FINAL REPORT

COLLABORATIVE PROJECT CIAT-IBPGR

~~DNA~~ POLYMORPHISM AND

DNA FINGERPRINTING OF CASSAVA

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INTRODUCTION

When genomic DNAs from two genetically distinct individuals are digested with a restriction enzyme, electrophoresed, blotted onto a membrane, and probed with a radioactively-labelled DNA clone, polymorphisms in the hybridization patterns sometimes result due to sequence differences between the individuals. The development of restriction fragment length polymorphism (RFLP) technology has opened a door to detecting, monitoring and manipulating genetic variation in plants in a manner not previously possible (1). One of the most ready application of this technology is in assessing genetic variation in natural populations and exploring the evolutionary relationships among plant taxa (2).

DNA fingerprinting techniques are currently being used as a complement for isozyme characterization and to construct saturated genetic maps in many crops (3-6), mainly because it can cover an extended portion of the plant genome. There are several advantages of using DNA techniques to measure and monitor genetic variation. The ability to score DNA markers is largely unaffected by the environment (including seasonal fluctuations and geographical origin) and stage of development, problems often encountered with morphological and even protein markers. In addition DNA techniques can detect variation in noncoding as well as in coding regions of the genome.

"DNA Fingerprints" have been reported in a wide array of organisms. A major advance has been the discovery of minisatellite DNA sequences present in the human genome that are hypervariable in many species of animals and plants (7, 8). Recently, it was found that wild-type M13 bacteriophage also detect hypervariable minisatellites in human, animal and plant DNA (9).

In this study we used DNA techniques to estimate the level of DNA variation in 3 varieties of cassava belonging to P-IVAG project by using different probes which have been cloned at the BRU. Smallest Cla I/Bsm I fragment of the M13 bacteriophage including two regions with repeats capable to reveal "DNA fingerprints" in some angiosperms species (10) was also used in this study.

RESEARCH PROCEDURES

DNA Extraction

Isolation of nuclei from M Col 22 cultivar was carried out as described previously (11). Approximately 10 g of green leaf tissue was quickly frozen in liquid nitrogen and grinded to a fine powder in a mortar, then added to 20 ml of a chilled cell lysis and neutralization buffer (CLAN) composed of 50 mM Tris-HCl pH 7,8, 5 mM $MgCl_2$, 100 mM KCl, 0,5% PVP-40 (polyvinyl-pyrrolidone), 10 mM 2-Mercaptoethanol and 0,15% Nonidet P-40. The mixture was agitated on a magnetic stirer plate for 3-10 min at 4°C to allow the buffer to thaw, then filtered through two layers each of cheesecloth and miracloth to remove large debris. The filtrate was centrifuged at 1000 x g for 10 min at 4°C and the supernatant solution was discarded. The pellet was resuspended in 10 ml of CLAN by several passages through a 10 ml. (2mm diameter tip) plastic pipet. The volume was brought to 50 ml with prechilled corn saline buffer (CSB) containing 50mM tris-HCl pH 7,8, 5mM $MgCl_2$, 100 mM KCl and 1mM 2-Mercaptoethanol; and centrifuged at 100 xg for 5 min at 4°C. Nuclei were observed by light microscopy. Nuclear and genomic DNA isolation procedures were similar to that described by Dellaporta et al (12).

Genomic Library Construction

Nuclear DNA was divided into 5 fractions. Each fraction was digested with one of the following restriction enzymes (Pst I, Eco RI, Bam HI, Xba I, Hind III). Digested DNA was ligated into pUC19 plasmid (13). DH5-alpha bacterial cells were then transformed with ligated plasmid. Colonies of cells containing plasmids with cassava inserts were selected based on X-gal and IPTG screening procedures (14). Individual colonies were isolated and plasmid mini-preps were prepared (15). Insert sizes were determined relative to BstEII-digested lambda on 1,0% agarose gels.

Restriction digests, electrophoresis and Southern analysis

Three cassava cultivars (M Col 22, M Col 1505, CM507-37) from the P-IVAG project were used in this work. DNA from each cultivar was digested with the following 10 enzymes (Bam HI, Eco RI, Hind III, Pst I, Xba I, Hae III, Eco RV, Dra I, Taq I and Hpa II). Spermidine (4 mM) was added to digests to promote complete digestion. Electrophoresis of plant DNA (3 ug DNA per lane), Southern blotting and hybridization were carried out as described by Southern (16). Whole plasmids including cassava inserts between 0,2 Kb and 3,0 Kb were hexamer labeled with ^{32}P-dATP to high specific activities (1-10 x 10^8 cpm/ug) (17) and used as probes on filters of cassava DNA. Filters were washed at 65°C for 30 min each at 2 x SSC, 1 x SSC and 0,5 x SSC (all washes contained 0,1% SDS) and exposed to X-ray film with intensifier screens.

When M13 bacteriophage was used as probe, DNA from each cultivar was digested with Hae III enzyme. Ten micrograms of digested DNA was electrophorosed in 25-cm long 1% agarose gels. JM 105 cells were infected with M13 bacteriophage and 500 ug of DNA were extracted and purified with polyenthilenglycol as described (18).

Cla I/Bsm I fragment (Fig. 1) was eluted from the gel and hexamer labeled with ^{32}P-dATP as above. Filters were hybridized in 7% SDS, 1 mM EDTA (pH 8.0), 0.263M Na_2HPO_4 and 1% bovine serum albumin (fraction V); and washed twice each for 15 min. in 2XSSC, 0.1% SDS at room temperature followed by one 60 min. wash in the same solution at 50°C (19). Filters were exposed for one day with intensifier screens.

