Genetic Resources and Crop Evolution (2005) 52: 1057–1067
DOI 10.1007/s10722-004-6107-z

Microsatellites and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal

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Received 29 April 2003; accepted in revised form 10 January 2004

Key words: Cowpea, Germplasm resource, Genetic diversity, RAPD, Senegal, SSR, UPGMA, *Vigna unguiculata*

Abstract

Genetic diversity in local cowpea varieties and breeding lines from Senegal were studied using random amplified polymorphic DNA (RAPD) and microsatellite (SSR) techniques. Among the 61 RAPD primers used, twelve show polymorphism. Fifteen of the 30 microsatellite primer pairs were polymorphic, detecting one to nine alleles per locus. The RAPD and SSR data were analyzed both separately and in combination to assess relationships among genetic lines. Although RAPD provided information on levels of genetic diversity, microsatellite markers are most effective in determining the relationship among cowpea accessions and varieties. The SSR results support the genetic diversification of cowpea in Senegal and underscore their potential in elucidating patterns of germplasm diversity of cowpea in Senegal.

Abbreviations: IITA – International Institute of Tropical Agriculture

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], a northern African domesticate, is an important food source for more than 200 million people in Africa, India and South America. Due to its good protein quality, cowpea plays a major role in human nutrition and, consequently, it is referred to as "poor man's meat". Dry grain is the principal product consumed by humans, but leaves (in many parts of eastern Africa and Senegal), fresh green pods (humid regions of Asia and Caribbean), fresh peas (Southeastern US and Senegal), and roots (Sudan and Ethiopia) are also used for human consumption (Ehlers and Hall 1997). Seeds of cowpea mixed with soil or oil are used by Hausa and Edo Tribes to treat stubborn boils. Additionally, cowpea plays an important role in animal feed because all aboveground parts (leaves, stem, pods and grains) are used. Cowpea is cultivated alone or intercropped with sorghum, pearl millet, maize, cassava, or cotton. It can grow in soil of low fertility due to its high rates of nitrogen fixation (Eloward and Hall 1987), effective symbiosis with mycorrhizae (Kwapata and Hall 1985), and ability to withstand acid and alkaline soil conditions (Fery 1990) as well as considerable drought conditions and moderate shade.

Genetic diversity in cultivated cowpea has been assessed on the basis of morphological and physiological markers (Ehlers and Hall 1997). Additionally, biochemical markers, such as isozymes, have been used in order to determine genetic similarities among cowpea varieties

(Panella and Gepts 1992; Pasquet 1993, 2000). These markers may be influenced to various degrees by plant-endogenous and environmental factors and, thus, are not reliable tools for genetic diversity assessment. To enhance our understanding of genetic diversity and relatedness among species of *Vigna* and varieties of cowpea, techniques like restriction fragment length polymorphism (RFLP; Fatokun et al. 1993), random amplified polymorphic DNA (RAPD; Mignouna et al. 1998; Fall et al. 2003), DNA amplification fingerprinting (DAF; Spencer et al. 2000), amplified fragment length polymorphism (AFLP; Fatokun et al. 1997; Tosti and Negri 2002; Coulibaly et al. 2002), and microsatellites or simple sequence repeats (SSR; Li et al. 2001) have been used. These molecular markers are not influenced by endogenous and exogenous factors (Tanksley et al. 1989).

RAPD is based on DNA amplification with simple polymerase chain reaction (PCR) using a single arbitrary primer of 10 bp long without knowledge of target DNA sequence. Because of the short length of the primer and the low annealing temperature (35–40 °C), primer annealing to homologous region in the target DNA sequences is frequent. Microsatellites, on the other hand, are tandem repeated units of short nucleotide motifs that are 1–6 bp long. Produced by errors during DNA replication, microsatellites are distributed throughout plant and animal genomes (Tautz and Renz 1984). The variation in this region can be detected with PCR by developing primers for the conserved DNA sequence flanking the SSR. For this reason, SSR are powerful, reproducible and informative molecular markers for use in genome analysis. The hypervariability of SSR, their codominance and the simple protocol necessary for their analysis constitute their main advantages (Dib et al. 1996).

RAPD and SSR have become effective molecular markers in genome analysis of many legume crops (Doldi et al. 1997; Doyle et al. 1998; Peakall et al. 1998; Mengoni et al. 2000; Li et al. 2001), and in genetic mapping (Winter et al. 1999; Yu et al. 2000; He et al. 2001). The aim of this study is to assess the potential application of RAPD and SSR techniques in determining genetic diversity and relationships among cowpea breeding lines and local cultivars found in the Senegalese national germplasm. These varieties were selected with future aim of improving cowpea production for farmers with low income in Sahelian region. Currently, the cowpea diversity and relatedness among these genetic lines is poorly understood, a situation that needs to be improved because of the presence of a wealth of germplasm resource for this crop in the Senegal. Farmers currently classify their genetic lines on the basis of pod or seed characteristics, productivity, cycle duration, or even a person's name.

