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Full Length Research Paper

Investigation of genetic relationship among yellow mosaic virus resistant cowpea lines by using microsatellite markers

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Microsatellite markers were used to investigate the genetic basis of cowpea yellow mosaic virus (CYMV) resistance in 40 cowpea lines. A total of 60 simple sequence repeat (SSR) primers were used to screen polymorphism between stable resistance (GC-3) and susceptible (Chrodi) genotypes of cowpea. Among these, only 4 primers were polymorphic and these 4 SSR primer pairs were used to detect CYMV resistant genes among 40 cowpea genotypes. The polymorphism information content (PIC) of these SSR markers ranged from 0.30 to 0.72. A dendrogram of these genotypes based on microsatellite polymorphisms generally agreed with the CYMV resistant phenotype of these lines. All the genotypes could be divided into two major groups, separated at 45% similarity. The resistant group comprised of 18 cowpea lines with 77 to 100% similarity, in which 10 genotypes shared 100% similarity. Also, the two resistant lines were classified in a separate group with one susceptible line and joined with resistant group at 47% similarity. The susceptible group consisted of two subgroups with 71 and 77% similarity within each subgroup.

Key words: Cowpea yellow mosaic virus, resistance, genetic similarity, microsatellite, simple sequence repeat, markers, cowpea, polymorphism.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is an important grain legume crop in developing countries of the tropics and subtropics, especially in sub-saharan Africa, Asia, Central and South America (Singh et al., 1997). Its value lies in its high protein content (23 to 29%, with potential up to 35%) and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils (Steele, 1972). Cowpea is considerable as one of the most widely adapted and versatile crops which can tolerate to high temperatures and drought compared to other crop species. It can thrive in dry environments and produce the dry grain yield of up to 1000 kg/ha in a Sahelian environment with only 181 mm of rainfall and high evaporative demand (Hall and Patel, 1985). It is estimated that cowpea is now cultivated on at least 12.5 million hectares, with an annual production of over 3 million tons worldwide (Singh et al., 1997). Cowpea productivity is greatly affected by a number of biotic factors such as fungi, bacteria and viruses. Viral diseases are considered to be a major limiting factor for low productivity of cowpea in the tropical and sub-tropical countries (Mali and Thottappilly, 1986). More than 20 different viruses have been reported from various cowpea-growing areas worldwide (Thottappilly and

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Abbreviations: CYMV, Cowpea yellow mosaic virus; **SSR,** simple sequence repeat; **PIC,** polymorphism information content; **DNA,** deoxyribonucleic acid; **PCR,** polymerase chain reaction; **RAPD,** random amplification of polymorphic deoxyribonucleic acid; **AFLP,** amplified fragment length polymorphism; **CCS,** Chaudhary Charan Singh; **HAU,** Haryana agricultural university; **CTAB,** cetyl trimethyl ammonium bromide.

Rossell, 1985). Among these viruses, CYMV causes the most serious disease of cowpea. It may cause 80 to 100% yield reductions (Chant, 1960; Gilmer et al., 1974; Williams, 1977). Microsatellites or SSRs are co-dominant markers that are routinely used in many industrial and academic laboratories. Microsatellites are the most widely used markers, occur at high frequency and appear to be distributed throughout the genome of higher plants.

These are deoxyribonucleic acid (DNA) sequences that consist of two to five nucleotide core units such as $(AT)_n$, $(CTT)_n$ and $(ATGT)_n$, which are tandemly repeated. The regions flanking the microsatellites are generally conserved among genotypes of the same species, allowing the selection of polymerase chain reaction (PCR) primers that will amplify the intervening SSR in all genotypes. Variation in the number of tandem repeats (n) results in different PCR product lengths. These repeats are highly polymorphic even among closely related cultivars, due to mutations causing variations in the number of repeating units. They detect a large number of alleles; level of heterozygosity is high and follows Mendelian inheritance (Wu and Tanksley, 1993). Microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Chen et al., 1997; Li et al., 2000), genotype identification and variety protection (Senior et al., 1998), seed purity evaluation and germplasm conservation (Brown et al., 1996), diversity studies (Xiao et al., 1996), paternity determination and pedigree analysis (Ayres et al., 1997), gene and quantitative trait locus analysis (Blair and McCouch, 1997), and marker-assisted breeding (Ayres et al., 1997; Weising et al., 1998). For identification of molecular markers linked to agronomically important genes, SSR is also one of the best choices as compared to random amplification of polymorphic deoxyribonucleic acid (RAPD) and amplified fragment length polymorphism (AFLP) in a more polymorphic information or more cost effective manner, respectively (Lee 1995; Kelly and Miklas, 1998; Young, 1999). Therefore, this study was done to apply microsatellites markers for differentiation and estimation of genetic relationships within and between yellow mosaic virus resistance and susceptible cowpea lines.

MATERIALS AND METHODS

Plant materials

The present investigation was conducted using pure lines population of cowpea created in the Forage section, Plant Breeding Department. Ninety cowpea lines were used to screen CYMV resistance under the field condition of Forage section, Plant breeding Department, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana (India) in July 2005. After the screening of 90 cowpea genotypes, 20 clearly resistant lines and 20 susceptible lines were selected for further analysis using SSR markers. Genomic DNA was isolated from the young leaves of 3 to 4 week old seedlings of cowpea lines using cetyl trimethyl ammonium bromide (CTAB) extraction method of Murray and Thompson (1980) modified by Saghai-Maroof et al. (1984) and Xu et al. (1994).

