Vatke is a major constraint to cowpea production. S. gesnerioides parasitism causes severe chlorosis, wilting, and stunting of susceptible hosts, resulting in yield losses estimated in the millions of bushels annually (Aggarwal and Ouédraogo 1989; Muleba et al. 1996, 1997; Singh and Emechebe 1997). Control of the parasite by chemical and cultural treatments is difficult and expensive and, therefore, significant effort has been put into the identification of natural sources of genetic resistance within cowpea cultivars and to the selection and breeding of improved lines (Singh and Emechebe 1997).

The first S. gesnerioides resistant cowpea varieties identified were Suvita-2 (Gorom local) from Burkina Faso and 58–57 from Senegal (Aggarwal et al. 1984; Berner et al. 1995). Subsequently, additional sources of resistance were identified including B301, a landrace from Botswana (Singh and Emechebe 1990a), 872, a landrace from Niger, APL1, a landrace from Nigeria (Lane et al. 1997a), and two breeding lines developed at IITA, IT81D-994 and IT82D-849 (Singh and Emechebe 1991). Although resistant in Burkina Faso, when grown in Nigeria, Suvita-2 proved to be susceptible to S. gesnerioides parasitism (Aggarwal et al. 1984, 1986). Parker

and Polniaszek (1990) and Singh and Emechebe (1990a, b) subsequently showed that differences exist in the pathogenicity of S. gesnerioides populations from different locations in West Africa leading to our current understanding (Table 1) that at least five different races of S. gesnerioides are present in West and Central Africa (Lane et al. 1997b). Well-adapted high-yielding cultivars resistant to all five races of S. gesnerioides are under development but not widely available (Singh 1999). Among cultivars in use, Suvita-2 and IT81D-994 are resistant to races 1, 2, and 4, whereas B301 and IT82D-849 are resistant to races 1, 2, 3, and 5, (Lane et al. 1994, 1997b; Muleba et al.1996).

Genetic segregation analysis currently indicates the existence of three independent dominant genes conferring resistance to S. gesnerioides in cowpea (Atokple et al. 1995; Singh and Emechebe 1990a, 1997; Touré et al. 1997). These have been designated Rsg1, Rsg2, and Rsg3 in varieties B301, IT82D-849, and Suvita-2, respectively. Although they are good sources of genetic resistance, cowpea varieties such as B301 and 58–57 have poor seed and agronomic qualities (Atokple et al. 1995; Lane et al. 1997a). Using traditional breeding and selection methods, attempts to create commercially acceptable cultivars free of undesirable genetic traits and resistant to all five races of S. gesnerioides have not yet been successful. If only, backcross breeding methods are applied, several generations of backcrossing, taking up to seven or more field seasons, may be necessary to obtain lines carrying the desired character.

The use of DNA marker systems, such as random-amplified polymorphic DNAs (RAPDs) (Williams et al. 1990), amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995), and simple sequence repeat (SSRs) (Akkaya et al., 1992) has contributed greatly to the development of genetic linkage maps for many important crop species, including cowpea (Fatokun et al. 1992, 1993, 1997; Young et al. 1992; Myers et al. 1996; Menendez et al. 1997). In combination with the bulked segregant analysis (BSA) (Michelmore et al. 1991), the use of RAPDs, AFLPs, and SSRs have made it possible to rapidly identify molecular markers within plant genomes linked to agronomically important genes (Lee 1995; Kelly and Miklas 1998; Young 1999). The development and use of molecular marker technologies has also facilitated the subsequent cloning and characterization of disease, insect, and pest resistance genes from a variety of plant species (Hammond-Kosack and Jones 1997; Ronald 1998; Meyers et al. 1999). These studies have led to a greater understanding of the basis for plant-pathogen interactions and the process of plant disease resistance.

Here we report the identification of AFLP markers linked to two loci conferring race-specific resistance to S. gesnerioides. The potential for use of these markers in a marker-assisted selection (MAS) program (Knapp 1998; Simpson 1999; Young 1999) is also discussed.

Table 1 Parental lines and their resistance response to various races of Striga gesnerioides (S susceptible, R resistant)

Cowpea cultivar/ landrace/line	Striga gesnerioides races				
	1	2	3	4	5
Tvx 3236	S	S	S	S	S
Suvita 2	R	R	S	R	S
B301	R	R	R	S	R
IT81D-994	R	R	S	R	?
IT82D-849	R	R	R	S	R
IT84S-2246-4	S	S	S	S	S
Tvu 14676	?	?	R	?	?
IT84S-2049	S	S	S	S	S
524B	?	?	?	?	?

