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An intraspecific genetic map of velvetbean (*Mucuna* sp.) based on AFLP markers

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Abstract Velvetbean (*Mucuna* sp., *n*=11), a self-pollinated species, is an important legume used in tropical agricultural systems in rotation with other crops for nematode management and/or soil improvement. A genetic map of velvetbean was constructed in order to identify potential molecular markers linked to important morphological and agronomic traits that would be particularly useful for developing and improving the species. Traits such as seed coat color, pod color, and pod pubescence were among the main parameters observed in a process of genetic diversity estimation. Two slightly divergent velvetbean accessions, PI364362 and 'Edgar Farm White', a land race from Alabama, were used to make an intraspecific F_1 hybrid. Amplified fragment length polymorphism analysis (AFLP) detected an average of six polymorphic fragments per primer pair between the two parents. As expected for dominant markers, the sum of all AFLP bands from both parents was generally observed to be present in the AFLP profiles of the F_1 progeny, indicating full penetrance and the dominant nature of AFLP markers. An F_2 population was generated by self-pollinating a single F_1 plant. Using 37 AFLP primer pairs, we detected 233 polymorphic markers of which 164 (70.4%) segregated in 3:1 Mendelian ratios, while the remaining 69 (29.6%) both segregated and were scorable. The genetic linkage map constructed from this population comprised 166 markers, including two morphological traits (pod color and pod pubescence). Twenty linkage groups were found with an average distance between markers of 34.4 cM, covering a total of 687.9 cM. The linkage groups contained from 2 to 12 loci each and the distance between two consecutive loci ranged from 0 to 21.8 cM. The newly designated morphological traits pod color (*pdc*) and pod pubescence (*pdp*) co-segregated with each other at a distance of 4.2 cM. Two DNA markers designated ACGCAG2 and ACTCTG1 were located in the same group as *pdc* and *pdp*. The AFLP linkage map provides opportunities for use in marker-assisted selection and in the detection of loci controlling morphologically important traits.

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Introduction

The velvetbean (*Mucuna* sp.) is an important tropical legume. It is a self-pollinated crop (Duke 1981). Reports demonstrate that velvetbean has been successful in decreasing nematode populations and/or improving the physical properties of soil (Miller 1902; Piper and Tracy 1910; Ferris 1917; Piper and Morse 1938; Weaver et al. 1993). These characteristics make velvetbean a legume of excellent choice for rotation or inter-cropping systems.

Genetic variability has been reported within the cultivated species using molecular tools (Capo-chichi et al. 2001). The genus *Mucuna* (Adans) covers perhaps 100 species of annual and perennial legumes, including the annual velvetbean (Buckles 1995). Within the genus, there are numerous hybrids (Piper and Tracy 1910; Bailey 1947; Burkill 1966). The most commonly cited species include *M. deeringiana* (Bort) Merrill, *M. utilis* Wallich (Bengal velvetbean), *M. pruriens* (L.) DC, *M. nivea* (Roxb.) DC, *M. hassjoo* (Piper and Tracy) Mansl. (Yokohama velvetbean), *M. aterrima* Holland (Mauritius and Bourbon velvetbean), *M. capitata* (Sweet), and *M. diabolica* Keuchenius (Bailey 1947; Duke 1981). Comparing the gametic chromosome number, previous researchers reported *Mucuna* sp. to have an *n*=11, except for *M. benetti* (Muell) and *M. gigantea* (Willd) DC, which possess *n*=14 (Sastrapradja et al. 1974). The occurrence of

n=14 in *Mucuna* raises a question as to whether *n*=11 or *n*=14 is the basic complement of the genus. Sastrapradja et al. (1975) found that the seedling morphology features for both *M. benetti* and *M. gigantea* fall into the same grouping with *M. acuminita* (Merrill) and *M. macrophylla* (Miq.) and yet the chromosome number of the last two species is *n*=11.