Molecular markers

Sixty SSR primers were used to investigate the genetic basic of 40 cowpea lines against cowpea yellow mosaic virus resistance in cowpea. The SSR primers specific for cowpea (*Vigna unguiculata*) and moth bean (*Vigna aconitifolia*) were obtained from Life Technologies Pvt. Limited. The sequences of primers used in this study are given in Tables 1 and 2.

Microsatellite marker analysis

Sixty SSR primer pairs were used to detect polymorphism between standard resistant (GC-3) and susceptible (Chirodi) cowpea genotypes. The polymorphic markers were then used to carry out PCR for all individuals of 40 cowpea lines to detect the resistant genes. PCR for the amplification of template DNA was performed in PTC 100 TM thermo-cycler (MJ Research Inc., Watertown, MA, USA). Total volume of PCR reactions mixture was made to 20 $\propto$l, which contained 1x PCR buffer, 200 µM dNTPs, 0.5 µM of primer (both), 2 mM $MgCl_2$, 1.5 unit of *Taq* polymerase and 50 ng of template DNA. PCR conditions for the microsatellite analysis included an initial pre-denaturation step of three minutes at 94°C and following 30 cycles of 92°C for 2 min (denaturing), 55°C for 1.5 min (annealing) and 72°C for 1.5 min (extension), with the final step of extension carried out at 72°C for 10 min. The PCR products were separated on 3% agarose gel electrophoresis. The PIC of each microsatellite was calculated based on allele pattern of all the genotypes as described by Weir (1996). $PIC = 1-\Sigma P_i^2$, where P_i is the frequency of the i^{th} allele in the examine test lines. NTSYSpc (version 2.0) was used to calculate the genetic similarity (Jaccard's coefficient), principal coordinate, and cluster analyses (Unweighted Paired Group Method Using Arithmetic Averages).

RESULTS

Polymorphism of microsatellites in 40 cowpea lines

To detect polymorphism between resistant and susceptible cowpea genotypes, genomic DNA of standard resistant variety (GC-3) and susceptible variety (Chirodi) were first used as template for PCR amplification using SSR markers. Among 60 SSR markers used, 40 cowpea specific SSR primer pairs showed amplification, 18 primer pairs out of 20 moth bean SSR primers failed to give amplification and only two primer pairs (AGB1 and AGB16) showed amplification. These 42 SSR primer pairs produced 110 amplified fragments, in which only 9 polymorphic bands were obtained. The number of alleles ranged from one (monomorphic primer pair) to seven (data not shown). Out of 42 SSR primer pairs which showed amplification, four SSR markers gave clearly polymorphic bands on the 3% agarose gel (Figure 1). These 4 primer pairs with their polymorphic alleles, monomorphic alleles and PIC listed in the Table 3, were used to analyze 40 cowpea genotypes. The number of polymorphic alleles ranged from 2 to 3 with the average of 2.25. The polymorphism

Table 1. Summary of cowpea SSR primer pairs specific for cowpea.

Primer		5'-3' Sequence	SSR sequence	Predicted size (bp)*
VM1	F	CACCCGTGATTGCTTGTTG	$(TC)_{20}$	135
	R	GTCCCCTCCCTCCCACTG		
VM2	F	GTAAGGTTTGGAAGAGCAAAGAG	$(AG)_{32}$	162
	R	GGCTATATCCATCCCTCACT		
VM3	F	GAGCCGGGTTCAATAGGTA	$(AG)_{27}$	171
	R	GAGCCAGGGCAGAGGTAGT		
VM4	F	AGTAAATCACCCGCACGATCG	$(CT)_{20}$	248
	R	AGGGGAAATGGAGAGGAT		
VM5	F	AGCGACGGCAACAACGAT	$(AG)_{32}$	188
	R	TTCCCTGCAACAAAAATACA		
VM6	F	GAGGAGCCATATGAAGTGAAAAT	$(AG)_{26}$	248
	R	TCGGCCAGCAACAGATGC		
VM7	F	CGCTGGGGGTGGCTTAT	$(AG)_{13}$	158
	R	AATTCGACTTTCTGTTTACTTG		
VM8	F	TGGGATGCTGCAAAGACAC	$(AG)_{16}$	295
	R	GAAAACCGATGCCAAATAG		
VM9	F	ACCGCACCCGATTTATTTCAT	$(CT)_{21}$	271
	R	ATCAGCAGACAGGCAAGACCA		
VM10	F	TCCCACTCACTAAAATAACCAACC	$(AC)_3(CT)_{10}(AC)_3$	278
	R	GGATGCTGGCGGCGGAAGG		
VM11	F	CGGGAATTAACGGAGTCACC	$(AT)_4..(AC)_{12}$	195
	R	CCCAGAGGCCGCTATTACAC		
VM12	F	TTGTCAGCGAAATAAGCAGAG	$(AG)_{27}$	157
	R	CAACAGCAGACGCCCAACT		
VM13	F	CACCCGTGATTGCTTGTTG	$(CT)_{21}$	135
	R	GTCCCCTCCCTCCCACTG		
VM14	F	AATTCGTGGCATAGTCACAAGAGA	$(AG)_{24}$	144
	R	ATAAAGGAGGGCATAGGGAGGTAT		
VM15	F	CGGCTGCAGCAAACAAGAG	$(AG)_4..(GT)_{10}$	162
	R	AAACCCGTGCAAGAAACCAA		
VM16	F	TCCTCGTCCATCTTCACCTCA	$(CT)_7…(CT)_7$	203
	R	CAAGCACCGCATTAAAGTCAAG		
VM17	F	GGCCTATAAATTAACCCAGTCT	$(CT)_{12}$	152
	R	TGTGTCTTTGAGTTTTTGTTCTAC		
VM18	F	AGCCGTGCACGAATGAT	$(GA)_{13}$	257
	R	TGGCCTCTACAACAACACTCT		

Table 1. Contd.