Materials and methods

Plant material, growth conditions, and field experiments

The plant populations used in this study and their resistance/susceptibility phenotypes are presented in Table 1. Suvita-2 (Gorom local) is an improved cultivar from Burkina Faso. B301 and Tvu14676 (B203) are cultivars from Botswana. Tvx3236, IT81D-994, IT82D-849, and IT84S-2246 are breeding lines developed at IITA. IT84S-2049 and 524B were provided by A.E. Hall (University of California, Riverside). IT84S-2049 is an IITA breeding line reported to have multiple disease and pest resistance (Menéndez et al. 1997) and is genetically similar to the S. gesnerioides-susceptible line IT84S-2246. Cultivar 524B is a California black-eye type developed from a cross between cv. CB5 and CB3 which together encompass the genetic variability in cowpea in California. The resistance reaction of 524B to S. gesnerioides had not previously been tested.

The four resistant cultivars, Suvita-2, B301, IT81D-994, and IT82D-849, were crossed with the susceptible cultivar Tvx3236 to produce F_1 hybrids. Four F_2 segregating populations were generated by self-fertilizing the F_1 individuals. Parental, F_1, and F_2 individuals from the four populations were grown in the field under S. gesnerioides infestation at the Institut de l'Environment et de Recherches Agricoles (INERA) research station in Kamboinse, Burkina Faso. The F_2 progenies from each population were planted in rows, with the distance between rows being 1.5 m and approximately 1.5 m separating the individual plants. Emergence of S. gesnerioides was scored every 2 days starting 3 weeks after planting and continuing until complete senescence of the plant (120 days after planting). Plants were pulled from the ground and their roots checked for attached and pre-emergent S. gesnerioides seedlings. Plants allowing parasite attachment, development, and emergence were classified as susceptible. Those free of infection or showing minimal levels of S. gesnerioides attachment and no emerged seedlings were grouped as resistant. In order to determine the genotypes of the F_2 plants, we grew 25 F_3 seeds of each individual F_2 plant under S. gesnerioides infestation and monitored them as indicated above for the F_2 plants. A sample of 150 individuals was formed from each of the segregating F_2 populations.

In a separate experiment, the parents and 150 F_3 lines obtained from F_2 plants of a cross betweenTVU14676 (resistant) and IT84S-2246 (susceptible) were grown under S. gesnerioides race 3 infestation in a field in Bakura, Nigeria. The parents and F_3 lines were planted in five replications of a randomized complete block design. Each field plot (replication) consisted of two 5-m-long rows spaced 1.5 m apart. Within-row plant spacing was 30 cm, and this allowed the planting of 32 F_3 progeny from a single F_2 plant per plot. Nine weeks after planting the plots each of which contained a single F_3 line or parent line, the plants were evaluated for S. gesnerioides reaction. The mean number of surviving plants and plants with emerged S. gesnerioides (averaged over five replications) were determined for each parent and F_3 line. The inherit-

ance of resistance in a given cross was determined by comparing observed to expected phenotypic ratios and testing the significance of the difference by Chi squared analysis.

DNA extraction

Total genomic DNA was isolated from leaf tissues of individual plants using the method of Varadarajan and Prakash (1991). Prior to DNA isolation of the plants produced in Burkina Faso, the tissues were dessicated following the procedure of Chase and Hills (1991), and the dried samples were kept at 4°C. The DNA concentration in samples was measured spectrophotometrically at A_{260} using a Varian DMS200 spectrophotometer. Concentrated DNA samples were maintained in ethanol at 4°C and, as necessary for AFLP analysis, working solutions (100 ng/µl) were prepared in TE buffer (10 mM TRIS-HCl, 1 mM EDTA, pH 8.0) as needed. Dilute solutions were maintained at −20°C. A total of 116 individuals from the plant population screened against race of S. gesnerioides yielded DNA samples suitable for AFLP analysis, and these samples were used in the studies described below.

AFLP analysis

Sixteen pair-wise combinations of γ-[^{32}P]-ATP labeled (New England Nuclear Life Sciences Products, Boston, MA) EcoRI and MseI primers were used in AFLP analysis to screen for the degree of polymorphism between resistant and susceptible parents used to generate the five cowpea populations. Based on these studies, IT82D-849 and Tvx3236 were determined to contain the highest degree of genetic polymorphism among populations screened with race 1, and the IT82D-849×Tvx3236 cross was chosen for further detailed analysis along with the IT84S-2246–4×Tvu 14676 cross representing race 3 resistance. Eventually, 40 EcoRI and MseI primer combinations were tested with progeny from the IT82D-849 x Tvx3236 and IT84S-2246–4×Tvu 14676 crosses and scored in bulked samples and F_2 individuals (as described below). AFLP analysis was performed essentially as described by Vos et al. (1995) using the AFLP™ Analysis System I AFLP Starter Primer Kit (Gibco BRL-Life Technologies, Gaithersburg, MD) with minor modifications. Each reaction used 500 ng rather than 250 ng of genomic DNA, and the recommended dilutions used after the ligation of the adapters (1:10) and following the pre-amplification reaction (1:50) were cut in half.