DNA Sequencing

Dideoxy method described by Sanger et al., (20) using bacteriophage T7 DNA polymerase was used.

RAPD

Random amplified polymorphism DNA technique recently described (21, 22) was used. The primer E11 (GAGTCTCAGG) was obtained from OPERON. Amplification reactions were performed in volumes of 25 µl containing 10mM Tris-HCl, pH 8,3, 50 mM KCl, 2mM $MgCl_2$, 0.001% gelatin, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.2µM primer, 25 ng of genomic DNA and 0,5 unit of Taq DNA polymerase. Amplification was performed in a thermal cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 36°, 2 min at 72°. Amplification products were analyzed by electrophoresis in 1,5% agarose gels and detected by staining with ethidium bromide.

RESULTS

Digestion with restriction enzymes

DNA isolated from green leaf tissue was digested with ten restriction enzymes. The majority of the DNA remains high molecular weight when digested with methylation - sensitive enzymes (MspI, ApaI, Pst I) (Figure 2, lanes m, o, p). These enzymes are

sensitive to cytosine methylation in the 5' location (23). Comparative digests with C-methylation - insensitive enzymes including Eco RI, Eco RV, Taq I, Hinf I shows a distribution of fragment sizes between 0,2 and 10 Kb (Figure 2, lanes d, f, l, n). In all cases, cassava DNA was cut more effectively by C-methylation - insensitive enzymes than was by C-methylation-sensitive enzymes. A comparison of cutting efficiencies of C-methylation-sensitive and insensitive restriction enzymes would suggest that cassava DNA is highly methylated.

The role of DNA methylation in eucaryotes and especially plants is poorly understood (24). There is evidence that methylation plays a role in gene regulation but there are many examples of a negative correlation between methylation and gene expression (25). DNA methylation in cassava must be studied in detail.

Cloning of DNA in pUC19 vector

Digested DNA from M Col 22 cultivar from the P-IVAG project was cloned in polylinker site of pUC19 plasmid. Five kinds of fragments; Pst I, Bam HI, Xba I, Eco RI and Hind III fragments, were cloned. After transformation of bacterial cells, the colonies of cells containing plasmids with cassava inserts were selected based on X-gal hydrolysis by B-galactosidase synthesis in LB medium supplemented with ampicillin. White colonies containing recombinant plasmids (Figure 3) were isolated, grown in LB medium and stored at -20°C in 30% glycerol until required.

Size of inserts

200 white colonies were grown in LB medium for twelve hours and plasmids mini-preps were prepared. Purified plasmids were digested in order to separate the insert cloned from the plasmid. The inserts were separated by electrophoresis on 1,0% agarose gels and their sizes were determined relative to BstE II-digested lambda.

Five kind of inserts were obtained, between 0,2 Kb and 7 Kb (Figure 4), which is the optimal size range for detecting polymorphic fragments and to conduct fingerprinting analysis.

Dot blot Hybridization

Genomic clones were preselected for low and repetitive copy number sequences by hybridizing with ^{32}P-labelled total genomic DNA. Results of this hybridization are shown in Figure 5 where differences in the hybridization intensity are notable. Strongly hibridizing clones were omitted from further screening (clones 14, 24) and the remaining clones were considered to represent either a single or low copy sequence and used for RFLP studies.

Southern blot analysis

The DNA from three cassava lines cultivars M Col 22, M Col 1505 and CM 507-37, was individually digested with each of the following restriction endonucleases: Bam HI, Dra I, Eco RI, Eco RV, Hae III, Hind III, Hpa II, Pst I, Taq I, Xba I. Digestions proceeded for over 16 hours to ensure completion. DNA was depurinated (0,25 M HCl, 15 min), denatured (1,5 M NaOH - 1,5 M NaCl) and transferred from the gel onto nylon membrane. Whole plasmids including cassava inserts were labeled and hybridized to the nylon filters.

Hind III, Pst I, and Xba I probes showed similar ability to detect polymorphism between three cultivars studied here whereas in Eco RI and Bam HI probes this ability was low. When M Col 22 was compared with both M Col 1505 and CM 507-37 cultivars, the level of polymorphism reached 60% but it was between 5% and 30% when M Col 1505 was compared with CM 507-37 (Table 1).

Tables 2 to 7 compare the ability of ten different restriction enzymes (seven were six cutters and three were four cutters) to detect polymorphism.

Two enzymes Eco RI and Eco RV displayed high level of polymorphism when both Hind III and Xba I probes were used (Tables 4 and 6). However, when Pst I probes were used Hae III restriction enzyme detected 35% of polymorphism vs 30% and 10% displayed by Eco RI and Eco RV respectively (Table 5).

Dra I and Hpa II did not detect polymorphism between M Col 1505 and CM507-37. Further, polymorphism displayed by these two enzymes between M Col 22 and the other two cultivars was very low. Higher polymorphism between M Col 22 and both M Col 1505 and CM507-37 was detected by Eco RV enzyme when Hind III probes were used (Table 4). Polymorphism between M Col 1505 and CM507-37 was low in all cases. However, Eco RV and Eco RI enzymes also detected higher level of polymorphism between these two cultivars (Table 7).

Table 1. Source of probes compared in their ability to detect polymorphism at least with one restriction enzyme.

Probe	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Pst I	60	5	60
Xba I	60	20	55
Hind III	55	30	55
Eco RI	40	20	40
Bam HI	30	5	30

Table 2. Percent polymorphism with Bam HI Probes.