VM19	F R	TATTCATGCGTGACACTA TCGTGGCACCCCCTATC	$(AC)_7…(AC)_5$	241
VM20	F R	GGGGACCAATCGTTTCGTTC ATCCAAGATTCGGACACTATTCAA	$(GT)_{17}$	246
VM21	F R	TAGCAACTGTCTAAGCCTCA CCAACTTAACCATCACTCAC	$(AT)_{17}$	179
VM22	F R	GCGGGTAGTGTATACAATTTG GTACTGTTCCATGGAAGATCT	$(AG)_{12}$	217
VM23	F R	AGACATGTGGGCGCATCTG AGACGCGTGGTACCCATGTT	$(CT)_{16}$	174
VM24	F R	TCAACAACACCTAGGAGCCAA ATCGTGACCTAGTGCCCACC	$(AG)_{15}$	144
VM25	F R	CCACAATCACCGATGTCCAA CAATTCCACTGCGGGACATAA	$(TC)_{18}$	240
VM26	F R	GGCATCAGACACATATCACTG TGTGGCATTGAGGGTAGC	$(TC)_{14}$	294
VM27	F R	GTCCAAAGCAAATGAGTCAA TGAATGACAATGAGGGTGC	$(AAT)_5..(TC)_{14}(AC)_3$	207
VM28	F R	GAATGAGAGAAGTTACGGTG GAGCACGATAATATTTGGAG	$(TC)_{20}$	250
VM29	F R	CGTGACACTAATAGTAGTCC CGAGTCTCGGACTCGCTT	$(TC)_{11}$	295
VM30	F R	CTCTTTCGCGTTCCACACTT GCAATGGGTTGTGGTCTGTG	$(TC)_{10}$	140
VM31	F R	CGCTCTTCGTTGATGGTTATG GAAAAAGGGAGGAACAAGCACAAC	$(CT)_{16}$	200
VM32	F R	GCACGAGATCTGGTGCTCCTT AGCGAAAACACGGAACTGAAATC	$(AG)_{10}$	177
VM33	F R	GCACGAGATCTGGTGCTCCTT CAGCGAGCGCGAACC	$(AG)_{18}(AC)_8$	270
VM34	F R	AGCTCCCCTAACCTGAAT TAACCCAATAATAAGACACAT	$(CT)_{14}$	216
VM35	F R	GGTCAATAGAATGGAAAGTGT ATGGCTGAAATAGGTGTCTGA	$(AG)_{11}(T)_9$	127
VM36	F R	ACTTTCTGTTTTACTCGACAACTC GTCGCTGGGGGTGGCTTATT	$(CT)_{13}$	160

Table 1. Contd.

VM37	F R	TGTCCGCGTTCTATAAATCAGC CGAGGATGAAGTAACAGATGATC	$(AG)_5(CCT)_3\ (CT)_{13}$	289
VM38	F R	AATGGGAAAAGAAAGGGAAGC TCGTGGCATGCAGTGTCAG	$(AG)_{10}(AC)_5$	135
VM39	F R	GATGGTTGTAATGGGAGAGTC AAAAGGATGAAATTAGGAGAGCA	$(AC)_{13}(AT)_5(TACA)_4$	212
VM40	F R	TATTACGAGAGGCTATTTATTGCA CTCTAACACCTCAAGTTAGTGATC	$(AC)_{18}$	200

* The predicted size was determined from the sequencing results for the isolated clones. F, R: Forward and reverse sequences.

information content varied from 0.30 to 0.72 with the average of 0.54.

Genetic diversity of the cowpea lines

As the previous study, SSR markers showed high levels of polymorphism in cowpea (Li et al., 2001), the present study could also use SSR marker to distinguish CYMV resistant lines in cowpea. In fact, four polymorphic microsatellites were able to distinguish 13 to 17 resistant lines out of the 20 resistant genotypes (data not shown). Among these 4 SSR markers, moth bean designed SSR marker, AGB1 (AG1/AF48383), produced 17 genotypes amplified 100 bp allele out of 20 susceptible genotypes analysed; Three genotypes HC98-08, HC2-62 and FS-68 did not have 100 bp allele amplifiction (Figure 2). In the other group of 20 resistant genotypes, 15 genotypes without 100 bp allele were obtained; three genotypes HC98-50, HC98-63 and HC1-10 (R7, R10 and R16, respectively) amplified 100 bp allele. A dendrogram of the 40 cowpea lines was constructed by the Unweighted Paired Group Method Using Arithmetic Averages on the basis of the genetic similarity (Jaccard's coefficient) (Figure 3). In the population of 20 CYMV resistant lines and 20 CYMV susceptible line of cowpea, it is expected that the lines shared the common genetic similarity within resistance and susceptible genotypes. In fact, groupings of the 40 cowpea lines based on microsatellite polymorphisms generally agreed with the resistant phenotype of these lines.