Bulk design and bulked segregant analysis

Bulked segregant analysis was carried out essentially as described by Michelmore et al. (1991). Homozygous-resistant and homozygous-susceptible bulks were prepared from F_2 individuals by pooling aliquots containing equivalent amounts of total DNA (approximately 10 µg) from each of 12 resistant and 12 susceptible F_2. Following analysis of the various bulks for the presence or absence of the various AFLP markers, individual F_2 progeny within each bulk were analyzed to determine linkage as described below.

Marker segregation, linkage analysis, and mapping

Chi-squared (χ^2) tests were performed to examine the goodness-of-fit between the expected Mendelian ratio for the F_2 populations (3:1; resistant:susceptible) and the segregation data for the AFLP markers and the resistance/susceptibility trait. The segregation of AFLP markers and linkage analysis were performed on 116 F_2 individuals from the IT82D-849×Tvx3236 population and 150 F_2 individuals from the IT84S-2246–4×Tvu 14676 population. Linkage analysis between the AFLP markers and the S. gesnerioides resistance loci was performed using the MAPMAKER 3.0 program (Lander et al. 1987). The "group" command was used to determine linkage groups, pair-wise comparisons, and grouping of markers. A LOD score of 3.0 or above and a maximum recombination frequency of 30% were specified. To determine the most likely order within a linkage group we used the "compare" command, and the best order was accepted based on a log-likelihood difference of two or more. The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequency into map distances in centiMorgans (cM).

In order to place the AFLP markers identified in this study on the existing map of the cowpea genome (Menéndez et al. 1997), we tested the EcoRI and MseI primer combinations giving rise to the polymorphic band in the IT82D-849×Tvx3236 or the IT84S-2246–4×Tvu 14676 population, on the parents and 88 recombinant inbreds resulting from a cross between IT84S-2049 and 524B used to create a genetic linkage map of 181 markers. Markers were placed on the map using MAPMAKER 3.0 based on the conditions previously described by Menéndez et al. (1997).

Results

Identification of AFLP markers linked to the S. gesnerioides race 1 resistance locus Rsg2–1

Resistance to S. gesnerioides race 1 is inherited as a single dominant trait in all four populations derived from crosses involving the Striga-resistant lines Gorom, B301, IT81D-994, and IT82D-849 and Tvx3236, a Striga-susceptible line (Table 2). Examination of the level of polymorphism between each pair of parental lines (Tvx3236×Gorom, Tvx3236×B301, Tvx3236×IT81D-994, and Tvx3236×IT82D-849) available in this study using AFLP analysis and 16 combinations of EcoRI and MseI primers determined that Tvx3236×IT82D-849 had the highest level of polymorphism (data not shown). This latter population was

Table 2 Segregation ratios for resistance to S. gesnerioides race 1 (Burkina Faso) and race 3 Nigeria)

Population	Population/ genotype		Number of plants per genotype	Ratio	Chi square	P value
Tvx3236×Gorom[a]	F_2	R–	116	3:1	0.57	0.40–0.50
		rr[c]	33			
Tvx3236×B301[a]	F_2	R–	224	3:1	0.40	0.60–0.70
		rr	68			
Tvx3236×IT81D-994[a]	F_2	R–	164	3:1	0.40	0.60–0.70
		rr	49			
Tvx3236×IT82D-849[a]	F_2	R–	212	3:1	0.90	0.30–0.40
		rr	62			
IT84S-2246–4× Tvu 14676[b]	F_2	R–	111	3:1	0.138	0.70–0.80
		rr	39			

[a] Population screened against S. gesnerioides from Burkina Faso
[b] Population screened against S. gesnerioides from Nigeria
[c] R–, Homozygous and heterozygous resistant, respectively; rr, homozygous susceptible