The size, shape, and color of the seed are the principal points of difference. Velvetbean seeds vary from nearly white to marbled brown, brown, and black. Sastrapradja et al. (1972) observed considerable variation in the seed coat color in *M. pruriens* DC ranging from white to black, either in simple or mottled coloration. Piper and Morse (1938) reported that varieties which commonly produce marbled seeds may also produce entirely white or colored seeds. This suggests that populations with marbled seeds are heterogeneous. Lubis et al. (1980) pointed out that multigenic factors were involved in the production of the colors, as a result of a cross between two different species distinct in their seed coat color.

The pod traits include differences in size, shape, shape of ridges on the valves, and pubescence. Pod pubescence has been reported to be one of the most variable features found in any legume (Piper and Tracy 1910). The velvetbean pod pubescences used in this study are of two distinct types: one being covered with a dense, black velvety pubescence while the other type consists of a short, gray pubescence (Piper and Morse 1938). The morphology of pod hairs in *M. pruriens* can be used to identify whether a taxon is cultivated or wild (Aminah et al. 1974; Lubis et al. 1979). In general, wild *Mucuna* taxa are characterized by their large size, coarse texture and irritant hairs. The mode of inheritance of the pod hairs showed that two genes, designated R and N, were responsible for determining the trait (Lubis et al. 1979). The pods of some varieties are 2–3 in. long, while those of others may reach a length of 5–6 in.. Calgari et al. (1993) observed a range of pod length of 5–8 cm for *M. deeringiana*, 9–13 cm for *M. pruriens* and *M. aterrima*.

AFLP analysis is a powerful DNA fingerprinting technique for detecting genetic variability and genome mapping (Zabeau and Vos 1993; Vos et al. 1995; Maughan et al. 1996; Maheswaran et al. 1997). It is highly informative and efficient for the early identification of traits. The utilization of AFLP markers in genetic linkage mapping (Ballvora et al. 1995; Becker et al. 1995; Mackill et al. 1996) and analyses of genetic pools (Folkertsma et al. 1996; Travis et al. 1996) has facilitated progress that would otherwise have taken much longer. Molecular marker-based linkage maps have been useful for identifying and localizing important genes controlling both qualitatively and quantitatively inherited traits in a wide range of species (Tanksley et al. 1989).

For mapping, intraspecific crosses have advantages over interspecific crosses, because many breeding programs use intraspecific variability, particularly for polygenic characteristics, and the problems of low fertility and low recombination rates often found in interspecific crosses are reduced (Lefebvre et al. 1995). The objective of this study was the construction of a genetic linkage map of velvetbean to be used in genetic studies, as well as in programs involving the breeding of the species. The availability of these genetic maps will be useful for the detection of loci contributing to quantitative trait expression, and will eventually increase the efficiency of breeding and selection programs, both of which are at early stages of development.

Materials and methods

Plant material

Traits were measured for two years; one year for the F_1 and the second year for the F_2. An intraspecific F_2 population was generated by self-pollination of a single F_1 plant from a cross between velvetbean accessions 'Edgar Farm White', a land race from Alabama and PI 364362, an exotic line. The two parents differed for seed coat color, pod color, and pod pubescence. These traits are among the main parameters observed in a process of genetic diversity. The female parent (Edgar Farm White) produced black pods which were generally covered with velvet-like black hairs and had seeds with a white seed coat, while the pods of the male parent (PI 364362) were greenish, covered with gray hairs and had a black seed coat. The F_2 population consisted of 82 plants, all of which were maintained in the field under standard culture conditions for phenotypic evaluation. F_2 plants were scored for their pod pubescence and pod color phenotypes.