Two major groups separated at 45% similarity. Among 20 resistant cowpea lines analyzed, 18 lines shared 77 to 100% similarity, in which 10 cowpea lines are 100% similarity. These 10 genotypes were HC98-30, CS88, HC98-45, HC98-58, HC98-64, HC1-3, HC2-9, HC2-11, CPD26-0 and HC1-14 (R1, R3, R4, R9, R11, R12, R13, R14, R15 and R18, respectively). The other two resistant lines were classified in a separated group with one susceptible line and joined with resistant group at 47% similarity. The susceptible group consisted of two subgroups with 71% similarity for 7 cowpea lines (HC3-25, HC3-39, HC3-22, HC3-30, HC3-2, HC3-31, HC3-29), and 77% similarity among 12 cowpea lines (HC97-39, HC9B-28, HC2-59, HC2-72, HC2-85, HC98-08, HC2-62, HC2-61, HC2-69, HC3-40, HC2-87 and HC3-21). The same pattern of similarity between resistant and susceptible groups was observed in two-dimensional (Figure 4) and three-dimensional principle coordinate analysis (Figure 5). There were also two separated groups of resistant and susceptible genotypes on the dendrogram with resistant genotypes HC1-19, HC98-33, HC1-10, HC1-11, HC1-14 and HC1-15 (R20, R2, R16, R17, R18 and R19, respectively) lying closer to each other in one group, and the susceptible genotypes HC2-62, HC2-72, HC2-85, HC2-87, HC3-22, HC3-25, HC3-30, HC3-31, HC3-39 and HC3-40 (S6, S8, S9, S11, S14, S15, S17, S18, S19 and S20, respectively) were gathered in another group. HC98-48, HC98-51, HC98-63, FS-68 and HC3-29 (R6, R8, R10, S10 and S16) were separated with these two groups.

DISCUSSION

Microsatellite markers have been used to detect polymorphism in many important crop species. They have shown high levels of polymorphism in rice (*Oryza sativa* L., Chen et al., 1997), wheat (*Triticum aestivum* L., Devos et al., 1995), barley (*Hordeum vulgare* L., Liu et al., 1996), oat (*Avena sativa* L., Li et al., 2000), maize (*Zea mays* L., Senior et al., 1998), sorghum (*Sorghum bicolor* (L.) Moench, Brown et al., 1996), soybean (*Glycine max* (L.) Merr., Akkaya et al., 1992), beans (*Phaseolus* and *Vigna*, Yu et al., 1999), *Brassica* species (Szewc-McFadden et al., 1996), alfalfa (*Medicago* spp., Diwan et al., 1997), sun-flower (*Helianthus annuus* L., Brunel, 1994), tomato (*Lycopersicon esculentum* Mill., Smulders et al., 1997), and cowpea (*Vigna unguiculata* L. Walp, Li et al., 2001).

Table 2. Summary of SSR primer pairs specific for moth bean.

Primer	Accession	Primer sequence (5'-3')	SSR sequence	Predicted size (bp)*
AGB1	AG1 AF48383	CATGCAGAGGAAGCAGAGTG GAGCGTCGTCGTTTCGAT	$(GA)_8GGTA(GA)_5GGGGACG(AG)_4$	132
AGB2	GATS11 AF48384	CACATTGGTGCTAGTGTCGG GAACCTGCAAAGCAAAGAGC	$(CT)_8CA(CT)_2GTTT\ (CT)_4$	306
AGB3	GATS11B AF48384	CCCACACATTGGTGCTAGTG AGCGCAATGCTACTCGAAAT	$(CT)_8$	160
AGB4	GATS54 AF48384	GAACCTGCAAAGCAAAGAGC TCACTCTCCAACCAGATCGAA	$(GA)_5AACAGAGT\ (GA)_8$	114
AGB5	GATS91 AF48384	GAGTGCGGAAGCGAGTAGAG TCCGTGTTCCTCTGTCTGTG	$(GA)_{17}$	229
AGB6	BM3 AF48384	CAGAAGTGCTTATCCCCGAG TGAAATCTTCCCCTCCTTCA	$(GAA)_3GATGAA\ (GCA)_2(GAA)_4$	193
AGB7	BM6 AF483844	AGGGTTTACACACGACAGGC GGTTGATATGCCCTCATGGT	$(GAAAA)_3$	153
AGB8	BM16 AF483845	CACCGGGAGTGGCTGACA GTTTGGGGCGGAGTTCGA	$(CA)_{21}TA(CA)_5$	149
AGB9	BM20 AF483846	ATCCGTAGAGAGGTGAACGG ATGAGTGCAGTTTGGTGCAG	$(CAGA)_3GACA\ (CAGA)_{12}$	146
AGB10	BM25 AF483847	CGCCTCCAACGGTCTTCT CAAGCAGGTGCGAATCCA	$(CA)_{17}CG(CA)_2$	227
AGB11	BM48 AF483848	GCCGTTGAGCTGGAGAGCA CCTTCTTCTTGAGCCCGCTG	$(GA)_5$	232
AGB12	BM53 AF483849	AACTAACCTCATACGACATGAAA AATGCTTGCACTAGGGAGTT	$(CT)_{21}(CA)_{19}(TA)_9$	287
AGB13	BM67 AF483850	CCAATGCTGCCACACAGATA CGCCCTTATGATCCAGTCCT	$(CA)_{31}(CG)_5\ (CA)_{10}$	289
AGB14	BM68 AF483851	TTCGTTCACAACCTCTTGCATT TGCTTGTTATCTTGCCCAGTG	$(CA)_6TA(CA)_4\ (TA)_4\ (CA)_5$	170
AGB15	BM79B AF483852	CATGGAGGTAGAGGATAATAAGGAG CATTAGAGCCGCCACTTG	$(GA)_{28}$	125
AGB16	BM98 AF483853	GCATCACAAAGGACTGAGAGC CCCAAGCAAAGAGTCGATTT	$(CA)_8(CT)_3$	247
AGB17	BM114 AF483854	AGCCTGGTGAAATGCTCATAG CATGCTTGTTGCCTAACTCTCT	$(TA)_8(GT)_{10}$	234
AGB18	BM137 AF483855	CGCTTACTCACTGTACGCACG CCGTATCCGAGCACCGTAAC	$(CT)_{33}$	155
AGB19	BM138 AF483856	TGTCCCTAAGAACGAATATGGAATC GAATCAAGCAACCTTGGATCATAAC	$(GT)_{13}$	203