Table 1. List of 15 cowpea [*Vigna unguiculata* (L.) Walp.] varieties used in this study and their pedigree.

Varieties	Pedigree
58-74	Local variety
Ndiaga Aw	Local variety
Diongoma	58-57 × IT81D-1137
CB5	Calif. Blackeye × Iron
Baye Ngagne	Local variety
Melakh	IS86-292 × IT83s-742-13
Bambey 21	5/8 of 58-40 + 1/4 of 66-74 + 1/8 of 58-50
66-35	Local variety
58-57	Local variety (Podor)
Mougne	58-74 × Pout
Mouride	58-57 × IT81D-1137

Materials and methods

Material

Eleven cowpea varieties (Table 1) from the Senegalese National Germplasm Collection were selected for this study on the basis of high yield quality. The accessions represent six cowpea breeding lines and five local varieties. Ten individuals per accessions were sampled. Seeds were grown under greenhouse conditions and leaves were harvested from one-week old seedlings.

DNA isolation

Genomic DNA was isolated from leaves of individual plants according to Fulton et al. (1995) with slight modification. After precipitation, DNA was washed with 70% ethanol, dried on speed vacuum, resuspended in 100 μL TE, and quantified with a spectrophotometer. Bulked DNA representing equal amounts from the ten individual plants was used for each variety following Yang and Quiros

(1993), Price et al. (2000) and Tosti and Negri (2002).

RAPD amplification

Amplifications were performed with the PCR method. 1.5 U of lyophilized *Taq* (Amersham Pharmacia Biotech, San Francisco, California, USA) was used per PCR reaction. The PCR reaction was carried out in 2 mM $MgCl_2$, 25 ng of genomic DNA, 1 μM of each primer, and 200 μM of each dNTP in a final volume of 25 μL. Sixty-one primers were used (all Operon A, B, and F, plus primer V18 of the V series; Genosphere Biotechnologies, Paris, France). The reactions were placed in a thermocycler PTC-100 programmed for pre-denaturation step of 3 min at 94 °C followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C and a final extension of 5 min at 72 °C. Amplification products were resolved on 1.8% agarose gel using 1× BTE at 140 V for 4 h. A control sample that contains all amplification components except genomic DNA was used in all experiments. Additional controls include amplifications of duplicate individuals per variety, and the experiments involved in primer testing and PCR condition refinements.

Microsatellites amplification

The 30 microsatellite primer pairs (Table 2) used here were based on those published for cowpea by Li et al. (2001) and one, VM22, obtained from moth bean (*Vigna aconitifolia*; Hong et al. 1993). Primers were synthesized by Operon Technologies (Qiagen, California). DNA amplification using PCR was carried out in a PCT 100 (M. J. Research Inc., USA) under the same conditions described for RAPD amplification except that 50 ng of DNA was used in this case. Amplification was performed following Li et al. (2001) and the annealing temperature varied according to the T_m the primer. The PCR products were separated on 10% polyacrylamide gel and 1× BTE at 80 V for 12 h (EC 105-Apparatus Corporation St. Peterburg, Florida, USA). Polyacrylamide and agarose gels were stained for 30 min with 1 mg/mL of ethidium bromide and washed for 15 min in distilled water. The stained gels were exposed to UV light and images were photographed using BIO-CaptMW software (Vilber Lourmat, Marne-La-Vallée, France). A control sample that contains all amplification components except genomic DNA was used in all experiments.

Scoring and analysis of RAPD and SSR data

The reproducible bands were scored on the basis of their presence/absence across the genetic lines and were given a binary code (1/0). Bands present across all genotypes (invariable markers) were excluded from the analysis because they are not informative. Matrices of raw data were generated for RAPD and SSR results separately and used for further analyses. Combined analyses of the two data sets were also performed. Both simple matching (SM, Sokal and Michener 1958) and Dice (Dice 1945) algorithms were used to estimate similarities among the genetic lines. The simple matching algorithm considers both presence and absence of markers in calculating degrees of similarity. The Dice algorithm is identical to the Nei and Li's measure (Nei and Li 1979, equation 21); both calculate the degree of affinities on the basis of only shared present attributes as a proportion of the total. These similarity matrices based on RAPD, SSR and combined RAPD/SSR were subjected to the unweighted pair-group method (UPGMA) for grouping the genotypes. The NTSYS-pc computer program (Rohlf 2000) was used for generating the similarity matrices and the UPGMA clustering.