The AFLP protocol

Total genomic DNA was isolated from fresh green leaf material according to the shortened 2xCTAB procedure of Doyle and Doyle (1990). DNA concentration was estimated by comparison of the fluorescence intensities of ethidium bromide-stained samples to those of λDNA standards on 1% agarose gels. The AFLP method was performed essentially as described in Vos et al. (1995), with minor modifications. The templates for the AFLP reactions were prepared using approximately 250 ng of genomic DNA from the parents and each of their progeny for restriction digestion with *Eco*RI and *Mse*I and for the ligation of adapters. The digestions were carried out in a final volume of 25 μl at 37°C for 2 h, then all samples were heated to 70°C for 15 min to inactivate the restriction enzymes. *Mse*I and *Eco*RI adapters were subsequently ligated to the digested DNA fragments by adding 24 μl of adapter ligation solution and 1 μl of T4 DNA ligase to the digested samples. The ligation reactions were incubated for 2 h at 20°C and then up to overnight at 4°C. Digested-ligated DNA fragments were then used as templates for the preamplification step.

Preamplification reactions were performed in a 25.5 μl volume containing 2.5 μl 10xPCR buffer for AFLP (100 mM TrisHCl pH 8.3, 15 mM $MgCl_2$, 500 mM KCl), 20 μl pre-amp primer mix, 2.5 μl template DNA, and 0.5 U Taq DNA polymerase. PCR amplification consisted of 20 cycles of 94°C (denature) for 30 s, 56°C (annealing) for 60 s, and 72°C (extension) for 60 s. Selective amplifications were performed using various combinations of *Eco*RI and *Mse*I primers, both with three selective nucleotides. Primers were purchased from Life Technology as individual oligonucleotides. A volume of 5.3 μl PCR reaction contained 1 μl of preamplification product, 0.3 μl of *Eco*RI primer, 1 μl of *Mse*I primer, and 3 μl TaqMix. The TaqMix for 50 samples consisted of 30 μl 10xPCR buffer, 18 μl $MgCl_2$, 100.5 μl dd H_2O, and 1.5 μl Perkin Elmer Taq polymerase. This second amplification was carried out by programming a touch-down cycle profile (Don et al. 1991) as follows: 94°C for 30 s, 65°C (-0.7°C/cycle) for 30 s, and 72°C for 60 s during 12 cycles, until reaching the optimal annealing temperature of 56°C. At this temperature, 23 more cycles

were achieved to complete the second amplification. The products were held at 4°C.

Gel analysis

AFLP reaction products were resolved on denaturing gels containing 8% Long Ranger polyacrylamide (9.5 g of urea, 2.7 ml 10xTBE, 3.6 ml Long Ranger solution, and 7.5 ml ddH_2O). Each gel consisted of 23.3 g of gel solution with 150 μl of 10% ammonium persulfate solution and 15 μl of tetramethylenediamine as the catalysts. Following the amplification reaction, the PCR products were mixed with 3 μl of formamide stop/loading buffer. This mixture was heated at 94°C for 3 min, then immediately chilled to 4°C before a 0.8 μl aliquot of each sample was loaded on the gel using an 8-channel Hamilton syringe (Hamilton Company, Reno, Nev.). Electrophoresis was conducted on a Li-Cor Long ReadIR DNA sequencer (Li-Cor, Lincoln, Neb.) using 1xTBE, freshly prepared from a 10xTBE stock solution with run parameters of 1,500 V, 40 mA, 25 W, signal channel 3, motor speed 3 or 4, a plate temperature of 45°C and 16-bit pixel depth for collection of the TIFF image files. Square-tooth combs with 48 wells were used.

Scoring of AFLP fragments

The AFLP fragments (size: 75–500 bp) were scored as dominant, i.e. presence versus absence of bands, and therefore for the parents, F_1 and F_2 generations markers were assigned to either parental allele for map construction. Markers originating from each parent were scored according to the standard coding system using A, B, C, D and H of JoinMap version 3.0 (Van Ooijen and Voorrips, 2001). Ambiguous genotypes were resolved by assigning a blank score (-) to the individual locus for map construction. The AFLP markers were designated by the primer pair used, followed by a number reflecting the fragment position on the gel (e.g., AAGCTG4= *Eco*RI+AAG/*Mse*I+CTG, band 4). The numbers given the markers are in ascending molecular weight order. Chi-square (χ^2) tests of goodness-of-fit were performed on segregation data for all markers and the morphological traits evaluated; pod color at maturity and pod pubescence color. The seed coat color used as one of the criteria for selecting the parents did not segregate in the F_2 individuals, therefore this trait was discarded. Each marker was tested for segregation ratios expected under the disomic inheritance patterns. Only markers segregating in a 3:1 ratio were mapped using the JoinMap version 3.0