Table 2. Contd.

AGB20	BM139 AF483857	TTAGCAATACCGCCATGAGAG ACTGTAGCTCAAACAGGGCAC	$(CT)_{25}$	115

*The predicted size was determined from the sequencing results for the isolated clones.

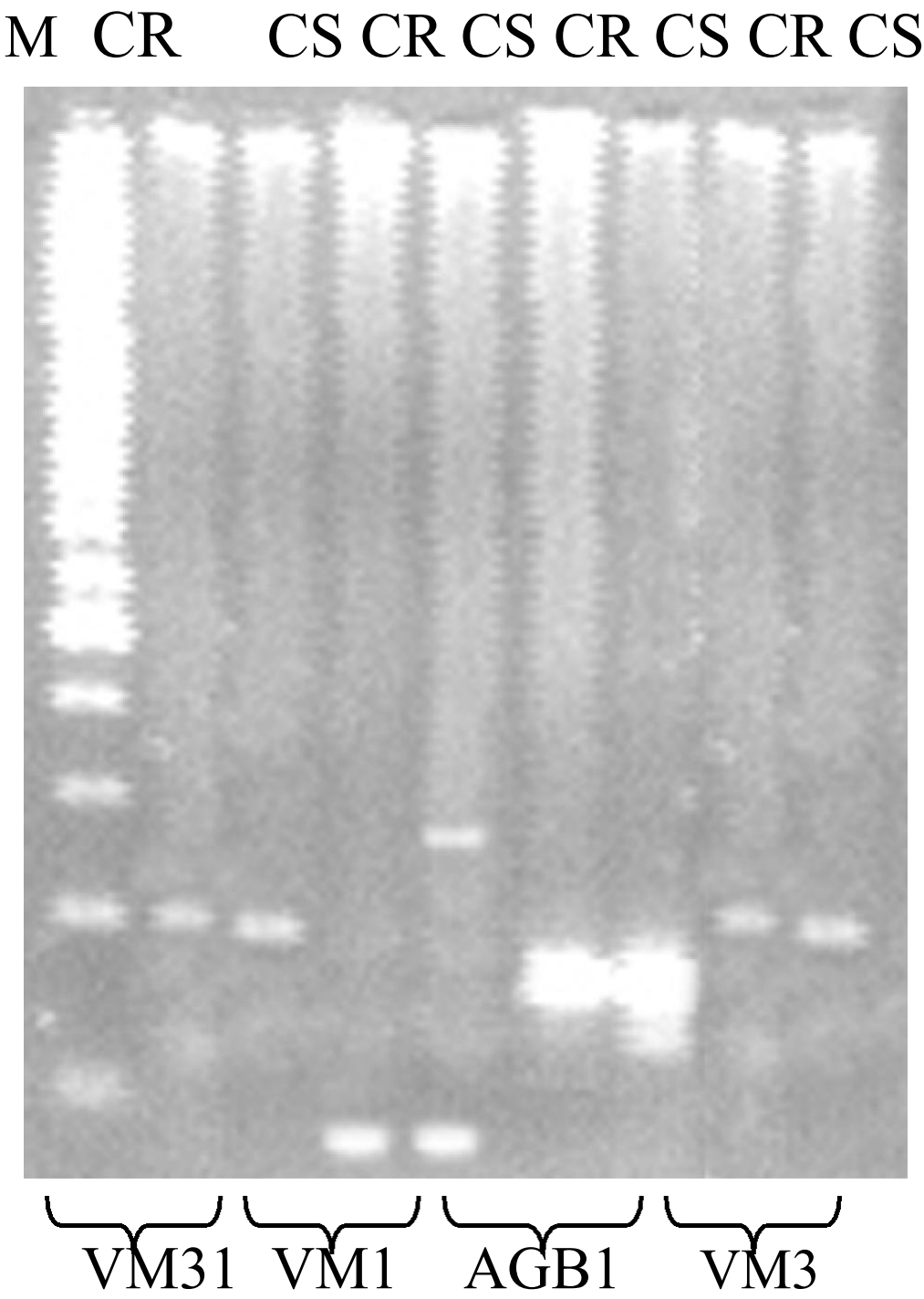

Figure 1. Polymorphic pattern of VM31, VM1, AGB1 and VM3 PCR products between standard resistant and susceptible varieties. Lane M: 100 bp ladder; CR: check (standard) resistant variety (GC-3), CS: check (standard) susceptible variety (Chirodi).

Table 3. Polymorphism in cowpea genotypes revealed by SSR primers.