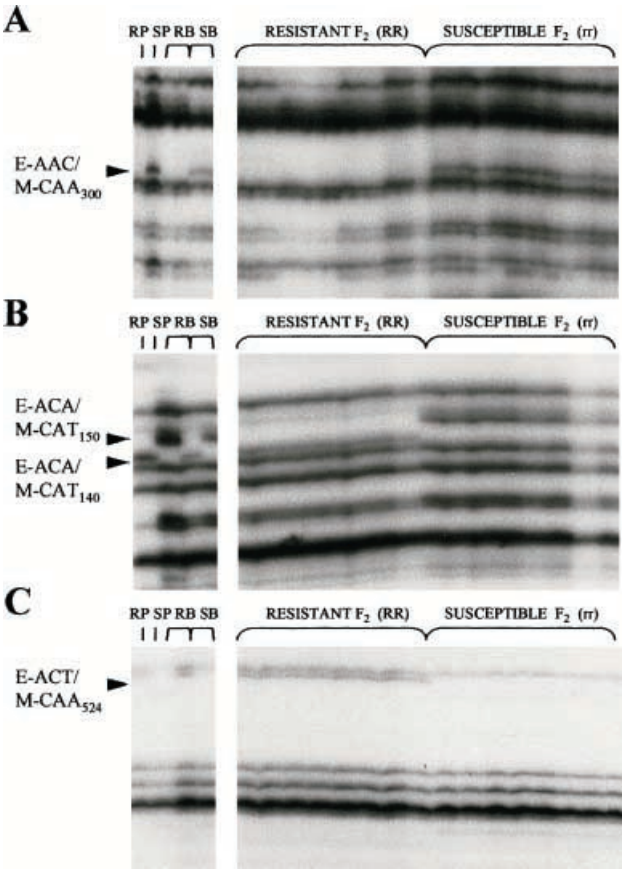

Fig. 1A–C Autoradiographs showing AFLP markers linked to Striga gesnerioides race 1 resistance gene Rsg2–1. Shown are the results of an AFLP analysis of DNA taken from resistant parent (RP) IT82D-849, susceptible parent (SB) Tvx 3236, resistant (RB) and susceptible (SB) DNA bulks from F_2 progeny and DNA from individual homozygous resistant (RR) or susceptible (rr) F_2 progeny using different AFLP primer combinations. A The marker E-AAC/M-CAA$_{300}$ (indicated by arrowheads), linked in trans to the resistance locus Rsg2–1, was amplified by primer combination E-AAC+M-CAA. B The markers E-ACA/M-CAT$_{150}$ and E-ACA/M-CAT$_{140}$ (indicated by arrowheads) were amplified by the primer combination E-ACA+M-CAT and are linked in trans and in cis, respectively, to the Rsg2–1. C The marker E-ACT/M-CAA$_{524}$ (indicated by arrowhead), linked in cis to Rsg2–1, was amplified by primer combination E-ACT + M-CAA

subsequently used to identify molecular markers linked to Rsg2. Homozygous-resistant and homozygous-susceptible bulks (each containing pooled DNA from 12 F_2 individuals) were tested together with the two parental lines using a total of 40 selective AFLP primer combinations. The number of fragments amplified with each primer combination ranged from 58 to 102, with the mean number of informative markers being approximately 80. The greatest number of detectable fragments (92–102) were produced with the MseI+3 primers, M-CAA, M-CAT, and M-CTT, combined with any of the eight EcoRI+3 primers (e.g., E-AAC, EACA, etc.) tested. The number of polymorphic bands between the two parents ranged from 15 to 21, with a mean number of 18 depending on the primer pair used. The size of the fragments varied between 75 bp and 600 bp.

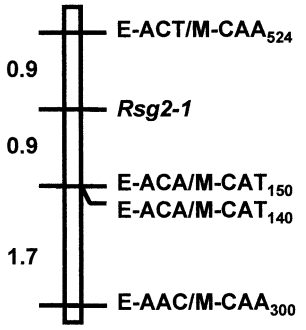

Fig. 2 Map showing the linkage of AFLP markers E-AAC/M-CAA$_{300}$, EACA/M-CAT$_{150}$, E-ACA/M-CAT$_{140}$ and E-ACT/M-CAA$_{524}$ to S. gesnerioides race 1 resistance gene Rsg2–1 obtained by analysis of F_2 progeny from the cross of IT82D-849×Tvx 3236. Map distances are shown in centiMorgans

Three primer combinations detected a polymorphism between the parental lines and the resistant and susceptible bulks. Markers were identified that were linked either in cis or trans relative to the Rsg2 resistance allele. Markers segregating with the resistance phenotype were linked in cis relative to the Rsg2 resistance allele and were observed only in resistant individuals, whereas markers segregating with susceptibility were linked in trans and were present only in susceptible individuals. Primer combination E-AAC/M-CAA revealed an approximately 300-bp product present in Tvx3236 (the susceptible parent) and in the susceptible bulks. This marker, linked in trans to the Rsg2 allele, was designated E-AAC/M-CAA$_{300}$. The E-AAC/M-CAA$_{300}$ marker was not observed in any of the 24 resistant F_2 individuals comprising the two resistant bulks but was present in all 24 susceptible individuals comprising the two susceptible bulks (Fig 1 A).