Results

RAPD and SSR Genotype markers

Among the 61 RAPD primers, 12 (A1, A13, A14, B6, B10, B11, B12, B15, F4, F13, F16, V18) showed genetic variability among cowpea varieties. Only two primers (A14 and F4) could discriminate among all the cowpea varieties examined here. A total of 666 reproducible bands were scored and 114 (17%) were polymorphic. Figure 1A illustrates the RAPD band pattern obtained with primer A14. Fifty percent of the 30 pairs of microsatellite primers were polymorphic; the others were monomorphic (data not shown). The number of alleles detected varied from one to nine, with a

Table 2. List of microsatellite primer pairs used in this study. Microsatellite were isolated from cowpea (Li et al. 2001) except VM22 from moth bean (*V. aconitifolia*) (Hong et al. 1993).

Primer codes	Primer sequences	Repeat	T_m(°C)
VM2	5′ GTA AGG TTT GGA AGA GCA AAG AG 3′ 5′ GGC TAT ATC CAT CCC TCA CT 3′	(AG)32	60
VM3	5′ GAG CCG GGT TCA ATA GGT A 3′ 5′ GAG CCA GGG CAC AGG TAG T 3′	(AG)27	60
VM5	5′ AGC GAC GGC AAC AAC GAT 3′ 5′ TTC CCT GCA ACA AAA ATA CA 3′	(AG)32	57
VM10	5′ TCC CAC TCA CTA AAA TAA CCA ACC 3′ 5′ GGA TGC TGG CGG CGG AAG G 3′	(AC)3(CT)10(AC)3	64
VM11	5′ CGG GAA TTA ACG GAG TCA CC 3′ 5′ CCC AGA GGC CGC TAT TAC AC 3′	(TA)4 .. (AC)12	63
VM12	5′ TTG TCA GCG AAA TAA GCA GAG A 3′ 5′ CAA CAG ACG CAG CCC AAC T 3′	(AG)27	61
VM13	5′ CAC CCG TGA TTG CTT GTT G 3′ 5′ GTC CCC TCC CTC CCA CTG 3′	(CT)21	63
VM14	5′ AAT TCG TGG CAT AGT CAC AAG AGA 3′ 5′ ATA AAG GAG GGC ATA GGG AGG TAT 3′	(AG)24	62
VM17	5′ GGC CTA TAA ATT AAC CCA GTC T 3′ 5′ TGT GTC TTT GAG TTT TTG TTC TAC 3′	(CT)12	58
VM19	5′ TAT TCA TGC GCC GTG ACA CTA 3′ 5′ TCG TGG CAC CCC CTA TC 3′	(AC)7.. (AC)5	61
VM22	5′ GCG GGT AGT GTA TAC AAT TTG 3′ 5′ GTA CTG TTC CAT GGA AGA TCT 3′	(AG)12	58
VM23	5′ AGA CAT GTG GGC GCA TCT G 3′ 5′ AGA CGC GTG GTA CCC ATG TT 3′	(CT)16	62
VM25	5′ CCA CAA TCA CCG ATG TCC AA 3′ 5′ CAA TTC CAC TGC GGG ACA TAA 3′	(CT)18	60
VM26	5′ GGC ATC AGA CAC ATA TCA CTG 3′ 5′ TGT GGC ATT GAG GGT AGC 3′	(TC)14	60
VM27	5′ GTC CAA AGC AAA TGA GTC AA 3′ 5′ TGA ATG ACA ATG AGG GTG C 3′	(AAT)5 . . . (CT14) . (AC)3	57
VM28	5′ GAA TGA GAG AAG TTA CGG TG 3′ 5′ GAG CAC GAT AAT ATT TGG AG 3′	(TC)20	57
VM29	CGTGACACTAATAGTAGTCC 3′ 5′ CGA GTC TCG GAC TCG CTT 3′	(TC)11	60
VM30	5′ CTC TTT CGC GTT CCA CAC TT 3′ 5′ GCA ATG GGT TGT GGT CTG TG 3′	(TC)10	61
VM31	5′CGC TCT TCG TTG ATG GTT ATG 3′ 5′ GTG TTC TAG AGG GTG TGA TGG TA 3′	(TC)16	61
VM33	5′ GCA CGA GAT CTG GTG CTC CTT 3′ 5′ CAG CGA GCG CGA ACC 3′	(AG)18 . (AC)8	63
VM34	5′ AGC TCC CCT AAC CTG AAT 3′ 5′ TAA CCC AAT AAT AAG ACA CAT A 3′	(CT)14	55
VM35	5′ GGT CAA TAG AAT AAT GGA AAG TGT 3′ 5′ ATG GCT GAA ATA GGT GTC TGA 3′	(AG)11.(T)9	58
VM36	5′ ACT TTC TGT TTT ACT CGA CAA CTC 3′ 5′ GTC GCT GGG GGT GGC TTA TT 3′	(CT)13	60
VM37	5′ TGT CCG CGT TCT ATA AAT CAG C 3′ 5′ CGA GGA TGA AGT AAC AGA TGA TC 3′	(AG)5 . (CCT)3 . (CT)13	60
VM38	5′ AAT GGG AAA AGA AAG GGA AGC 3′ 5′ TCG TGG CAT GCA GTG TCA G 3′	(AG)10.(AC)5	60
VM39	5′ GAT GGT TGT AAT GGG AGA GTC 3′ 5′ AAA AGG ATG AAA TTA GGA GAG CA 3′	(AC)13 . (AT)5 . (TAC)4	59
VM40	5′ TAT TAC GAG AGG CTA TTT ATT GCA	(AC) 18	59

Table 2. Continued.