Linkage analysis of markers and map construction

Linkage analyses and segregation tests were performed with the computer software package JoinMap version 3.0. A χ^2 test for skewness was performed with a threshold level for significance of 0.5%. Markers dominantly scored were tested against the 3:1 ratio. Morphological markers codominantly scored were tested against the 1:2:1 ratio, referring to homozygous PI364362: heterozygous: homozygous 'Edgar Farm White'. For linkage analysis, markers were assigned to linkage groups by increasing the LOD score for grouping in steps of one LOD unit. The calculations of the linkage maps were done by using all pairwise recombination estimates smaller than 0.45 and Kosambi's mapping function (Kosambi 1944).

Results

Transmission of AFLP markers to F_1 progeny

To evaluate the usefulness of AFLP markers in genetic mapping using intraspecific reference mapping populations, analyses were performed using genomic DNA isolated from different strains of two velvetbean accessions to determine their polymorphism levels. We first examined the transmission of AFLP markers to F_1 progeny to determine their suitability for genetic mapping analysis. Twelve AFLP primer pairs amplified fragments from the original parents and the F_1 individuals, which led to a total of 72 polymorphic fragments between the parents and 75 between the parents and the F_1 progeny. Polymorphic markers ranged from 2 (for E-ACT/M-CTC and E-ACG/M-CAG) to 12 (for E-ACT/M-CAT) with an average of 6 per primer pair between the parents, and from 2 (for E-ACT/M-CTC and E-ACG/M-CAG) to 12 (for E-ACT/M-CAT) with an average of 6.2 per primer pair between the parents and the F_1 progeny (Table 1).

As expected for dominant markers, the sum of all AFLP fragments from both parents were generally observed to be present in the AFLP profiles of the F_1

Table 1 Level of polymorphic markers between parents, between parents and F_1 progeny, and transmission of AFLP markers to F_1 progeny using 12 primer combinations. *Eco* RI + 3:3′ end selective nucleotides of the primers are complementary to the *Eco* RI and *Mse* I sites, respectively

Primer combinations		Polymorphic markers between parents	Polymorphic markers between parents and F_1 progeny
*Eco*RI+3	*Mse*I+3		
AAG	CAA	4	4
AAG	CAG	3	3
AAG	CAT	6	6
AAG	CTC	10(2) [a]	8
AAG	CTG	7	9(2) [b]
AAG	CTT	9	9
ACC	CAA	7	10(3)
ACG	CAG	2	2
ACT	CAG	4	4
ACT	CAT	12	12
ACT	CTC	2	2
AGT	CAG	6	6
Total		72	75
Mean		6	6.2
Maximum		12	12
Minimum		2	42

[a] Markers were not transmitted from the parents to the F_1

[b] Non-parental markers probably due to recombination or markers that were heterozygous in the parents and segregated in the F_1

Table 2 Number of AFLP amplification products generated with 37 different primer combinations and number of markers segregating 3:1 over the primer combinations tested