Primer combination E-ACA/M-CAT detected codominant markers, which we have designated as E-ACA/M-CAT$_{140}$ and E-ACA/M-CAT$_{150}$(Fig 1B). E-ACA/M-CAT$_{150}$ (corresponding to a 150-bp amplification product) is linked in trans with the Rsg2 resistance allele and is present in susceptible individuals. On the other hand, E-ACA/M-CAT$_{140}$ (corresponding to a 140-bp amplification product) is linked in cis and present only in resistant plants. Plants heterozygous at this locus exhibit both fragments. Finally, primer combination E-ACT/M-CAA amplified a 524-bp fragment, linked in cis to the Rsg2 resistance allele, that segregated with resistance in the bulks and F_2 individuals (Fig 1 C). This marker was designated E-ACT/M-CAA$_{524}$.

To determine the degree of linkage between the three AFLP markers and Rsg2, we analyzed 116 F_2 individuals from the Tvx3236×IT82D-849 cross using the three primer combinations described above. The E-AAC/M-CAA$_{300}$ and E-ACT/M-CAA$_{524}$ markers were dominant and segregated according to a 3:1 ratio, whereas the E-ACA/M-CAT$_{140/150}$ markers segregated in the expected 1:2:1 fashion. The linkage analysis performed by using MAPMAKER 3.0 (Lander et al. 1987) showed that all markers belonged to the same linkage group and are or-