SSR primers	Total no. of alleles	Polymorphic alleles	Monomorphic alleles	Percent polymorphism	PIC value
VM1	3	2	1	66.7	0.72
VM3	2	2	0	100.0	0.30
VM31	2	2	0	100.0	0.45
AGB1	4	3	1	75.0	0.67
Average	2.75	2.25	0.5	85.43	0.54

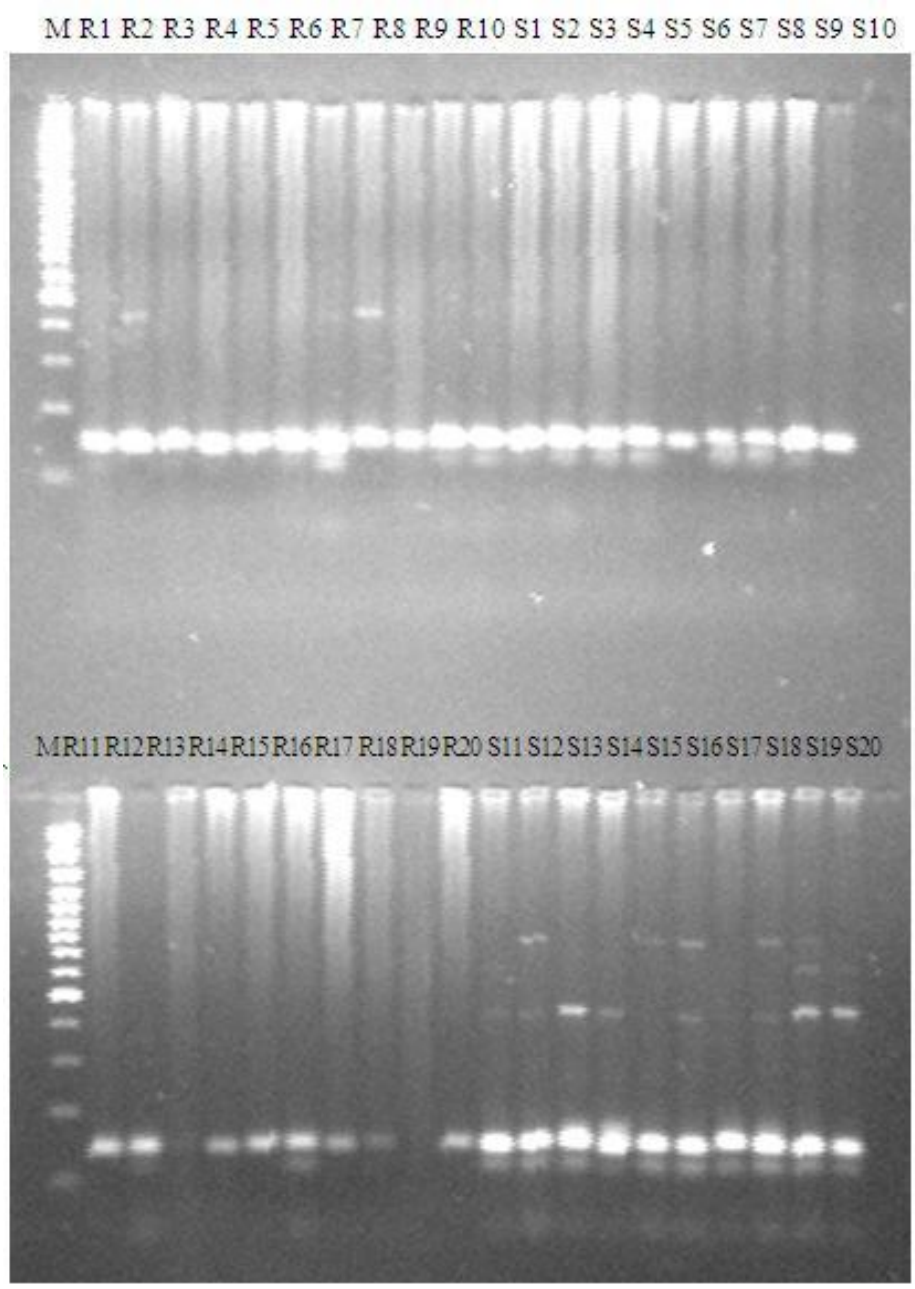

Figure 2. Electrophoresis pattern of PCR amplified fragments of 20 susceptible and 20 resistant genotypes with SSR marker AGB1, Lane M: 100bp DNA ladder; Upper lanes R1-10: resistant genotypes, S1-10: susceptible genotypes; Lower lanes R11-20: resistant genotypes; S11-20: susceptible genotypes.