Primer codes	Primer sequences	Repeat	T_m(°C)
	5′ CTC TAA CAC CTC AAG TTA GTG ATC		
VM68	5′ CAA GGC ATG GAA AGA AGT AAG AT 3′	(GA)15	60
	5′ TCG AAG CAA CAA ATG GTC ACA C 3′		
VM70	5′ AAA ATC GGG GAA GGA AAC C 3′	(AG)20	59
	5′ GAA GGC AAA ATA CAT GGA GTC AC 3′		
VM71	5′ TCG TGG CAG AGA ATC AAA GAC AC 3′	(AG)12.(AAAG)3	61
	5′ TGG GTG GAG GCA AAA ACA AAA C 3′		

5.2 average. Amplification with VM11, VM19, VM25, VM28, VM31, VM35, VM36, VM39, and VM68 primers revealed the highest number of alleles. Primer VM22, designed on the basis of the protein kinase cDNA sequence of moth bean [*V. aconitifolia* (Jacq.) Maréchal] (Hong et al. 1993), amplified five DNA fragments of different size; four of them were intense but one was weak (figure not shown). Correlation between the repeat number of SSR and the allele number was not detected in this study. Amplification with VM30 is presented in Figure 1B.

The RAPD method resulted in more amplified fragments than the SSR for all the cowpea varieties examined except for variety 58-74 (Figure 2). Variability among varieties in RAPD was high, with variety 66-35 having slightly over twice the number of bands observed in 58-74 (103 vs. 50); this was not the case in SSR (Figure 2). The standard error calculated for the varieties from the RAPD and SSR data is ± 5 and ± 1.4 SSR, respectively. Varieties 66-35 and Mounge appeared to be most variable With RAPD, whereas 58-74 was least variable (Figure 2). Varieties CB5, 58-74 and Melakh, least variable in RAPD, generated similar number of bands in the two approaches. However, it is to be noted that high variability in RAPD was not correlated with increase in number of SSR bands (Figure 2).

Genetic relationship among populations

UPGMA dendrograms for RAPD, SSR, and combined RAPD/SSR data showing the genetic relationship among cowpea breeding lines and local varieties are presented in Figure 3. In the RAPD study, there were slight differences between the Dice and SM dendrograms concerning the position of Melakh and CB5 lines. However, the changes in the position of these two lines were minor and do not affect the conclusions. Consequently, the SM-based dendrogram is presented (Figure 3A). This dendrogram shows the local variety 58-74 very distant from the rest of the genotypes at a coefficient of 0.56. Variety 58-74 was followed by 66-35 as separate entity with coefficient levels of 0.65. Remaining varieties group together very closely with coefficient values ranging from 0.64 to 0.79. Within the latter group, Mougne and Mouride form a distinct cluster followed by Melakh and then the rest of the cultivars.

The dendrograms resulting from SSR with Dice and SM differ only in terms of whether Mougne and Mouride formed a subcluster or a grade. SM-based dendrogram is presented here. The SM analysis shows two very distinct clusters. One small cluster containing the local varieties Mougne and 66-35 to which 58-74 was linked with a coefficient of 0.70 (Figure 3B). Within the larger cluster, two subgroups are evident (0.83 coefficient). One includes a strongly clustered CB5 (variety from California) and Bambey 21 (a breeding line from three local varieties) to which the local variety Ndiaga Aw is linked at low coefficient. The second subcluster contains the local varieties Baye Ngagne clustering with a Melakh plus 58-57 group, and Diongoma in a cluster with Mouride. The latter two varieties were obtained from a cross between 58-57 and IT81D-1137, a variety selected in IITA in Nigeria.

The dendrogram based on combined analysis of SSR and RAPD resembled for the most part the one based on RAPD. The simple matching SSR/RAPD dendrogram shows two main groups (Figure 3C). One group contains the local varieties 58-74 and 66-35, and is distant from the rest of the genotype at a coefficient of 0.58. The second group can be divided into two subclusters. One subcluster contains Mougne and Mouride and is distinct from the others at a coefficient of 0.66, whereas the second represents Melakh as an individual line linked to the rest of the genetic lines.