Primer combinations		Total number of polymorphic markers	Markers segregating 3:1	Markers showing segregation ratio distortion
*Eco*RI+3	*Mse*I+3			
AAC	CAA	8	6	2
AAC	CAC	4	4	0
AAC	CAG	11	4	7
AAC	CAT	8	8	0
AAC	CTA	12	8	4
AAC	CTC	14	10	4
AAC	CTG	6	1	5
AAC	CTT	7	4	3
AAG	CAA	3	3	0
AAG	CAC	5	4	1
AAG	CAG	3	3	0
AAG	CAT	6	5	1
AAG	CTA	10	6	4
AAG	CTC	8	8	0
AAG	CTG	5	4	1
AAG	CTT	6	2	4
ACC	CAA	10	7	3
ACC	CAC	5	4	1
ACC	CAG	7	4	3
ACC	CAT	5	4	1
ACC	CTA	8	8	0
ACC	CTC	2	1	1
ACC	CTG	2	2	0
ACG	CAA	1	1	0
ACG	CAG	2	2	0
ACG	CTA	10	5	5
ACG	CTC	9	6	3
ACG	CTG	3	3	0
ACG	CTT	8	6	2
ACT	CAA	3	2	1
ACT	CAC	11	6	5
ACT	CAG	4	4	0
ACT	CAT	12	8	4
ACT	CTA	3	2	1
ACT	CTC	2	2	0
ACT	CTG	7	5	2
ACT	CTT	3	3	0
Total		233	164	69
Percent		100	70.4	29.6

progeny, except for two of the ten markers generated by E-AAG/M-CTC, indicating full penetrance and the dominant nature of AFLP markers. The F_1 progeny should possess one set of chromosomes from PI364362 and the other from 'Edgar Farm White', thus allowing amplification of all AFLP fragments from both parents. The polymorphic paternal and maternal fragments were expected to show a dominant Mendelian pattern of inheritance in the F_1 progeny. Few cases of segregation of polymorphic AFLP markers were observed in the F_1 individuals, indicating that the majority of the polymorphic loci were homozygous in the parents. The majority of the AFLP markers, therefore, represent individual-specific markers. Those segregating in the F_1 progeny are probably either heterozygous in the parents, or non-parental fragments formed between allelic AFLP products from both paternal and maternal parents. The results also indicated that chromosomes of both parents paired properly, and most of their AFLP markers followed Mendelian inheritance. The reproducibility of each marker was assessed by using DNA templates from different individual plants of each parent and F_1 hybrid, isolated at different times. The results of this analysis showed that these markers are reproducibly amplified.

Segregation of AFLP markers and morphological traits in the F_2

Segregation of AFLP markers was observed for all polymorphic AFLP loci between the original parents, including the non-parental fragments in the F_1 progeny. A great majority of the AFLP markers followed Mendelian segregation in the F_2, indicating that these AFLP markers behave as heritable traits. A total of 233 polymorphic markers were generated by 37 primer pairs (Table 2). The number of polymorphic markers in the F_2 population, regardless of their origin, varied according to the primer pair used. Polymorphic markers ranged from 1 (for E-ACG/M-CAA) to 14 (for E-AAC/M-CTC) with an average of 6.3 per primer pair (Table 2). Paternal markers ranged from 0 (for E-ACT/M-CTC and E-AAC/M-CTG)