Bam HI Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	30	10	30
Dra I	-	-	-
Eco RI	35	10	35
Eco RV	10	-	10
Hind III	-	-	-
Hae III	-	-	-
HpaII	-	-	-
Pst I	-	-	-
Taq I	10	-	10
Xba I	-	-	-

Table 3. Percent polymorphism with Eco RI probes.

Eco RI Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	20	-	20
Dra I	-	-	-
Eco RI	10	-	10
Eco RV	20	10	10
Hind III	30	10	30
Hae III	20	10	10
HpaII	-	-	-
Pst I	30	-	30
Taq I	10	-	10
Xba I	20	10	10

Table 4. Percent polymorphism with Hind III probes.

Hind III Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	20	-	20
Dra I	5	-	5
Eco RI	45	20	45
Eco RV	55	30	50
Hind III	5	-	5
Hae III	5	-	5
HpaII	5	-	5
Pst I	35	5	20
Taq I	5	-	5
Xba I	35	5	20

Table 5. Percent polymorphism with Pst I probes.

Pst I Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	20	-	20
Dra I	10	-	10
Eco RI	30	-	30
Eco RV	10	-	10
Hind III	30	-	30
Hae III	35	-	30
HpaII	-	-	-
Pst I	20	-	20
Taq I	-	-	-
Xba I	10	5	-

Table 6. Percent polymorphism with Xba I probes.

Xba I Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	20	-	20
Dra I	-	-	-
Eco RI	50	10	45
Eco RV	45	10	45
Hind III	35	5	35
Hae III	15	5	10
HpaII	-	-	-
Pst I	15	-	15
Taq I	5	10	5
Xba I	15	10	10

Table 7. Percent polymorphism with five kind of probes.

Probes	M Col 1505/M Col 22	M Col 1505/CM507-37	M Col 22/CM507-37
Bam HI	22	2	22
Dra I	3	-	3
Eco RI	34	8	34
Eco RV	29	10	25
Hind III	20	3	20
Hae III	15	3	11
HpaII	1	-	1
Pst I	20	1	17
Taq I	6	2	6
Xba I	16	6	8

Different patterns of polymorphism were detected with our genomic clones. A Pst I clone, P21, detected polymorphism in M Col 22 when either Eco RI or Hind III restriction enzymes were used (Figure 6), but this clone did not discriminate between M Col 1505 and CM507-37. This clone can be classified as single copy sequence because only one or two bands were present, and suggests that polymorphism is due to base substitution. M Col 22 cultivar has an additional cleavage site inside both the Eco RI and Hind III fragments that reduce those fragments in size and produce another smaller fragment (Figure 6).

A Xba I clone X14, detected polymorphism in M Col 22 when the three cassava cultivars were digested with Pst I restriction enzyme (Figure 7), but it was not able to discriminate between CM 507-35 and M Col 1505 cultivars (Figure 7). Similar to P21 clone, polymorphism detected by X14 clone is due to an additional Pst I site in M Col 22. M Col 22 shows an additional cleavage site in the 4,5 Kb fragment of CM507-35 and M Col 1505, that reduces this fragment in size and produces a 1,0 Kb fragment in M Col 22 (Figure 7).

On the other hand, a Bam HI clone named B5, and a Xba I clone X6 detected another kind of polymorphism in M Col 22. When those clones were used as probes, additional bands were observed in M Col 22 when its hybridization pattern was compared to those showed by CM507-37 and M Col 1505 cultivars. The B5 probe revealed two additional bands in M Col 22 when the three cultivars were digested with Eco RV restriction enzyme (Figure 8, left arrows). The X6 probe showed a similar polymorphism to that displayed by B5 probe but only a 0,5 Kb additional fragment was detected in M Col 22 when digested with Eco RI enzyme (Figure 9). These results suggest the presence of a unique sequence present in M Col 22 but absent in CM507-37 and M Col 1505. This polymorphism is probably caused by an insertion in M Col 22. This point was clarified recently when we found a Pst I clone, P12, which hybridizes with DNA from M Col 22 but does not with DNA from other cultivars.

These results indicated that P12 insert contains a specific sequence present only in some cultivars (Fig. 10, Table 8). This fragment may be classified as "single dose restriction fragment" recently described (26).

DNA sequencing

P12 was sequenced partially. Two hundred eighty eight nucleotides were sequenced directly in pUC19 by using universal primers.
109 nucleotides and 179 were sequenced from the universal and reverse primers respectively.

UNIVERSAL SEQUENCING PRIMER

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          10          20          30          40          50
5'  CTTCTAGAGC GGACNGTGCT GCCCCTTCTT TCTAGACTTT GATGAAAGTT
3'  GAAGATCTCG CCTGNCACGA CGGGGAAGAA AGATCTGAAA CTACTTTCAA

          60          70          80          90          100
    GCTTGCGAAG TGCAATATGA TGATCGTAAA GACCATTTAA TCAAGCGCTG
    CGAACGCTTC ACGTTATACT ACTAGCATTT CTGGTAAATT AGTTCGCGAC

    ATCTAATA 3'
    TAGATTAT 5'
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REVERSE SEQUENCING PRIMER

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          10          20          30          40          50
5'  TGCAGGGAAA CTTACCTTAC CTTACCTACC TGGCTTGACA AAAGTAAATA
3'  ACGTCCCTTT GAATGGAATG GAATGGATGG ACCGAACTGT TTTCATTTAT

          60          70          80          90          100
    TGTCAAGTTT TGTGCATTCC ATCGAAATAC GTCATGAAAC CAACTCATGT
    ACAGTTCAAA ACACGTAAGG TAGCTTTATG CAGTACTTTG GTTGAGTACA