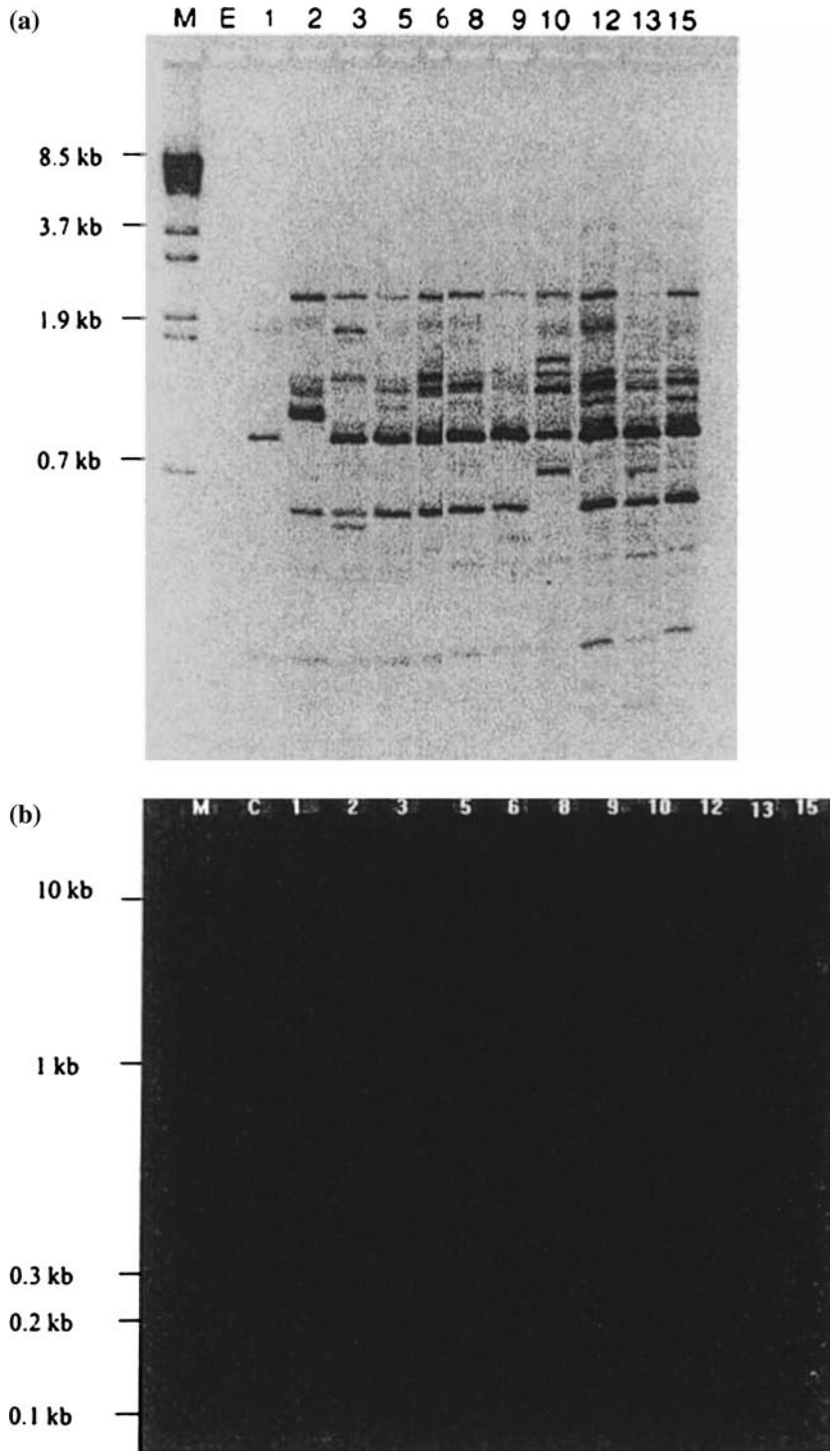

Figure 1. Amplification of RAPD markers using primer A14 (1A) and SSR using primer VM30 (1B) from 11 cowpea varieties. Lane M contains molecular weight marker of 100 Base-pair Ladder; lanes E and C is a control (without DNA). Lanes 1 through 15 represents the varieties studied: 58-74 (1); Ndiaga Aw (2); Diongoma (3); CB5 (5); Baye Ngagne (6); Melakh (8); Bambey 21 (9); 66-35 (10); 58-57 (12); Mougne (13); Mouride (15).