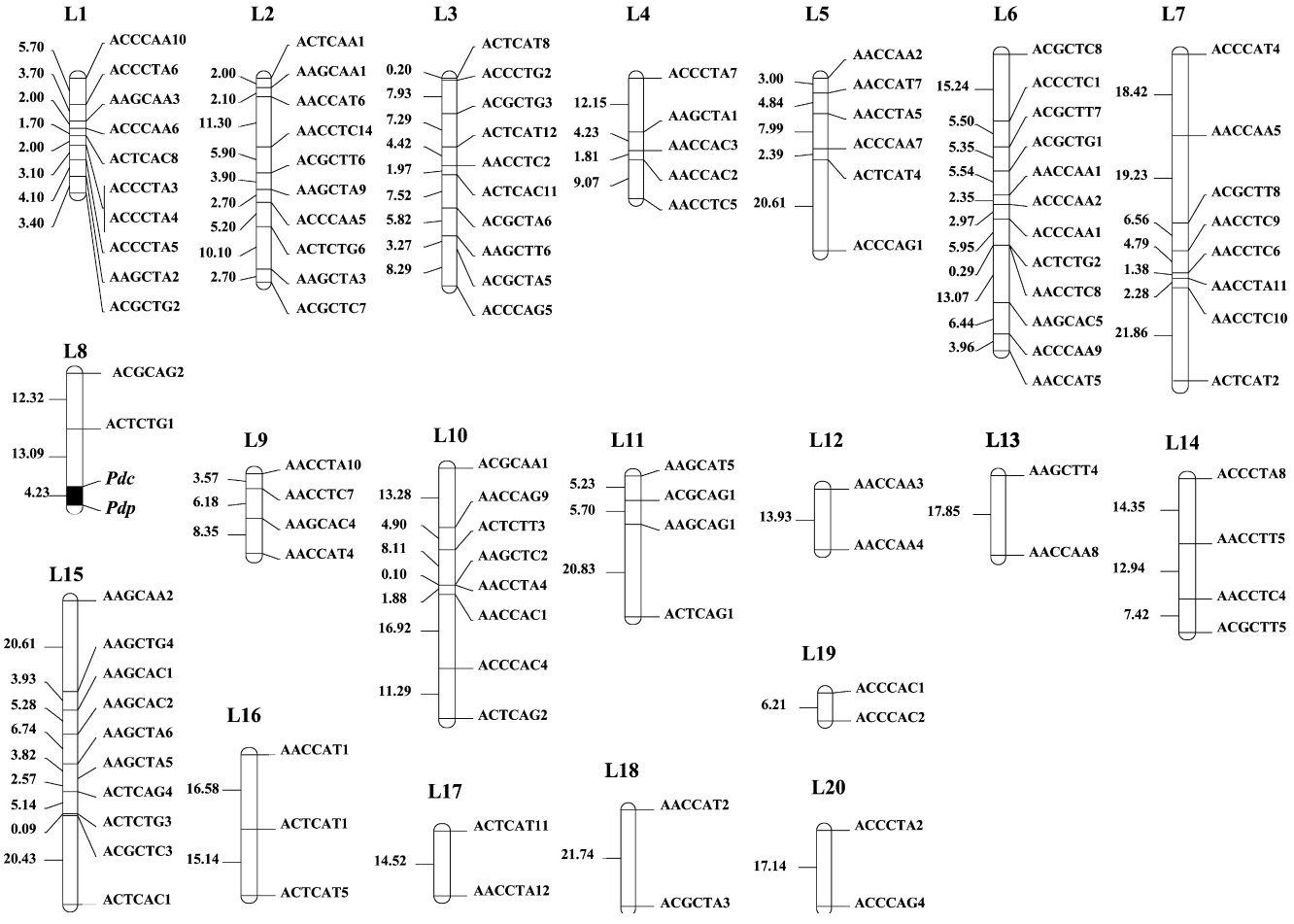

Fig. 1 AFLP linkage map of *Mucuna* based on a population of 82 F2 progeny. The names of markers are shown at the *right* and their map position (cM) at the *left*. Distances are in Kosambi cM

to 8 (for E-ACT/M-CAT) with an average of 2.7 per primer pair. Maternal markers ranged from 0 (for E-ACC/M-CTC, E-ACG/M-CAA, E-ACG/M-CTG) to 8 (for E-AAC/M-CTC) with an average of 2.9 per primer pair. Of the 233 markers, 100 (42.9%) were paternal, 106 (45.5%) were maternal and the remaining 27 (11.6%) were non-parental. Chi-square analysis indicated that 164 (70.4% of the total number of markers) markers were inherited in the expected 3:1 Mendelian ratio. The 27 non-parental markers were scorable and displayed different segregation patterns.