          110         120         130         140         150
    TGTGCCACTC CAGGACATCA TTGAAATTTA GATCAAGTCT GGAGAGATTA
    ACACGGTGAG GTCCTGTAGT AACTTTAAAT CTAGTTCAGA CCTCTCTAAT

          160         170
    ACTTCGTCGT CAAAGTCAAC AACTCATCA 3'
    TGAAGCAGCA GTTTCAGTTG TTGAGTAGT 5'
```

M13 - Cla/Bsm I Probe

By probing Southern, transfers of endonuclease digested and gel fractionated plant DNA extracts (including the nuclear, chloroplast and mitochondrial components) with the smallest Cla I/Bsm I fragment of the M13 bacteriophage, it has been demonstrated that it reveals minisatellites bearing endonuclease fragments in gymnosperms and angiosperms.
We isolated and used as probe this 0,781 Kb fragment (Fig. 1) in order to reveal "DNA fingerprint" of cassava. The filters were hybridized and washed as described above.

Four cultivars from Colombia (M Col 22, M Col 21, M Col 1505, M Col 638), five from Brazil (Br 885, Br 348, Br 12, Br 715, Br 534), two from Venezuela (Ven 331, Ven 82), two hybrids (CM507-37, CM91-3), one from Argentina (Arg 13) and one from Thailandia (Thai-1) were discriminated by this probe (Fig. 11). The origin of each cultivar is indicated (Table 8). This probe discriminated between 15 different cultivars used here, indicating that M13 is a good probe to carry out "DNA fingerprinting" studies in cassava.

Ribosomal gene-specific probes

Three ribosomal gene-specific probes from soybean (27), were also used in this study. pGmr1, pGmr3 and pXBr1 which include 100%, 50% and 5% of a single soybean rDNA repeat, respectively (Figure 12).

Seven different cultivars were digested with either Hae III or Taq I restriction enzymes and hybridized with these three ribosomal probes. When TaqI was used pGmr3 and pGmr-1 probes displayed some additional bands in M Br 348, M Arg 13, M Ven 331, M Ven 82 and M Col 22. However, patterns were very similar (Fig. 13,