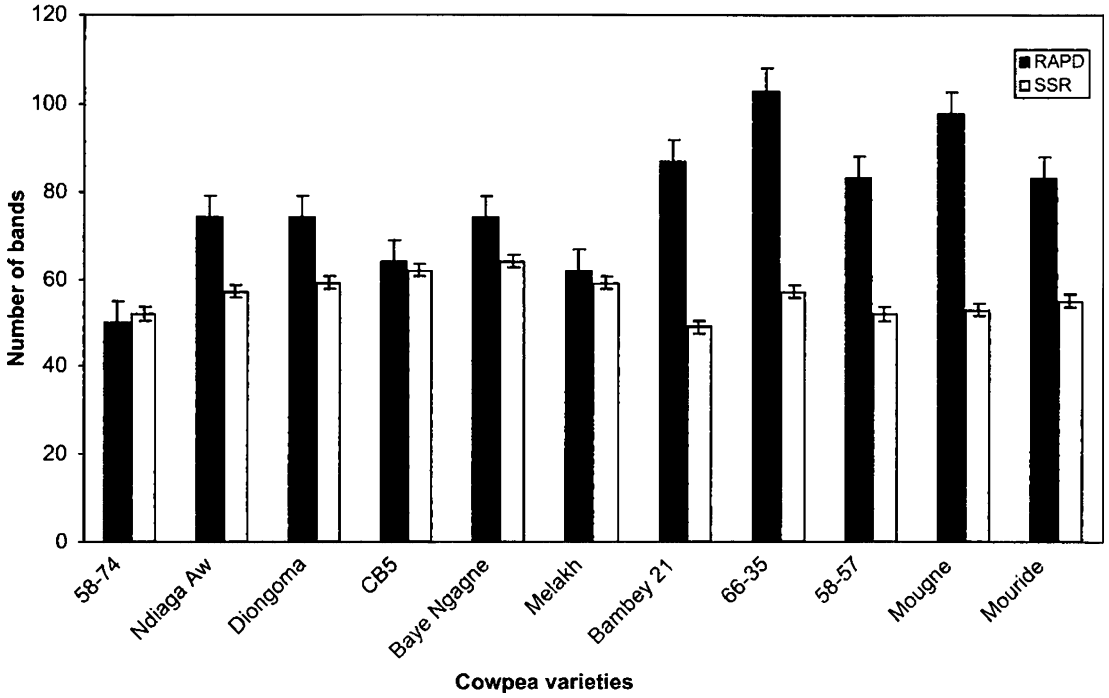

Figure 2. Total number of bands amplified with RAPD and SSR primers in each cowpea variety. Bars represent the standard error (SE). SE = ±5 for RADP and SE = ±1.4 for SSR.

Discussion

Naming cowpea varieties in Senegal by traditional farmers after pod or seed size, productivity, or names of certain people is an artificial and problematic system of classification and nomenclature. The same variety with the same morphological characters can have different names following the locality or ethnic groups. This situation hampers the reorganization of Senegal National Germplasm and the effectiveness of breeding programs in Senegal. The problem has a broader impact, knowing that the crop was domesticated in Africa and that the Senegal germplasm collection contains valuable genetic resources for cowpea breeding worldwide. Consequently, a better understanding of the genetic variation and a robust system of classification of the cowpea collection in Senegal with molecular markers is most needed.

Variability in SSR and RAPD

A relatively low level of variation was found in the Senegal cowpea varieties using both RAPD and SSR markers where only 17% and 12% of the bands, respectively, were polymorphic. These results are in agreement with the RAPD study on *V. unguiculata* (Tosti and Negri 2002). Similarly, isozyme studies suggested that genetic diversity in cultivated varieties was lower than that reported in others crops (Pasquet 1993, 2000). These findings underscore the potential low genetic diversity in cowpea in general, which might be the result of genetic bottleneck induced by domestication. This low genetic diversity at the molecular level is in contrast with the variation observed in seed color, seed coat pattern, plant type and seed size among cultivated varieties (Panella and Gepts 1992; Vaillancourt et al. 1993; Pasquet 2000). The microsatellite primer VM22 amplifies two weak bands and one intense band in local and breeding lines cowpea, except in CB5 (variety from California) where it showed five intense bands. This primer also amplified breeding lines from IITA and one wild cowpea (Li et al. 2001). These results suggest that the flanking region of VM22 primer pairs are conserved in *V. unguiculata* but the number of alleles varies among cowpea varieties. Our results confirm those obtained by Peakall et al. (1998) who showed that several soybean SSR

