

Final Project Report Submitted to
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"DNA Fingerprinting of Sweetpotato Genetic Resources"

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by

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**Final Report of USAID-HBCU Project
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"DNA Fingerprinting of Sweetpotato Genetic Resources"**

Summary

This research project had a goal to *develop methods for DNA fingerprinting of sweetpotato genotypes and employ this system to characterize the sweetpotato germplasm.* DNA fingerprinting technology can significantly enhance crop improvement efforts through unambiguous identification of germplasm accessions and identification of appropriate parents for breeding. Employing the polymerase chain reaction (PCR)-based "DNA Amplification Fingerprinting (DAF)" approach, we developed methods to produce informative and reliable fingerprint profiles of sweetpotato. Conditions for PCR were optimized, a superior system of gel electrophoresis that employs vinyl-polymer of polyacrylamide was identified and silver staining process of DNA detection was modified. We also developed a computer scanning system to digitize the DNA images facilitating data analysis and presentation. Many PCR primers were screened and those that detect high polymorphism among sweetpotato genotypes were identified. Using seven primers, DAF profiles were developed for seventy three accessions of sweetpotato collected from around the world, and thirty accessions representing US cultivars and their progenitors. Global sweetpotato collection exhibited a high genetic variability but accessions from certain geographic sources tended to cluster together. The US cultivars were relatively less diverse and formed a tight cluster in the principal coordinate analysis, indicating a narrow genetic base. Genetic relationships from phenograms based on Jaccard's coefficient showed that relationships among US cultivars and their progenitors to be consistent with the pedigree history. Those cultivars selected as somatic mutants showed close genetic similarity with the wild types and yet distinct in fingerprint profiles. The wild species *I. triloba* and tetraploid *I. batatas* appeared as outliers in the cladogram. DNA amplification fingerprinting technique can thus be gainfully employed to assess genetic variation in sweetpotato collections around the world. Improved germplasm collection activity is fostered by identifying those geographic areas with greatest genetic diversity. DNA fingerprints are valuable to breeders to identify divergent parental lines for hybridization and monitor somatic hybrids and somaclonal variation. Unambiguous identification of germplasm accessions is helpful in creating core subsets and to eliminate duplicates in the gene banks.

The primary goal of this research project is to *develop methods for DNA fingerprinting of sweetpotato genotypes and employ this system to characterize the sweetpotato germplasm*. DNA fingerprinting enables unambiguous identification of individual genotypes, and facilitates genetic characterization of the germplasm. In plants, DNA fingerprinting has relied largely on the identification of hypervariable DNA regions using minisatellite sequences, oligonucleotide repeats, or RFLP markers. When this research proposal was developed in 1991, it was proposed that human minisatellites will be employed to generate DNA fingerprints in sweetpotato. This approach employs Southern blotting, radioactive labeling and hybridization to detect DNA polymorphisms and is technically complex, time consuming, and expensive. DNA fingerprinting of sweetpotato using minisatellite probes often results in smears on Southern blots with very high backgrounds making it difficult for accurate interpretation. When this project was initiated in late 1992, a new and improved approach was available to identify individual-specific DNA profiles in plants based on the polymerase chain reaction (PCR). Termed random amplified polymorphic DNA (RAPD) or DNA amplification fingerprinting (DAF), the technique involves enzymatic amplification of random DNA using a single arbitrary primer, and is a simpler alternative to traditional molecular markers. The approach provides a powerful and rapid means to detect genetic polymorphisms. The technique is cost-effective, involves less labor, requires no radioactivity, and is well suited to analysis of large numbers of samples. The procedure requires small amounts of DNA, uses universal primers, and does not require cloning or prior knowledge of DNA sequences.