14). No differences were found when Hae III digests were hybridized with these probes (Fig. 13, 14). pXBr1 probe did not detect polymorphism (results not shown).

RAPD

Two cultivars M Col 1505 and M Col 22 and one wild *Manihot* species, *M. aesculifolia* were assayed for RAPD technique using a primer whose content in G-C was 60%. Conditions described in research procedures allowed us to discriminate among three genotypes (Figure 15) indicating the feasibility of this technique to carry out DNA fingerprinting studies.

Table 8.

Cultivar	Origin	P12
Brazil 715	South Br	+
Brazil 121	Northeastern Br	+
Brazil 534	Northeastern Br	+
Brazil 160	Northeastern Br	-
Brazil 885	Northeastern Br	-
Brazil 191	Northeastern Br	-
Brazil 348	Amazonas Br	-
Brazil 502	Amazonas Br	-
Brazil 5	ND	-
Brazil 12	ND	-
Brazil 309	ND	-
Ecuador 117	Ecuador	+
Ecuador 10	Ecuador	+
Ecuador 166	Ecuador	+
Peru 213	Peru	-
Peru 328	Peru	-
Venezuela 82	Orinoco	-
Venezuela 331	Orinoco	-
Argentina 13	Argentina	-
Col 21	North	+
Col 22	North	+
Col 1505	ND	-
CM 507-37	CIAT hybrid	-
Col 638	Llanos	+
Thailandia 1	Thai	+
Thailandia 8	Thai	-
Nigeria 5	Nig	-
4955-11	Thai-1 x Br5	+
4955-12	Thai-1 x Br5	+
*Chartaginensis	North of Colombia	+
*Grahami	Paraguay	+
*Aesculifolia	Mexico	+

N.D. Not determined
* Wild Manihot species

CONCLUSIONS

1. Cassava genomic DNA remained high in its molecular weight when digested with restriction enzymes sensitive to cytosine methylation in the 5' location, suggesting that cassava DNA is highly methylated.

2. Pst I and Xba I probes were the best probes to discriminate between M Col 22 and both CM507-37 and M Col 1505 cultivars.

3. Hind III were the best probes to discriminate between M Col 1505 and CM507-37 cultivars.

4. The best combination probe/enzyme to discriminate between M Col 22, M Col 1505 and CM507-37 cultivars was Hind III/Eco RV.

5. Eco RI and Eco RV restriction enzymes displayed highest polymorphism among the three cultivars tested.