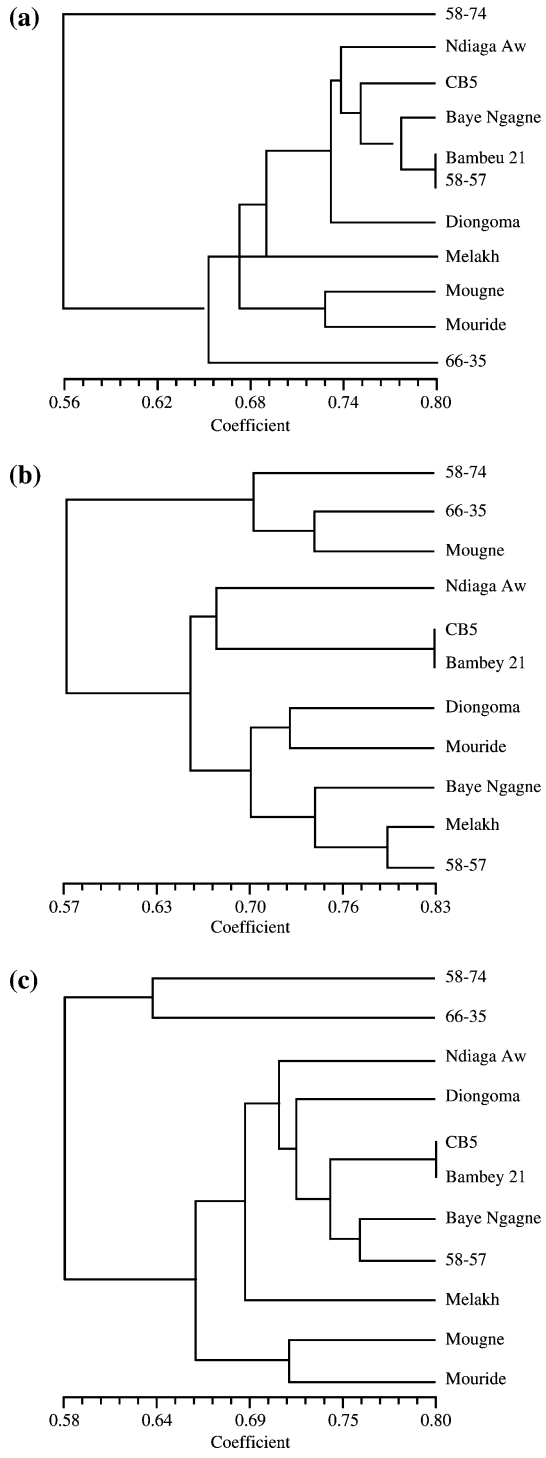

Figure 3. UPGMA-simple matching dendrograms showing the relationships among 11 cowpea varieties based on RAPD (A), SSR (B), and combined RAPD and SSR data (C).

primer pairs could amplify across legume genera. The number of alleles amplified in chickpea was between one and eight (Winter et al. 1999), in alfalfa nine to fourteen (Mengoni et al. 2000), in soybean eleven to twenty six (Rongwen et al. 1995), and in cowpea IITA two to seven (Li et al. 2001). In the present study, SSR primer pairs amplified one to nine alleles. This difference in number of alleles between the two studies on cowpea can be due to different varieties used and possibly to the 10% of polyacralamide used in our study in contrast with the 6% used by Li et al. (2001).

Relationships among cultivars

The strong grouping of Melakh and 58-57 with SSR data and their loose association in RAPD-based dendrograms can be explained on the basis of vegetative and reproductive characters. The two varieties possess many similar characters. They are both procumbent (prostrate), have indeterminate growth pattern, and possess green leaves. However, the flowers of 58-57 are bicolor or white, pods are short, and seeds are cream and brown-eyed. In contrast, the flowers of Melakh are white, the pods are long, and the seeds are white and brown eye. The results also suggest that the local variety 58-57, cultivated in the Senegal River Valley, has the same parents as Melakh. The parental varieties for Melakh are from Nigeria, suggesting a Nigerian, origin of this variety and an introduction to Senegal via River Valley. This hypothesis has previously been proposed by Sène (1966).

Analyses of SSR with both SM and DICE show Mougne, 66-35 and 58-74 appearing in one cluster. Mougne is derived from a cross between two local varieties in Senegal, 58-74 and Pout. The SSR results suggest that Mougne and 66-35 may share the same parents. Variety 58-74 appeared in the RAPD analysis very distinct from the rest of the cowpea varieties studied here and its genetic relationship to Mougne was not resolved (Figure 3A). Thus, SSR markers provide a better assessment than RAPD of the genetic relatedness among these genetic lines.

In SSR-SM analysis, the grouping together of Diongoma and Mouride and their subsequent linkage to 58-57 is well supported by the pedigree

of these varieties. Diongoma and Mouride were derived from the same parents (Table 1), and one of those parents is 58-57 (the other is IT81D-1137). Again, these genetic relationships were not apparent in the RAPD analyses here in spite of the larger number of characters obtained from RAPD (114 in RAPD vs. 74 SSR).

Additionally, Mougne and Mouride grouped together with RAPD and combined RAPD/SSR data using both SM or Dice algorithms but appeared in two distinct clusters in SSR-Dice and SM. Mougne is an improved variety from a cross between two cultivars from Senegal whereas Mouride is from a cross between one variety from Senegal and another from IITA (Nigeria). They do not possess similar morphological characters but only share resistance to chancre bacteria and susceptibility to insects. Therefore, their clustering with RAPD may be an artifact.