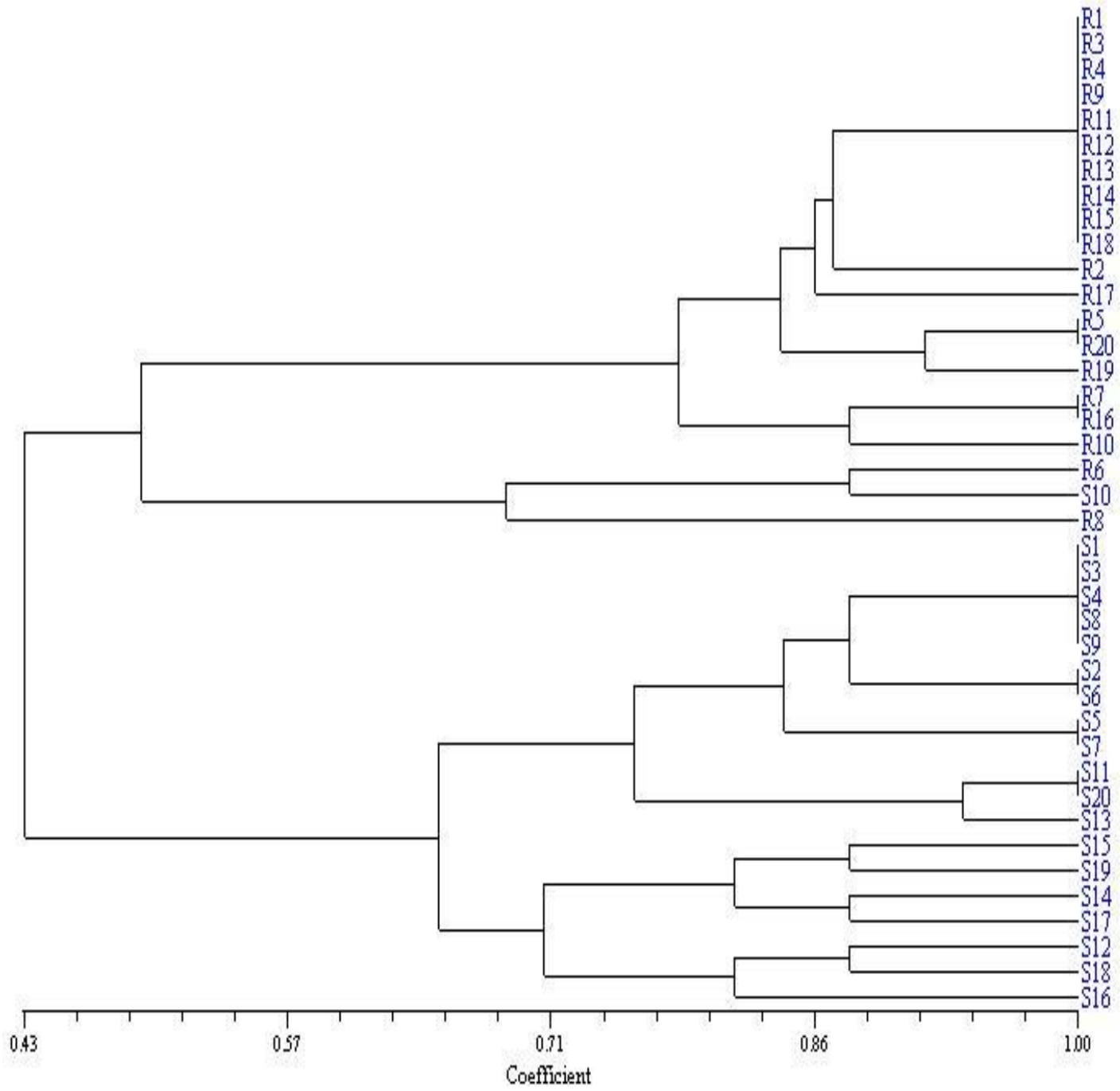

Figure 3. Phylogenetic relationship of the 40 cowpea lines constructed using four microsatellite polymorphisms.

The present study showed that microsatellite markers could also be used to distinguish CYMV resistant lines in cowpea. In fact, four polymorphic microsatellites were able to distinguish 13 to 17 resistant lines out of the 20 resistant genotypes. All the microsatellite primer pairs of cowpea could successfully amplify DNA from 40 cowpea lines. Furthermore, two microsatellite primer sets designed from the sequences of moth bean (AG1/AF48383 and BM98/AF483853; AGB1 and AGB16) were able to amplify DNA of cowpea in which AG1/AF48383 (AGB1) could distinguish 15 resistant lines among 20 resistant genotypes investigated. Therefore, microsatellite markers of cowpea could be used to detect CYMV resistant genes and map these genes to cowpea linkage map. In addition, these microsatellite primers could be used for comparative