We studied segregation for seed coat, pod and pod pubescence color at maturity. The F_1 hybrid produced brown pods, brown pod pubescence and seeds with a black seed coat. Seventy-three out of the 82 F_2 plants reached maturity and were used in this study. The F_2 plants produced pods which were variable in their color and pubescence but not in their seed coat color; all had a black seed coat. Thus, seed coat color did not segregate in the F_2. The F_2 hybrids can be classified into three classes based on pod color and four classes based on pod pubescence color. For pod color at maturity, 14 plants produced black pods (maternal phenotype), 40 produced brown pods (F_1 phenotype), and 19 produced greenish pods (paternal phenotype), approximately fitting the expected 1:2:1 ratio (χ^2=1.35, P>0.05). For pod pubescence, 14 plants produced pods with black pubescence (maternal phenotype), 32 with brown pubescence (F_1 phenotype), 8 with salmon or pink-like pubescence (off-type), and 19 with gray pubescence (paternal phenotype). Plants heterozygous for pod color (brown pods) segregated for pod pubescence and approximately fitted the expected 3:1 ratio (32 brown:8 salmon; χ^2=0.53, P>0.05). This may suggest that the formation of the new phenotype is the result of an interaction between gene pairs and linkage of pod color and pod pubescence. The 14 plants that produced black pods produced pods with black pubescence while the 19 plants that produced greenish pods produced pods with gray pubescence.

Linkage analysis and map construction

The 37 AFLP primer pairs detected 233 polymorphic markers of which 164 (70.4% of the total polymorphic markers) were inherited in expected 3:1 Mendelian ratios.

Of the 164 markers showing Mendelian transmission, 88 originated from PI364362 and 76 from 'Edgar Farm White' (Table 2). A linkage analysis was performed using all 164 markers plus two morphological traits (pod color and pod pubescence), making a total of 166 loci that constitute the data set. These 166 markers covered 687.9 cM based on the JoinMap analysis (Van Ooijen and Voorrips 2001). Linkage relationships of the 166 segregating markers were established at a 2.0 χ LOD χ 4.0 and a recombination fraction smaller than 0.5. Under these conditions, linkage groups could be classified into three categories: large groups (56–74 cM), medium groups (25–38 cM), and small groups (6–21 cM). There were 69 unlinked markers (29.6%). There were a number of small linkage groups with less than three markers. These included five groups with two markers, one group with three markers, and four groups with four markers. Of the 89 intervals, 37 (41.6%) were between 0 and 5 cM, 26 (29.2%) were between 5 and 10, 12 (13.5%) between 10 and 15, 8 (8.9%) between 15 and 20, and 6 (6.7%) between 20 and 25. The distribution revealed a strong skewedness. The markers were randomly distributed over the map and no clear clustering of markers was observed (Fig. 1). The average spacing between markers was 7.7 cM and the largest gap between two markers was 21.8 cM. AFLP markers amplified by the same primer pairs were randomly distributed over the map. The majority of the fragments amplified by the same AFLP primer pair were mapped to different linkage groups. For example, linkage groups L1, L4, L14, and L20 included markers derived from E-ACC/M-CTA.

Discussion

Genetic linkage maps have been produced in the past for many of the major crop species using intraspecific and/or interspecific mating systems. It is generally believed that the degree of polymorphism is lower in an intraspecific population than in an interspecific population. This may be explained by the high level of genetic variability within an interspecific population or the level of heterozygosity of the parents. However, intraspecific crosses have several advantages over interspecific crosses for breeding programs, particularly for polygenic characteristics, and also for the reduction of the problems of low fertility and low recombination rates associated with interspecific crosses. Consequently, the use of intraspecific crosses allows better map resolution than interspecific crosses (Lefebvre et al. 1995). Researchers have demonstrated that AFLP polymorphisms are an efficient means of generating molecular markers for gene mapping in intraspecific mating systems (Lefebvre et al. 1995; Saliba-Colombani et al. 2000). The AFLP technique was found to be the most advantageous method compared to RAPD and RFLP technologies since it allows the simultaneous analysis of a large number of DNA fragments per gel (Pejic et al. 1998).