We decided thus to employ the DAF approach to realize the objectives outlined in our earlier proposal because of the obvious advantages of this technique. As the DAF relies on PCR, a stochastic process, it was essential to optimize the reaction conditions. To ensure the reproducibility and reliability of the fingerprint profiles, and to minimize the artifactual variations, a series of studies was conducted to optimize the DAF procedure in sweetpotato. We have also identified a superior system of gel electrophoresis that employs vinyl-polymer of polyacrylamide and silver

staining to visualize the DNA. We have developed a computer scanning system to digitize the DNA images and thus facilitate data analysis and presentation. Using the optimized system, we have investigated the genetic relationships among more than 100 sweetpotato accessions including several U. S. cultivars, identified patterns of genetic diversity and fingerprinted all the tested accessions.

Goal 1: Optimize Conditions For DNA Amplification Fingerprinting Of Sweetpotato

Sweetpotato accessions maintained at the USDA/ARS Plant Introduction Station (Griffin, GA) were used for all the studies. Entire *in vitro* plantlets (2- 4 mo) were used for the isolation of DNA. Total genomic DNA was isolated essentially as described by Wilson *et al.*, (1992). Briefly, tissue (1 g) was ground to a fine powder in liquid nitrogen and added to 5 ml of isolation buffer (50 mM Tris/HCl, pH 8.0; 25 mM EDTA, pH 8.0; 0.35M sorbitol, 50 g/liter polyvinylpyrrolidone-40, 10 g/liter sodium bisulfite, and 0.2% (v/v) 2-mercaptoethanol), centrifuged at 2,000 X g (10 min) at 4°C, and the pellet was resuspended in 5 ml of extraction buffer (100 mM Tris/HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% (w/v) mixed alkyltrimethylammonium bromide and 1% (v/v) 2-mercaptoethanol). The suspension was incubated at 60°C for 60 min, extracted with an equal volume of chloroform : isoamyl alcohol (24 : 1), centrifuged at 5,000 X g for 10 min at room temperature, the aqueous phase transferred to a new tube and the DNA precipitated by the addition of 2/3 volume of cold isopropanol. DNA was removed using a glass hook, washed with 70% ethanol, resuspended in TE, precipitated with ammonium acetate:ethanol, and purified by phenol : chloroform extraction. DNA was quantified, and its purity determined, using spectrophotometry. The 260/280 ratios of the DNA averaged 1.6 to 1.8.

DNA Amplification. A single 8-mer primer was used in each PCR reaction to amplify segments of genomic DNA from a diverse group of germplasm accessions. Primer DNA of arbitrary sequences was synthesized on a Millipore DNA synthesizer. The total reaction volume for amplification was 25 µl containing various concentrations of template DNA, primer and MgCl₂, 5 units of Stoffel fragment or AmpliTaq (Perkin Elmer Cetus) (unless otherwise noted), dNTPs (200 µM each) (Promega Co., Madison, WI) with Cetus-supplied buffer (10 mM KCl, and 10 mM Tris-HCl for Stoffel Fragment; 10 mM Tris-HCl, 50 mM KCl and 0.1% (w/v) gelatin for AmpliTaq). Reaction mixtures were overlaid with two drops of

mineral oil. Amplifications were performed in a thermal cycler (Hybaid; National Labnet Co, Woodbridge, NJ) for 35 cycles after an initial denaturation at 96°C for ten minutes. Each cycle consisted of 5 sec at 96°C, 20 sec at 45°C and 30 sec at 72°C [Yu, 1992 #1858], and a final extension step of 5 min at 72°C. The well temperatures were monitored using a thermal probe inserted into a reaction tube containing mineral oil.

DNA Electrophoresis. Amplified DNA fragments were separated on a polyacrylamide-based vinyl polymer (PCR Purity Plus, #550, AT Biochem, Malvern, PA). We have reported elsewhere that this gel matrix is superior to polyacrylamide or agarose gels due to the increased mobility of the DNA fragments and better resolution of the DNA banding patterns (He et al. 1994). Electrophoresis was conducted in a vertical gel apparatus (Mini-Protean; Bio Rad, Richmond, CA). Gels (0.7 mm) were prepared as recommended by the supplier, using 3 ml of concentrated gel solution. Gels and electrophoresis buffers were prepared in TBE (100 mM Tris. HCl, 83 mM boric acid, 1 mM Na₂ EDTA, pH 8.3). After amplification, 5 µl of the reaction volume was loaded into the wells of the gel and electrophoresis was conducted at 200 volts until the front of the dye in the loading buffer (#556, AT Biochem) had migrated to the bottom of the gel.