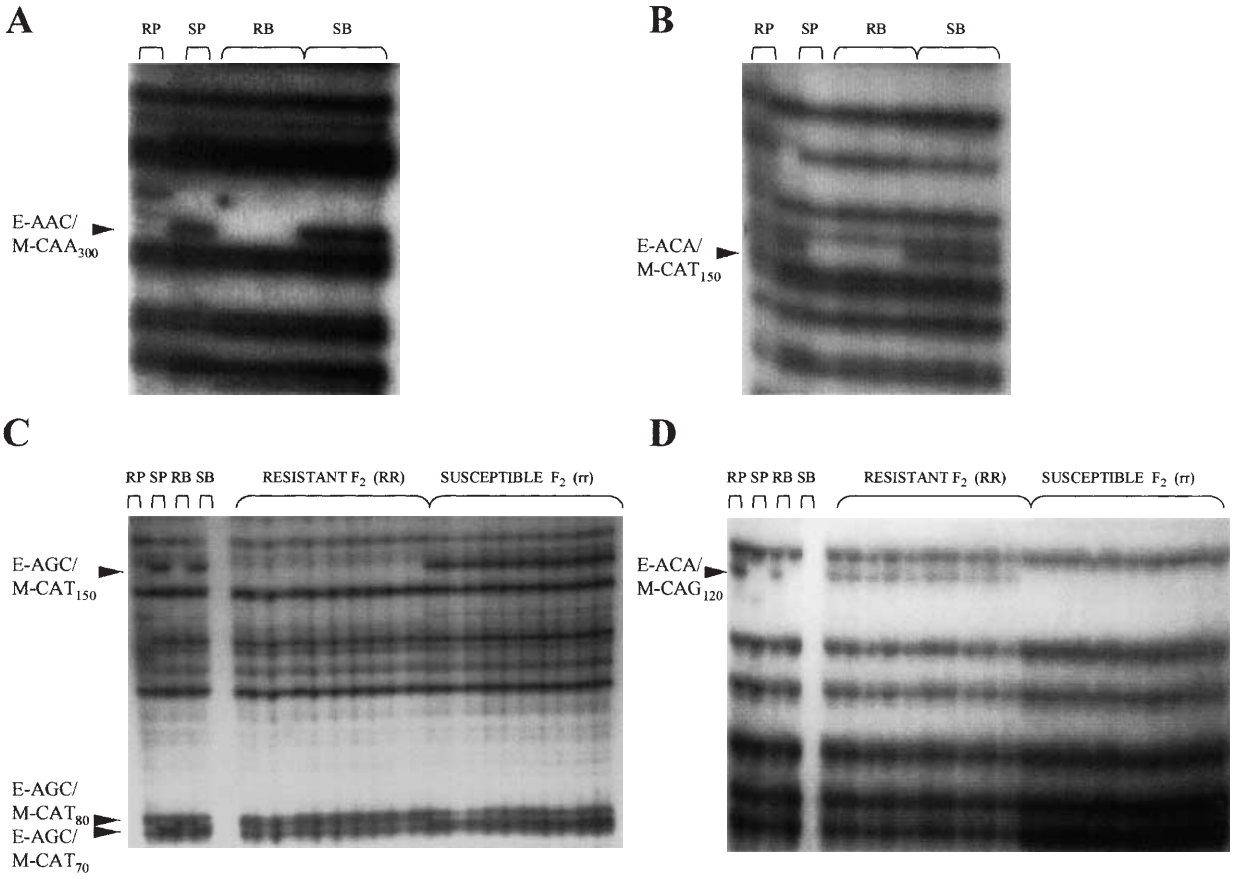

Fig. 3A–D Autoradiographs showing AFLP markers linked to S. gesnerioides race 3 resistance gene Rsg4–3. Shown are the results of an AFLP analysis of DNA taken from resistant parent (RP) Tvu 14676, susceptible parent (SB) IT84S-2246–4, resistant (RB) and susceptible (SB) DNA bulks from F_2 progeny and DNA from individual homozygous resistant (RR) or susceptible (rr) F_2 progeny using different AFLP primer combinations. A The marker E-AAC/M-CAA_{300} (indicated by arrowhead), linked in trans to the resistance locus Rsg4–3, was amplified by primer combination E-AAC+M-CAA. B The marker E-ACA/M-CAT_{150} (indicated by arrowhead), linked in trans to the resistance locus Rsg4–3, was amplified by primer combination E-AAC+M-CAT. C The markers E-AGC/M-CAT_{150} and E-AGC/M-CAT_{70} (indicated by arrowheads) were amplified by the primer combination E-AGC +M-CAT and are linked in in cis to Rsg4–3, whereas the marker E-AGC/M-CAT_{80} is linked in trans to Rsg4–3. D The marker E-ACA/M-CAG_{120} (indicated by arrowhead), linked in trans to Rsg4–3, was amplified by primer combination E-ACA+M-CAG

dered as shown in Fig. 2. Based on recombination frequency, the map distances between the AFLP markers and Rsg2 were determined to be 2.6 cM for E-AAC/M-CAA_{300}, 0.9 cM for E-ACA/M-$CAT_{140/150}$, and 0.9 cM for E-ACT/M-CAA_{524}. The gene locus is flanked by the markers E-ACT/M-CAA_{524} on one side and E-ACA/M-$CAT_{140/150}$ and E-AAC/M-CAA_{300} on the other side, with the entire linkage group spanning a total distance of 3.5 cM (Fig. 2). Thus, all three markers are tightly linked to the S. gesnerioides race 1 resistance gene Rsg2. We suggest the designation of Rsg2–1 for this locus to refer to its resistance to the S. gesnerioides race 1.

Identification of AFLP markers linked to Rsg4–3, a S. gesnerioides race 3 resistance locus

Analysis of F_2 progeny from a cross involving the Striga-susceptible line IT84S-2246–4 and the Striga-resistant line Tvu 14676 showed that resistance to S. gesnerioides race 3 from Nigeria was inherited as a single dominant trait (Table 2). The resistance gene present in Tvu14676 has been designated as Rsg4–3, although at the present time no data exists indicating whether or not this locus is the same as or different from Rsg1, Rsg2, or Rsg3.

Using the same approach described above for the identification of molecular markers linked to Rsg2–1, we performed AFLP analysis on parental DNA and bulked pools of DNA of F_2 plants from the IT84S-2246–4×Tvu 14676 cross. As shown in Fig. 3, linkage of six different markers to the resistance locus was identified. Two markers, E-AAC/M-CAA_{300} and E-ACA/M-CAT_{150}, were derived from primer combinations found effective in the analysis of the Tvx3236×IT82D-849 cross (Fig. 3A, B). These two markers were linked in trans to both Rsg2–1 and Rsg4–3. The primer combination E-AGC/M-CAT yielded three additional markers for the Rsg4–3 locus. Two of these markers, E-AGC/M-CAT_{150} (150 bp) and E-AGC/M-CAT_{70} (70 bp) were linked in trans with the resistance allele, and the third marker, E-AGC/M-CAT_{80} (80 bp), was linked in cis (Fig. 3 C). The final marker identified in this group was E-ACA/M-CAG_{120} (120 bp), obtained with the primer combinations