6. Dra I, Hpa II and Taq I restriction enzymes displayed lowest polymorphism among the three cultivars studied.

7. The smallest Cla I/Bsm I fragment of the M13 bacteriophage was able to discriminate eleven cultivars from different geographical origins indicating that this fragment is a good probe to carry out "DNA fingerprinting" studies in cassava.

8. Three ribosomal gene-specific probes from soybean were not good to discriminate between different cassava genotypes.

9. Three different genotypes were easily discriminated by using the RAPD technique, suggesting that this technique will be very useful in DNA fingerprinting studies of cassava for germplasm characterization.

10. A fragment called P12 was only present in fifteen genotypes out of thirty two genotypes tested. This fragment could be classified as a "single dose, restriction fragment", recently described.

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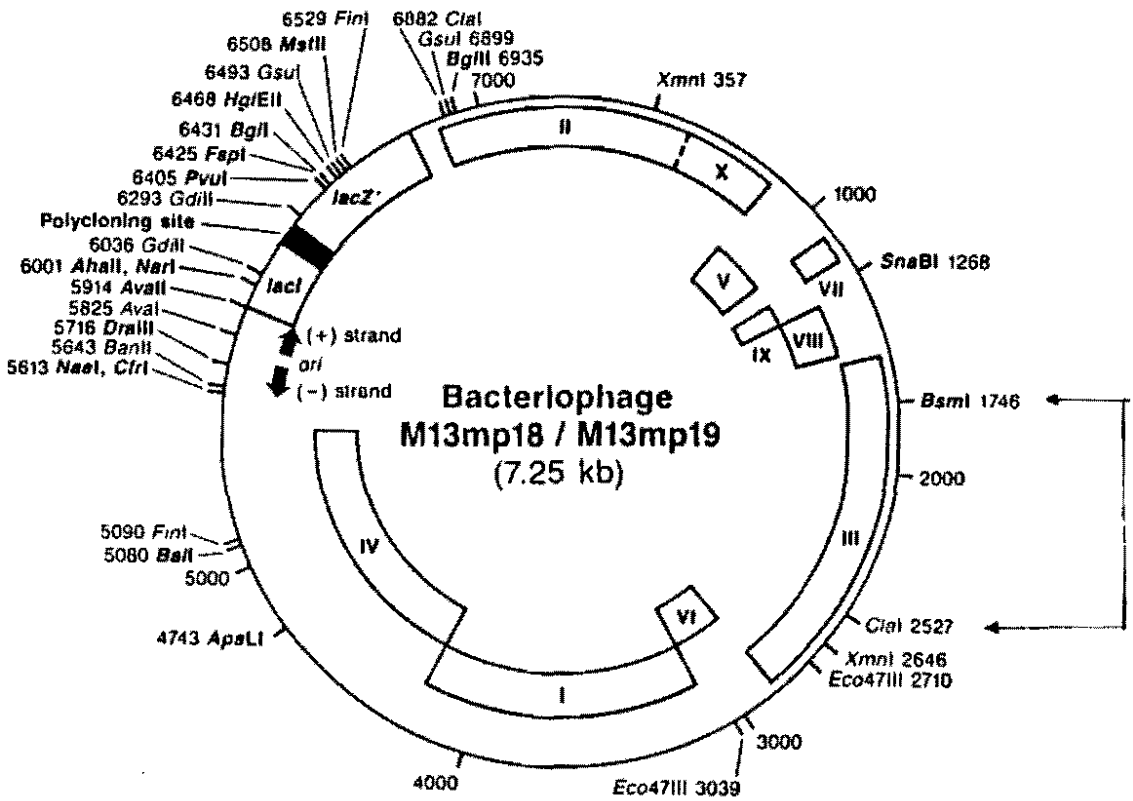

Figure 1. Restriction map of bacteriophage M13 vector. Smallest Cla I/Bsm I fragment from 1746 to 2527 was eluted from the gel and used as probe.

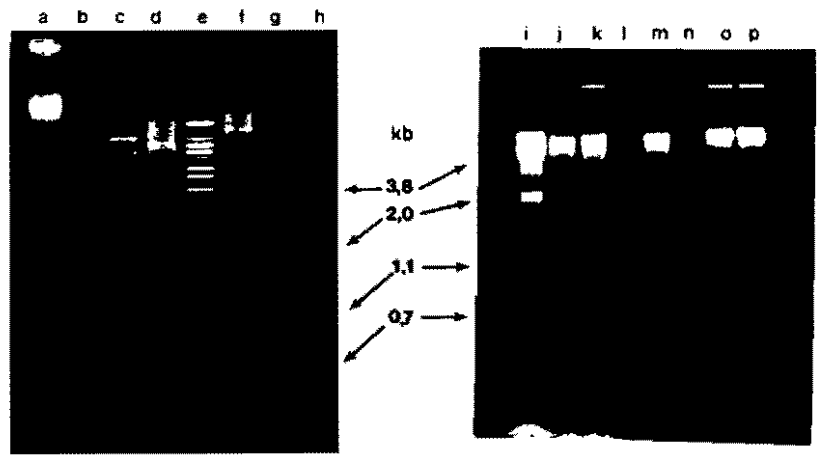

Figure 2. DNA from cassava leaves cleaved with different restriction enzymes. Molecular weights are in Kilobases.

a:	undigested	i:	Lambda - Pst I
b:	Hae III	j:	Bam HI
c:	Hind III	k:	Bam HI
d:	Eco RI	l:	Taq I
e:	Lambda-BstE II	m:	Msp I*
f:	Eco RV	n:	Hinf I
g:	Bgl II	o:	Apa I*
h:	Dra I	p:	Pst I*

* 5'-C-methylation sensitive restriction enzymes.

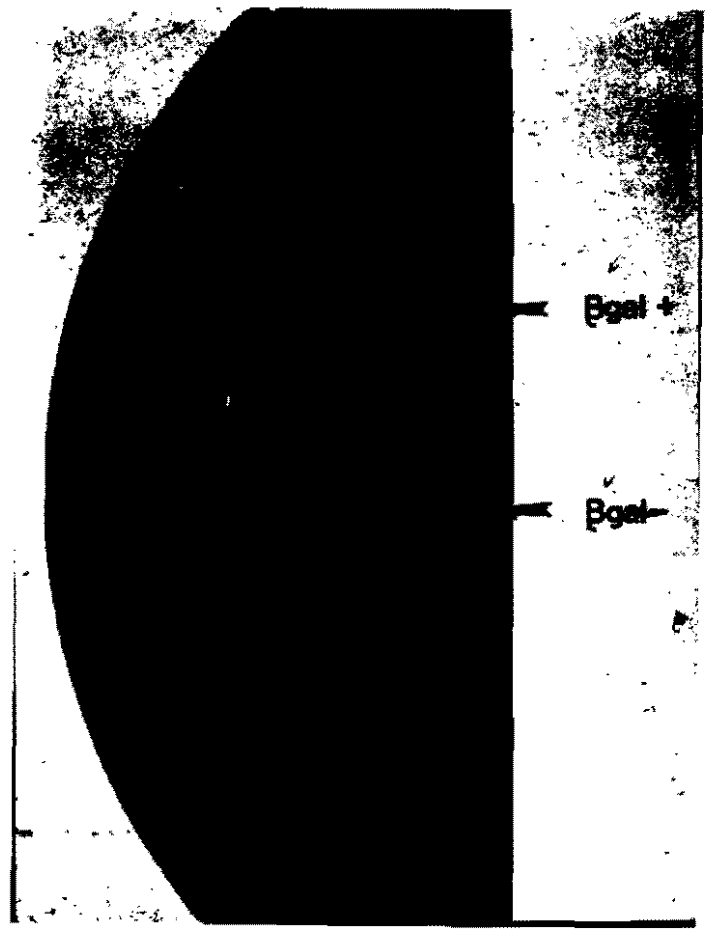

Figure 3. Selection of recombinant plasmids based on X-gal and IPTG screening procedures. White colonies (Bgal-) containing recombinant plasmids were isolated and its insert characterized.

Figure 4. Electrophoresis on 1% agarose gel of inserts after digestion. Size of inserts were between 0,2 Kb and 7 Kb. Molecular weights are expressed in Kilobases.

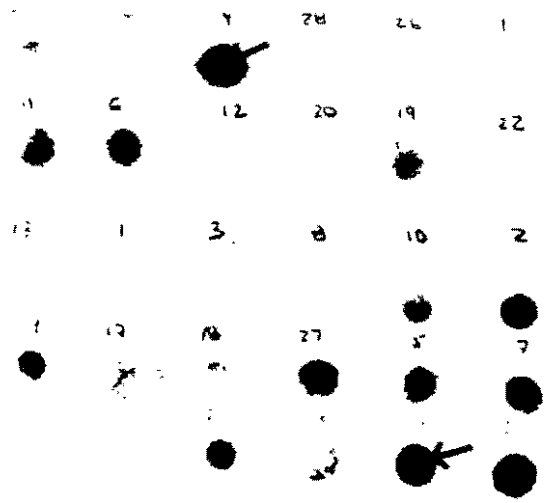