DNA visualization. Gels were silver stained procedure using a commercially prepared kit (Bio Rad #161-0443; Hercules, CA) and according to Bassam *et al.* (1991). These procedures were initially compared to detect DNA bands on vinyl-polymer of polyacrylamide gels. The procedure of Bassam *et al.* (1991) was subsequently modified to include an oxidization step using an oxidizer solution (Bio-Rad #161-0444) for 5 min at room temperature. The gels were photographed using the hand-held Polaroid camera with 667 film.

Amplification parameters were optimized by testing the following: (1) the template DNA concentration (0 to 34.6 ng/µl) using the primer concentration of 8.7 µM, (2) the concentration of primer (0 to 87 µM) with template DNA concentration of 6.9 ng/µl, and (3) Stoffel fragment v/s *AmpliTaq* DNA polymerase compared at increasing magnesium concentrations (2, 3, 4 and 5 mM). We also tested the non-recombinant *Taq* (Cetus) and other truncated *Taq* polymerases: *KlenTaq* 1 (AB Peptides, St. Louis, MO) and Δ *Taq* DNA polymerase version 2 (US Biochemicals, Cleveland, OH).

The reproducibility of the DAF was tested by comparing fingerprint profiles from amplifications of DNA samples isolated independently five times from the genotype, PI 531143. Variation in the amplification patterns due to differences in two thermal cyclers was also tested. The potential of DAF for identification of sweetpotato genotypes was tested by subjecting eight randomly chosen germplasm accessions to DAF analysis using select primers. Bands were scored on the enlarged pictures of gels obtained through an image analyzer.

Conditions for the efficient and reproducible detection of sweetpotato DNA amplification fingerprints were standardized. Results of studies testing many parameters of PCR reaction, DNA electrophoresis and detection in sweetpotato are summarized in Table 1.

Table 1: Optimum PCR reaction, gel electrophoresis and DNA detection conditions for obtaining informative and reproducible DNA fingerprint profiles in sweetpotato.

| Parameter | Variables Tested | Optimum Variable |
|-------------------|--|--|
| Template DNA | 0, 0.05, 0.1, ..., 34.6 $\mu\text{g}/\mu\text{L}$ | 0.2 to 13.8 $\text{ng}/\mu\text{L}$ |
| Primer | 0, 0.52, 1.09, ..., 87 μM | 2.18 to 29.98 μM |
| Taq DNA Polym. | Taq, AmpliTaq, Stoffel Frag. KlenTaq and ΔTaq | Stoffel Fragment |
| MgCl ₂ | 1 to 10 mM | 3, 4 or 5 mM |
| Silver staining | BioRad Kit and Bassam et al. (1991) | Bassam et al. (1991) with an oxidizing step |

Template DNA at concentrations of 0.2 $\text{ng}/\mu\text{L}$ to 13.8 $\text{ng}/\mu\text{L}$ resulted in the greatest number amplified bands; virtually no difference in the banding patterns was observed between lanes within this range which translates to 60 to 4200 haploid genome equivalents of template DNA per μL (data not shown). Template DNA at less than 0.1 $\text{ng}/\mu\text{L}$ (30 haploid genome equivalents) in the PCR reaction mix did not produce detectable DNA products. Concentrations greater than 13.8 $\text{ng}/\mu\text{L}$ (4200 haploid genome equivalents) resulted in the amplification of fewer products and selective loss of bands of higher molecular weight. The

DAF procedure generally requires a higher template DNA concentration than the RAPD protocol of Williams *et al.* (1990). However, the optimum concentration of sweetpotato DNA is higher than that for other species tested with the DAF approach (Caetano-Anollés *et al.* 1992). This may be because of either the large genome size (3.31 pg/haploid cell) or the genomic complexity of sweetpotato necessitating larger amounts of template DNA to ensure requisite matches between primer and annealing sites. DNA amplification was sensitive to changes in the primer concentration. At low primer concentrations (0.53 and 1.09 μM), there were fewer and fainter bands (data not shown). At primer concentrations from 2.18 to 29.98 μM the most informative and uniform DAF profiles were observed.