The level of polymorphism is directly dependent on the genetic relatedness between individuals. High values of similarity, ranging from 0.87 to 0.97, have been reported among velvetbean accessions, including the two parents used in this study (Capo-chichi et al. 2001). Since the populations we studied were derived from an intraspecific cross, we expected a low level of polymorphism compared to that found in interspecific crosses. As expected, the polymorphism rate between the two lines was indeed low. This was not surprising, because except for seed coat color, pod color, and pod pubescence they were morphologically very similar.

We have used AFLP technology to construct the first linkage map for velvetbean in an intraspecific population. Most of the AFLP fragments analyzed showed Mendelian segregation, indicating that these fragments are useful as molecular markers for velvetbean genome mapping and marker-assisted selection. However, because AFLPs generally act as dominant markers, only two alleles can be scored for each locus. It is obvious that by using only dominant instead of codominant markers, some polymorphisms remain undetected. Thus, depending on the level of heterozygosity of the parents, the efficiency of AFLP mapping will vary, but this technique appears to be a powerful way to quickly generate large numbers of markers useful for constructing genetic maps.

To evaluate the applicability of AFLP markers for the genetic linkage mapping of velvetbean, 12 primer pairs were tested to identify intraspecific variation. One of the most important factors determining the applicability of AFLP markers for linkage mapping analysis is reproducibility. Therefore, we tested the reproducibility by using DNA templates isolated at different times from different individual plants of each of the parents and the F_1 hybrids, in separate analyses. A high level of reproducibility was observed between individual samples. Overall, the results showed that the reproducibility with velvetbean genomic DNA was excellent for genetic map analysis.

The genetic map of *Mucuna* is made up of 166 markers including two morphological traits, and comprises 687.9 cM with an average distance between two markers of 34.4 cM. However, it is not saturated, because not all markers could be mapped. The existence of the minor linkage groups and the unlinked markers indicates that there are many large gaps with few markers (Kesseli et al. 1994). These small linkage groups and unlinked markers probably result from the small population size, coupled with the low map saturation. Hence the genome is not uniformly covered, which suggests that the AFLP markers identified are not random samples throughout an entire chromosome (Paglia et al. 1998). van Heusden et al. (2000) reported that AFLP markers can remain unlinked in cases where they result from two simultaneous segregating amplifications in duplicated regions, because two such loci will be analyzed as one. This may be explained by locus heterogeneity where several unlinked genes result in the same phenotype. We expect that the small linkage groups will be merged into larger groups

when more markers are assigned. Other types of markers such as SSR, RAPD, RFLP or AFLP generated with different primer pairs other than the *Eco*RI/*Mse*I primer sets used in this study should be used for larger genome coverage. For example, in pepper, AFLP markers revealed by *Pst*I/*Mse*I primer combinations showed a better distribution than those of *Eco*RI/*Mse*I (Lefebvre et al. 1995).

Morphological evidence was supported by AFLP analysis, in that the F_2 plants segregated for pod pubescence and pod color at maturity. Pod pubescence was not inherited in a Mendelian fashion; a new phenotype, the salmon color, appeared. Pod pubescence and pod color at maturity in the F_1 hybrids were quite different from those of each parent. This may imply codominance for the genes controlling pod pubescence and pod color. Our results show that these traits are useful in differentiating velvetbean accessions. Lubis et al. (1980) reported that the pod hair can be used to identify whether a taxon is cultivated or wild. Segregation for seed coat color was not found in the F_2 progeny and may be observed in later generations. Previous research reported that multigenic factors were involved in the production of seed coat color, following a cross between brown- and white-seeded plants in *Mucuna pruriens* (Lubis et al. 1980). We suggest that future attempts in velvetbean genome mapping should include the identification and eventual cloning of genes important for the determination of economic and biochemical values. In conclusion, more F_2 plants from the same F_1 need to be studied using different AFLP primers and other types of markers to increase the resolution of the genetic map.

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