Bambey 21, a variety derived from three parental stocks (Table 1) showed the strongest clustering in both RAPD and SSR data, but not with the same variety (Figure 3). Bambey 21 clustered at a 0.76 coefficient with 58-57 in RAPD analysis and at 0.83 with CB5. Bambey 21 and CB5 are morphologically very similar, differing only in seed color where the seeds of Bambey 21 are completely white whereas those of CB5 are white with a black eye. In contrast, the two differ in several morphological characters from 58-57. Therefore, the SSR data emerge again as an informative approach in discerning genetic relationships among cowpea varieties.

RAPD data analyses with Dice and SM failed to reveal important genetic and historical information about these varieties and ought not to be used in a genetic relationship program of cowpea. In contrast, molecular markers obtained from SSR are more effective in revealing relationships among the varieties of cowpea studied here when one considers both pedigrees and botanical features of the varieties. Compared with RAPD, SSR markers provided better segregation of the different groups of genetic lines. The difference between RAPD and SSR could be explained by the molecular basis of the two approaches. Variation reflected by RAPD resides in many different evolutionary events that occur in the annealing site of the primer and/or between them. Whereas the flanking regions of SSR are very conserved, the microsatellite mutation process is different both in allele lengths and motif size and, thus, could play an important role in the size of detected bands (Webster et al. 2002).

Microsatellites have been considered as effective molecular markers in revealing genetic variation in many legume crops including Chickpea [*Cicer arietinum* (L.), Winter et al. 1999], Alfalfa (*Medicago saliva*, Mengoni et al. 2000), Soybean [*Glycine max* (L.), Doldi et al. 1997; He et al. 2001], and cowpea [*V. unguiculata* (L.) Walp.; Li et al. 2001]. The present study showed that 50% of microsatellite primer pairs used could distinguish cowpea breeding lines and local varieties in Senegal. These results suggest that microsatellites are useful molecular markers in the classification of the Senegal National Germplasm of cowpea and in discerning their pedigrees. The results also show the potential use of microsatellites markers in the breeding programs of this important legume crop. The SSR approach is cost effective for cowpea because of the large number of SSR primers already available (Li et al. 2001). The effectiveness of SSR could be enhanced when it is used as part of a suite of markers that collectively can enhance the classification of cowpea genetic resources and contribute to its breeding programs.

The analysis of SSR with SM algorithm resulted in a dendrogram that is slightly more informative than that based on the DICE algorithm. This is most likely due to the nature of the SM algorithm where similarities based on both presence and absence of characters (Sokal and Michener 1958). Consequently, lack of SSR markers were important in resolving the relationships, implying that loss of SSR markers after the differentiation of some varieties was not a random event. Combined analyses of RAPD and SSR data did not effectively resolve relationship between varieties compared to the results obtained by SSR alone. This is most likely due to the larger number of RAPD characters compared to those from SSR (114 vs. 74), which skewed the matrix in favor of the RAPD clustering.

In conclusion, nuclear microsatellites were demonstrated to be a powerful tool for assessing genetic relatedness among cowpea varieties. RAPD or combination of RAPD and SSR data were not as effective in elucidating the genetic relationships among the local cowpea varieties in Senegal and the breeding lines. This finding is

intriguing because RAPD approach provided overall more variable markers than SSR (114 vs. 74), and in all varieties except 58-74, the number of markers in RAPD exceeded that of SSR (Figure 2). This was evident in the difference in standard error calculated for RAPD and SSR (± 5 vs. ± 1.4 SSR, respectively). Therefore, the effectiveness of these two approaches in discerning genetic relationships does not depend on the quantity of those molecular characters but on the quality. The randomness of RAPD amplification may be one of the factors that hamper their effectiveness in assessing accurate genetic relationships in this case. It is likely that RAPD sometimes amplifies regions of the same size (co-migrating bands) that are not homologous. Such non-homologous bands represent homoplasious characters that could negatively influence the analyses, resulting in misleading relationships. Species-specific SSR primers reduce the incidence of amplification of non-homologous regions, as it is the case in this cowpea study. RAPD, however, might be an effective approach for assessing the levels of genetic variability at the population level because of its broader genome coverage. The SSR results suggest the genetic diversification of cultivated cowpea in Senegal. Further investigation of the germplasm diversity of cowpea in that region is needed.

Acknowledgements

The authors are grateful to Virginia Polytechnic Institute and State University (USA) for supporting the visit of the senior author, and to Dr Myra Gordon for facilitating this collaborative research. We also thank Dr Ndiaga Cissé at the Centre National de la Recherche Agricole- Institut Sénégalais de Recherches Agricoles (CNRA-ISRA) for supplying the seed material used in this study. We also thank two anonymous reviewers for their constructive and valuable comments.

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