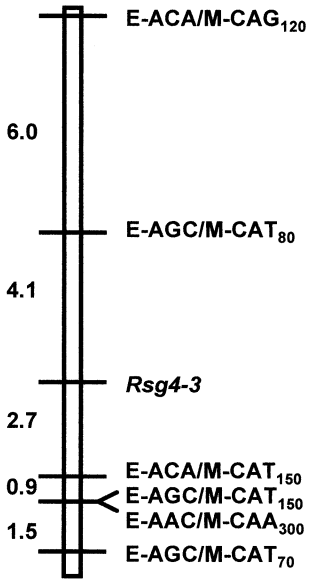

Fig. 4 Map showing the linkage of AFLP markers E-AAC/M-CAA_{300}, E-ACA/M-CAT_{150}, E-AGC/M-CAT_{150}, E-AGC/M-CAT_{70}, E-AGC/M-CAT_{80} and E-ACA/M-CAG_{120} to S. gesnerioides race 3 resistance locus (Rsg4–3) obtained by analysis of F_2 progeny from the cross of Tvu 14676×IT84S-2246–4 F_2 progeny. Map distances are shown in centriMorgans

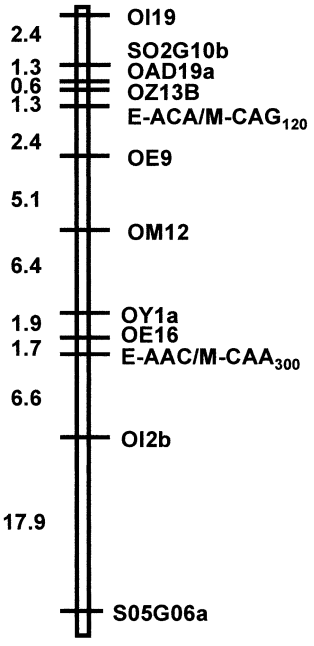

Fig. 5 Map showing the placement of AFLP markers linked to S. gesnerioides race 1 (Rsg2–1) and race 3 (Rsg4–3) resistance genes on partial map of linkage group 1 of the cowpea genetic map. Map distances are shown in centiMorgans

E-ACA/M-CAG. This marker was present in the resistant parent (Tvu14676) and the resistant bulk but absent in the susceptible parent (IT84S-2246–4) and the susceptible bulk (Fig. 3D).

Linkage analysis showed that the six markers were linked to Rsg4–3 and mapped to a contiguous region spanning 15.2 cM as shown in Fig 4. Based on the recombination frequency, the distance between each marker and the Rsg4–3 locus is as follows: 10.1 cM for E-ACA/M-CAG_{120}, 4.1 cM for E-AGC/M-CAT_{80}, 2.7 cM for E-ACA/M-CAT_{150}, 3.6 cM for E-AGC/M-CAT_{150} and EAAC-MCAA_{300}, and 5.1 cM for E-AGC/M-CAT_{70}.

Placement of Rsg2–1 and Rsg4–3 on the cowpea genetic linkage map

A genetic linkage map for cowpea was constructed by Menéndez et al. (1997) using an F_8 recombinant inbred population derived from a cross between IT84S-2049 and 524B, breeding lines developed in Nigeria and California, respectively. IT84S-2049 and IT84S-2246–4 are independent selections derived from a common parental line. For Rsg2–1 and Rsg4–3 to be placed on the cowpea genetic map, primer combinations detecting polymorphisms in our two mapping populations were used to analyze IT84S-2049 and 524B. The E-AAC/M-CAA_{300} and E-ACA/M-CAG_{120} markers were found to be polymorphic between the two parents, with the markers being present in IT84S-2049 and absent in 524B. By analyzing the segregation of the E-AAC/M-CAA_{300} and E-ACA/M-CAG_{120} markers in 88 individuals of the F8 recombinant inbred population, we mapped these markers to the bottom of linkage group 1 (Fig. 5). The two markers are approximately 17.5 cM apart, with E-ACA/M-CAG_{120} flanked by markers OZ13b (1.3 cM) and OE9 (2.4 cM) and E-AAC/M-CAA_{300} flanked by OE16 (1.7 cM) and OI2b (6.6 cM).

Discussion

Five races of S. gesnerioides are known to exist in West and Central Africa that are capable of parasitizing cowpea. Although resistance to parasitism by S. gesnerioides is known to exist in some selected breeding lines and commercially grown cultivars of cowpea (Berner et al. 1985), well-adapted, high-yielding cultivars resistant to all five races of S. gesnerioides are still not available to farmers. Inheritance studies have found evidence for at least three independent dominant genes, designated as Rsg1, Rsg2, and Rsg3, capable of conferring resistance to S. gesnerioides (Atokple et al. 1995; Singh and Emechebe 1997; Touré et al. 1997).