Figure 5. Dot blot hybridization of recombinant plasmids with ^{32}P-labelled genomic DNA. Ten nanograms of each plasmid were used in this experiment. Single and low copy sequences were selected according to hybridization intensity.

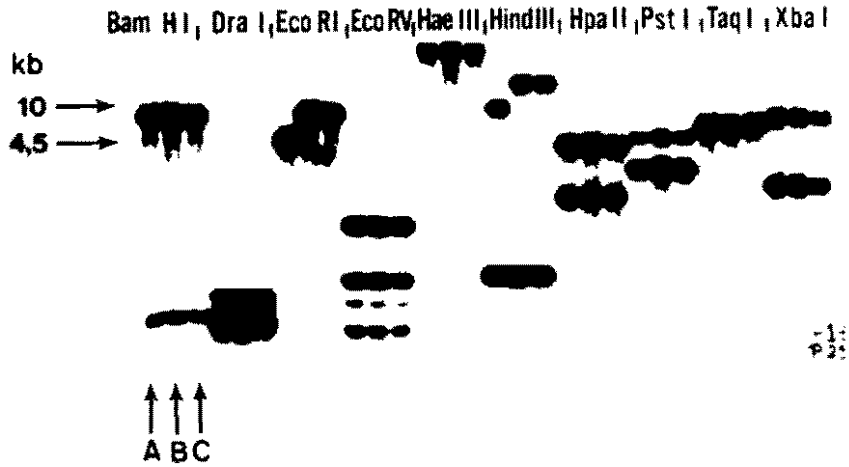

Figure 6. Variation at DNA level detected with probe P21. Southern blots were exposed by 2 days at -70°C with intensifier screens. A = M Col 22; B = M Col 1505; C = CM507-37. Restriction endonucleases Eco RI and Hind III detected DNA variation in M Col 22. Restriction endonucleases used to digest genomic DNAs are shown (listed above).

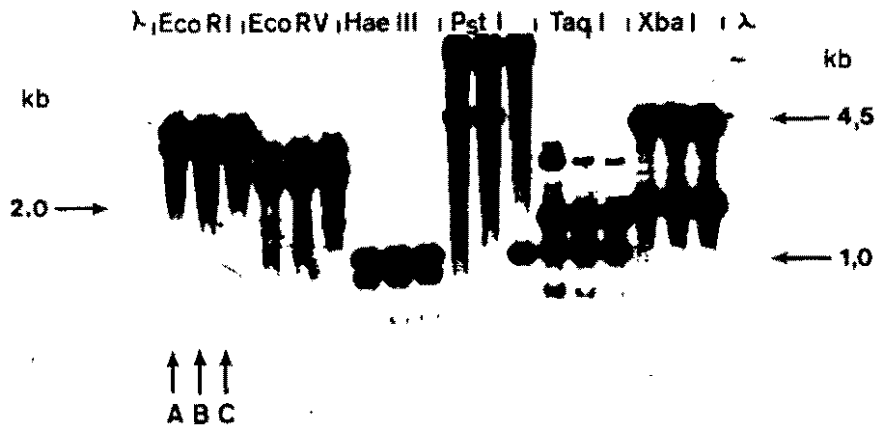

Figure 7. Variation at DNA level detected with probe X14. A = CM507-37; B = M Col 1505; C = M Col 22. Restriction endonuclease Pst I detected DNA variation in M Col 22 cultivar.

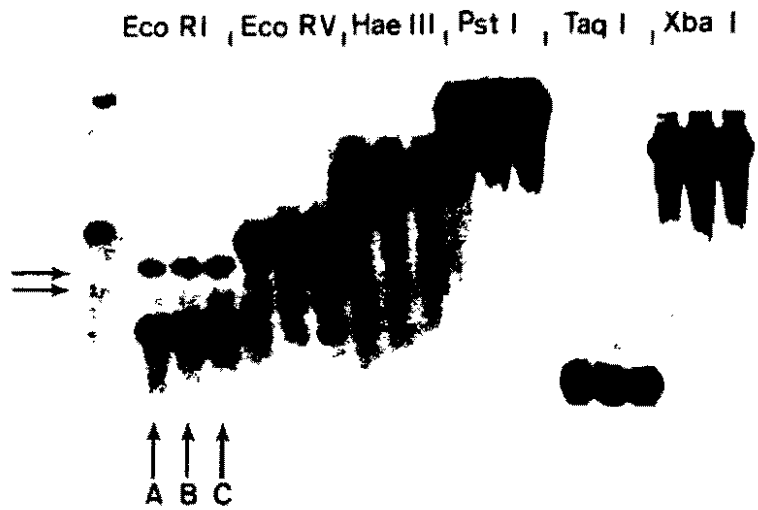

Figure 8. Identification of additional bands in M Col 22 (left arrows) with probe B5 when DNA was digested with Eco RV enzyme. A = CM507-37; B = M Col 1505; C = M Col 22.

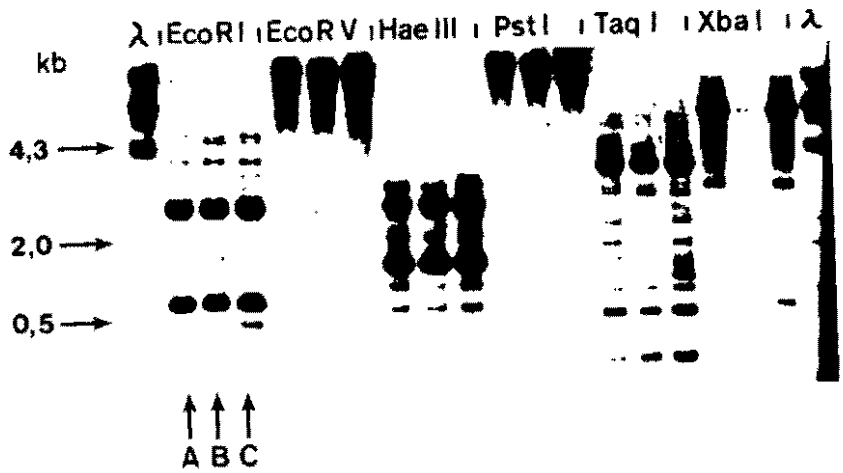

Figure 9. Identification of an additional band (0,5Kb) in M Col 22 with probe X6 when DNA was digested with Eco RI enzyme. A = CM507-37; B = M Col 1505; C = M Col 22.

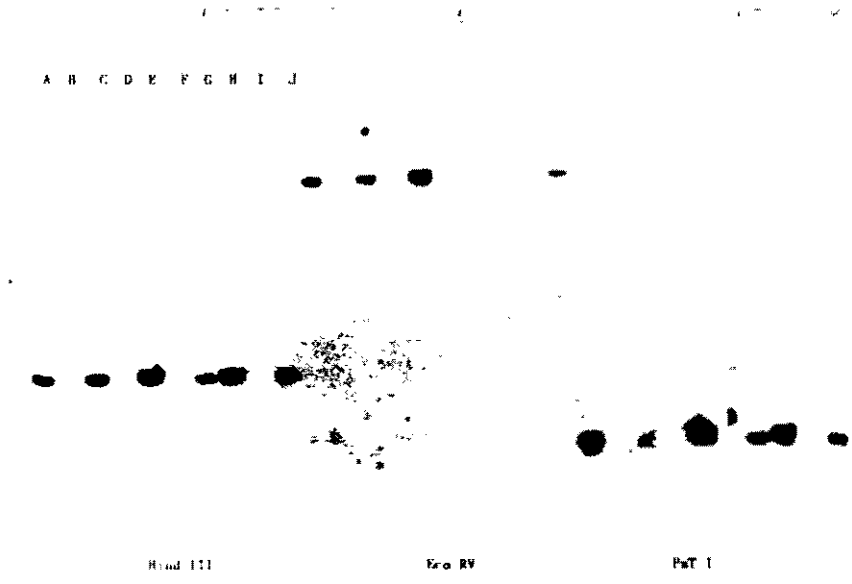

Figure 10. Identification of a specific sequence with probe P12. A = M Col 21; B = M Bras 191; C = M Bras 534; D = CM507-37; E = M Col 22; F = M Col 1505; G = M Grahami; H = M. Chartaginensis; I = M. Peru 328; J = M. Ecuador 117. Hybridization was detected in six of ten cultivars tested with any of the three restriction endonucleases. Restriction endonucleases used to digest genomic DNAs are shown (listed below).

Figure 11. Fingerprinting of different cassava cultivars digested with Hae III and hybridized with smallest Cla I/Bsm I fragment of the M13 bacteriophage.

a:	M Ven 331;	b:	M Ven 82;	c:	M Br 885;