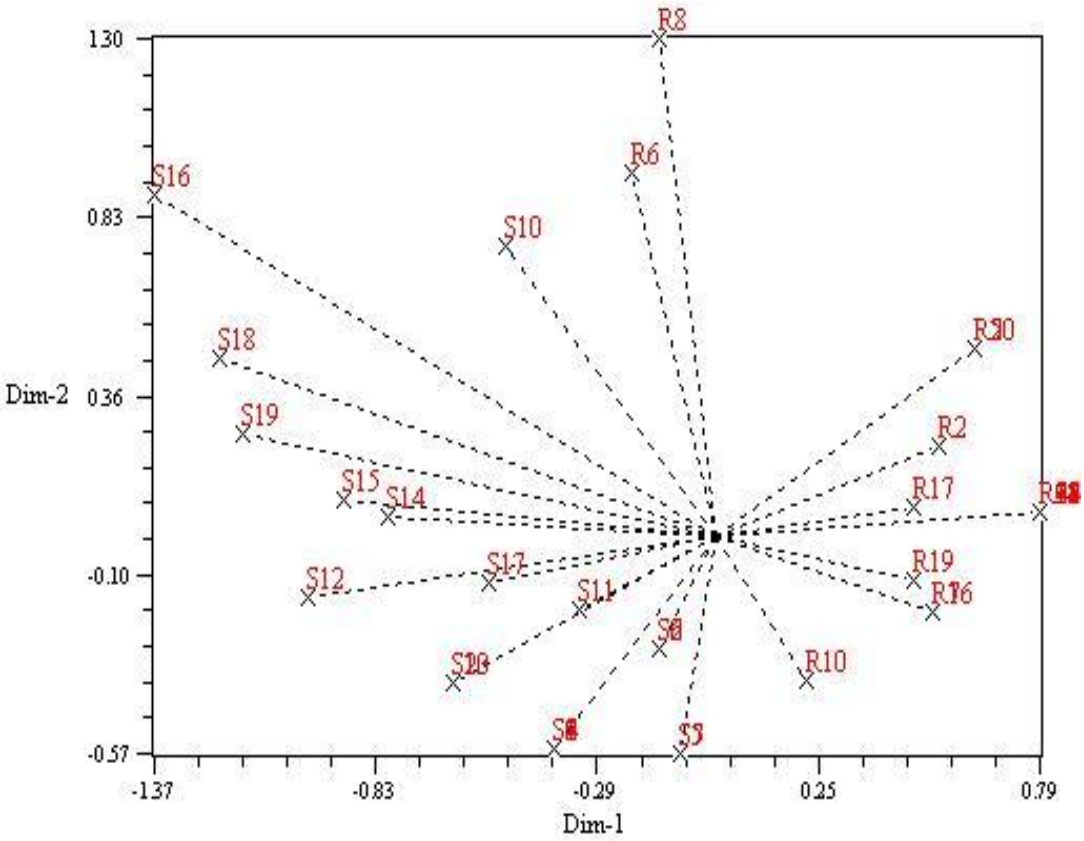

Figure 4. Two-dimension principle coordinates analysis of the 40 cowpea lines.

Figure 5. Genetic similarity among 40 cowpea genotypes revealed by three-dimensional view of dendrogram.

genome analysis between the different *Vigna* species. To differentiate the genetic bases of a number of cultivars in the same species, microsatellite markers could also be used.

Application of SSR markers have been used to estimate the genetic diversity of a large number of cultivars in rice (Yang et al., 1994), soybean (Rongwen et al., 1995), wheat (Plaschke et al., 1995), maize (Senior et al., 1998), and cowpea (Li et al., 2001). The number of alleles amplified per primer pair was from 3 to 25 for rice, 11 to 26 for soybean, 3 to 16 for wheat, 2 to 23 for maize and 2 to 7 for cowpea. In the present study, the same results were obtained as Li et al. (2001) reported earlier. It was observed that only one to seven alleles per primer pair were amplified from the 40 cowpea lines, but in present study, microsatellites bands were detected on 3% agarose gel electrophoresis. This showed that the level of microsatellite polymorphism in cowpea is much lower than other crops. The same reason as Li et al. (2001) did, the materials used in the present study were all from the pure line of cowpea created and maintained in HAU and thus had a relatively narrow genetic base. In a study of genetic diversity in soybean, 11 to 26 alleles per microsatellite primer pair were amplified from 96 soybean genotypes while this number was reduced to five to 10 alleles per primer pair in 26 cultivars from North American breeding programs (Rongwen et al., 1995). The other possible reason for the low level of microsatellite polymorphism is that the cultivated cowpea is relatively low in genetic diversity compared with other crops. It has been suggested that cowpea was only domesticated once (Pasquet, 1999), unlike *P. vulgaris* (Singh et al., 1991) or rice (Second, 1981). The low genetic diversity in cultivated cowpea may be a result of this narrow genetic base.

The low level of genetic diversity at the DNA level among cowpea breeding lines and cultivars could be increased by using its wild relatives to broaden the genetic base. Li et al. (2001) demonstrated that microsatellite markers were conserved among *Vigna* species. Hence microsatellite markers could provide a simple approach to assaying the introduction of such genetic material. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles (Smith et al., 1997). PIC values range from 0 (monomorphic) and 1 (very high discriminative, with many alleles in equal frequencies). Senior et al. (1998) reported that PIC is synonymous with the term “gene diversity” as described by Weir (1996). The PIC value of SSR markers in the present study was not very high and ranged from 0.30 to 0.72 but only four out of 42 SSR primer pairs gave polymorphism. The PIC values of SSR markers can be compared to results reported by Li et al. (2001) with PIC ranged from 0.02 to 0.73. Presently, event poly-acrylamide gels were used to detect the DNA alleles, the polymorphic information still could not compare with other crop species.

Groupings of the 40 cowpea lines based on microsatellite polymorphisms generally agreed with the CYMV resistant phenotype of these lines. Two major groups separated at 45% similarity (Figure 4). The resistant group including 18 resistant lines with 77 to 100% similarity. The other two resistant lines were classified in a separated group with one susceptible line and joined with resistant group at 47% similarity. The susceptible group consisted of two subgroups with 71 and 77% similarity within each subgroup. Comparison of the dendrogram produced by the present study with that constructed using 90 cowpea breeding lines and one wild relative done by Li et al. (2001), the genetic similarity concentrated into two groups of cowpea lines resistance to CYMV disease and susceptible ones, but not disperses discrepancies and incongruities as the earlier dendrogram. This agreement showed that microsatellite marker can be used to estimate the genetic bases of CYMV resistance in cowpea.

In conclusion, microsatellite markers are polymorphic in cowpea. They can be used to distinguish CYMV resistance lines of cowpea. A dendrogram constructed based on microsatellite polymorphism generally agreed with the reaction of cowpea lines with CYMV disease. The degree of the polymorphism is relatively low in cowpea compared with other crops.

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