Segregation analysis of the F_2 progenies of the four different populations of susceptible×resistant cowpea crosses used in the present study showed that a single dominant gene conferred resistance to the S. gesnerioides race 1 from Burkina Faso in all four populations. This finding confirms the earlier work of Aggarwal et al. (1984) and is consistent with field studies carried out at the International Institute for Tropical Agriculture (Anonymous 1987a) and the "Institut de l'Environnement et de Recherches Agricoles" (Anonymous 1987b)

in Burkina Faso demonstrating race 1 resistance in cvs. Gorom local (Suvita-2) and B301. Our studies extend these earlier findings, demonstrating that cvs. IT81D-994 and IT82D-849 also contain a single dominant gene conferring S. gesnerioides race 1 resistance. Allelism tests, currently underway, should resolve the relationship between the resistance allele(s) present in IT81D-994 and Rsg1, Rgs2, and Rsg3.

Previous studies of S. gesnerioides-cowpea interactions revealed that a single dominant gene in cvs. B301, Suvita-2, and IT82D-849 conferred resistance to S. gesnerioides race 2 from Mali (Touré et al. 1997) and race 3 from Niger/Nigeria (Singh and Emechebe 1990a; Atokple et al. 1995). However, Touré et al. (1997) found evidence for a susceptible allele to S. gesnerioides race 3 from Maradi, Niger in IT82D-849. Taken together, these results suggest that the same resistance genes in Suvita-2, B301, and IT82D-849 could be active on several Striga races (e.g., races 1 and 2 for Suvita-2 and races 1, 2, and 3 for B301 and IT82D-849). Whether the race 3 resistance allele Rsg4–3 (present in Tvu 14676) is also active against other races is also in need of further evaluation.

Using a combined strategy of AFLP analysis and bulked segregant analysis, we were able to identify three molecular markers tightly linked to the Rsg2–1 locus, that confers resistance to S. gesnerioides race 1 from Burkina Faso and six markers linked to the Rsg4–3 locus conferring resistance to S. gesnerioides race 3 from Nigeria. Of the various markers identified, two markers, E-AAC/M-CAA_{300} and E-ACA/M-CAT_{150}, were linked to both Rsg2–1 and Rsg4–3, suggesting that some clustering of resistance genes for Striga occurs within the cowpea genome. The existence of complex resistance loci, displaying either a multiallelic structure or clustering with each allele or gene leading to a different specificity, is now well-documented in the literature (see Pryor and Ellis 1993; Michelmore and Meyers 1998; Ronald 1998). The clustering of resistance genes effective against unrelated pathogens has also been described (Polzin et al. 1994; Witsenboer et al. 1995; Ronald 1998; Ashfield et al. 1998). It has also been suggested that the clustering of resistance genes may facilitate the generation of new specificities through gene duplication and mutation and that unequal crossing-over during recombination and/or gene conversion has contributed to resistance gene evolution (Hammond-Kosack and Jones 1997; Jones and Jones 1997; Michelmore and Meyers 1998). Thus, based on our findings, it is not unreasonable to assume that resistance genes for S. gesnerioides may be clustered within the cowpea genome. If this were the case, one might predict that some or all of the markers identified here will be immediately useful in the analysis of other populations of cowpea segregating for other race-specific resistances to S. gesnerioides. Equally intriguing is the possibility that loci conferring resistance to other parasitic plants might also be located within this cluster, since it is known that in addition to resistance to S. gesnerioides, B301 carries duplicate dominant genes for resistance to Alectra vogelii (Benth.), that are distinct from Rsg1 (Singh and Emechebe 1997).

At the present time the molecular basis for resistance in host plants to parasitism by Striga or any other parasitic angiosperm is not known. The placement of the E-AAC/M-CAA_{300} and E-ACA/M-CAG_{120} markers on the existing genetic linkage map for cowpea developed by Menéndez et al. (1997) opens up the possibility for eventually cloning and characterizing S. gesnerioides resistance genes by using one or more of the currently available methods for map-based cloning (Kumar 1999; Simpson 1999). Based on their recombination frequency and degree of linkage to either Rsg2–1 or Rsg4–3, the markers identified in this present study appear to be highly suitable for use in MAS programs (Ribaut and Hoisington 1998; Kumar 1999) aimed at the introgression of resistance Striga race 1 and 3 into promising breeding lines. To this end we are currently attempting to make these markers more informative tools by transforming them into sequence-characterized amplified regions (SCAR) (Paran and Michelmore 1993). Given the recent report by Lu et al. (2000) on the development of SCAR markers linked to the Or5 gene conferring resistance to broomrape (Orobanche cumana Wallr.) in sunflower, it is likely that we will soon know whether mechanisms for resistance to these noxious weeds follow the same paradigms recognized for other plant-pathogen interactions or whether plants have developed unique methods for warding off attack from other plant species.

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