d:	M Br 348;	e:	M Br 12;	f:	M Col 1505;
g:	CM91-3;	h:	CM507-37	i:	M Col 638;
j:	M Col 21;	k:	M Col 22;	l:	M Br 534;
m:	M Br 715;	n:	M Arg 13;	o:	M Thai 1

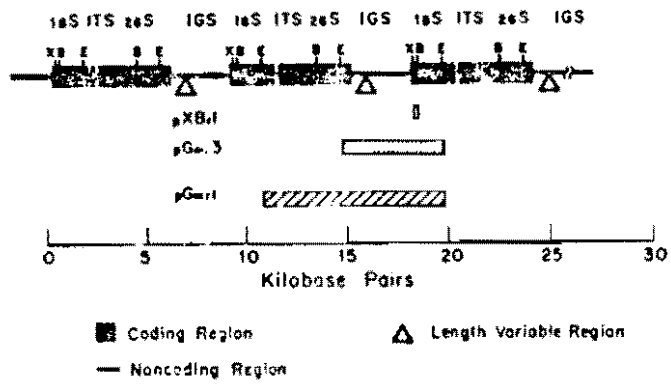

Figure 12. A typical array of plant ribosomal DNA. Taken from Zimmer et al. (27). This figure depicts the general features of the sequences that code for the large cytoplasmic ribosomal RNAs of higher plants. Ribosomal repeat units of 8-12 kbp exist in the genome in tandem arrays; each repeat consists of coding and noncoding regions (28). Indicated on the figure are diagnostic restriction endonuclease cleavage sites (X= Xba I; B= Bam HI; E= Eco RI). The Bam HI site in the 26S coding region in plant ribosomal gene arrays is not susceptible to cleavage in all arrays. Abbreviations on the figure denote the following: 18S = 18S rRNA coding region; ITS= internal transcribed spacer; 26S= 26S rRNA coding region; IGS= intergenic spacer. The position of the cloned probes (pxBrI, pGmr3, pGmr1) used for sequential probing is shown below the repeat unit.

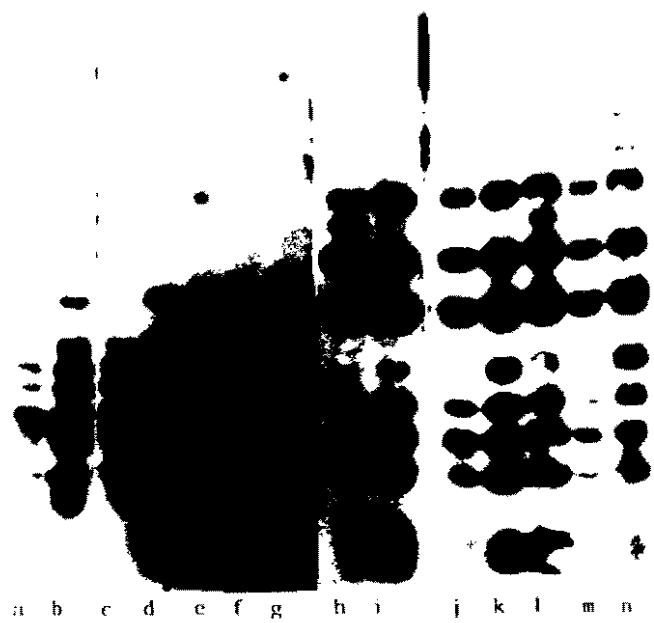

Figure 13. Hybridization with pGmr-1 probe of different cassava cultivars digested with either Hae III (lanes a-g) or Taq I (lanes h-n).

a,h	=	M Br 348
b,i	=	M Br 885
c,j	=	M Arg 13
d,k	=	M Ven 331
e,l	=	M Ven 82
f,m	=	M Ec 10
g,n	=	M Col 22

Figure 14. Hybridization with pGmr-3 probe of different cassava cultivars digested with either Taq I (lanes a-g) or Hae III (lanes h-n).

a,h	=	M Br 348
b,i	=	M Br 885
c,j	=	M Arg 13
d,k	=	M Ven 331
e,l	=	M Ven 82
f,m	=	M Ec 10
g,n	=	M Col 22

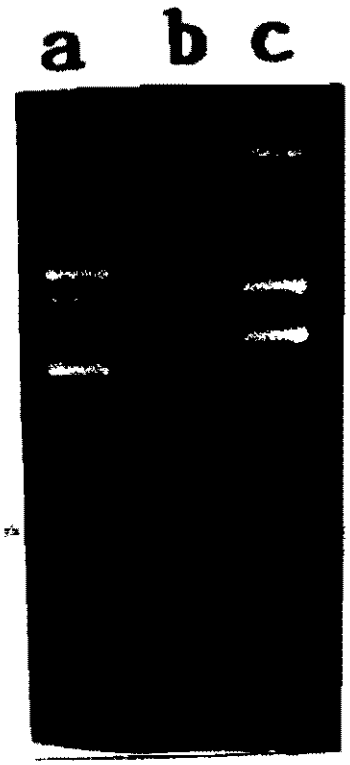

Figure 15. Amplification of cassava DNA. DNA was amplified from three different genotypes using a primer of arbitrary nucleotide sequence. Amplification products were resolved by electrophoresis in a 1,5% agarose gel which was stained with ethidium bromide and photographed.

a: M Col 1505;
b: M Col 22;
c: *M. aesculifolia*