The Stoffel fragment was more effective than the untruncated *AmpliTaq* DNA polymerase in producing a large number of DNA fingerprints (Fig. 1). *AmpliTaq* did not produce any amplification product at 2 mM, and resulted in three to four amplification products when the MgCl_2 concentration was increased (Fig. 1). The Stoffel fragment also did not amplify the template DNA at 2 mM MgCl_2 , but produced many, well-resolved bands with 3, 4 and 5 mM MgCl_2 (Fig. 1). Similar results were obtained when the study was repeated with 0 to 10 mM MgCl_2 (Table 1). Native *Taq* polymerase from *Thermus aquaticus* and the recombinant *AmpliTaq* have a low tolerance to increasing concentrations of magnesium when compared to the Stoffel fragment (Cetus product literature). The Stoffel fragment is a modified form of *AmpliTaq* DNA polymerase and is composed of a truncated fragment lacking the N-terminal 289 amino acid portion. The Stoffel fragment has higher thermostability than the native *Taq* or *AmpliTaq* polymerase with no associated 3' to 5', or 5' to 3' nuclease activities.

The amplification efficiency and patterns produced by ΔTaq was similar to that of the Stoffel fragment, while *KlenTaq* 1 did not produce satisfactory results (data not shown). The reasons for the superior performance of truncated *Taq* polymerases in DAF compared to the untruncated *AmpliTaq* are not clearly understood. The high denaturation temperatures (96°C) employed in our study, and the G+C rich templates and secondary structures characteristic of plant DNA, may make untruncated *AmpliTaq*—with its lower thermostability—less suitable for amplification.

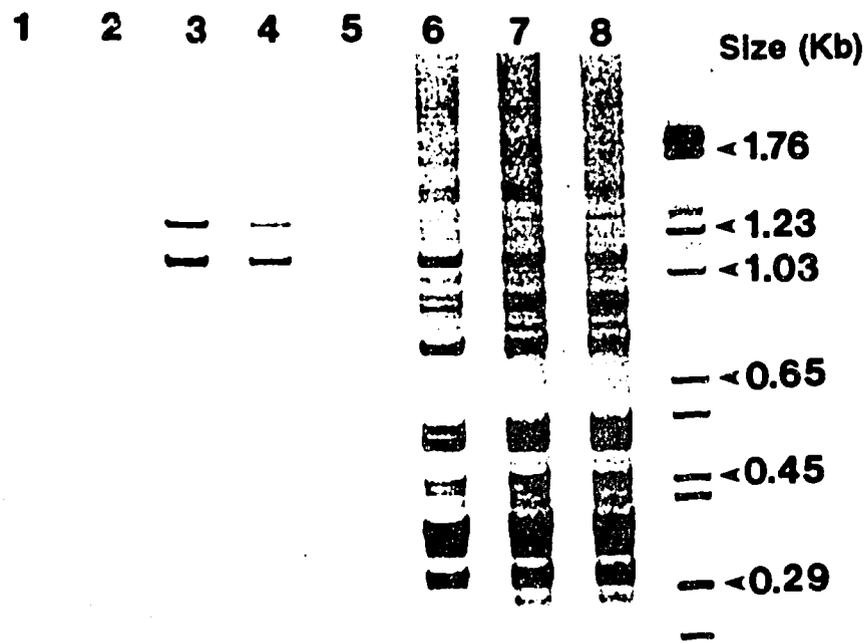


Figure 1: Comparison of *AmpliTaq* and Stoffel fragment for DNA amplification efficacy. Enzymes (0.2 units/ μ l) were tested at increasing concentrations of magnesium: lanes 1 to 4 represent *AmpliTaq* and 5 to 8 represent Stoffel fragment with Mg (2, 3, 4 and 5 mM). Reaction mixes contained template DNA (accession 27987; 3.84 ng/ μ l) and primer CGCACACC (7 μ M).

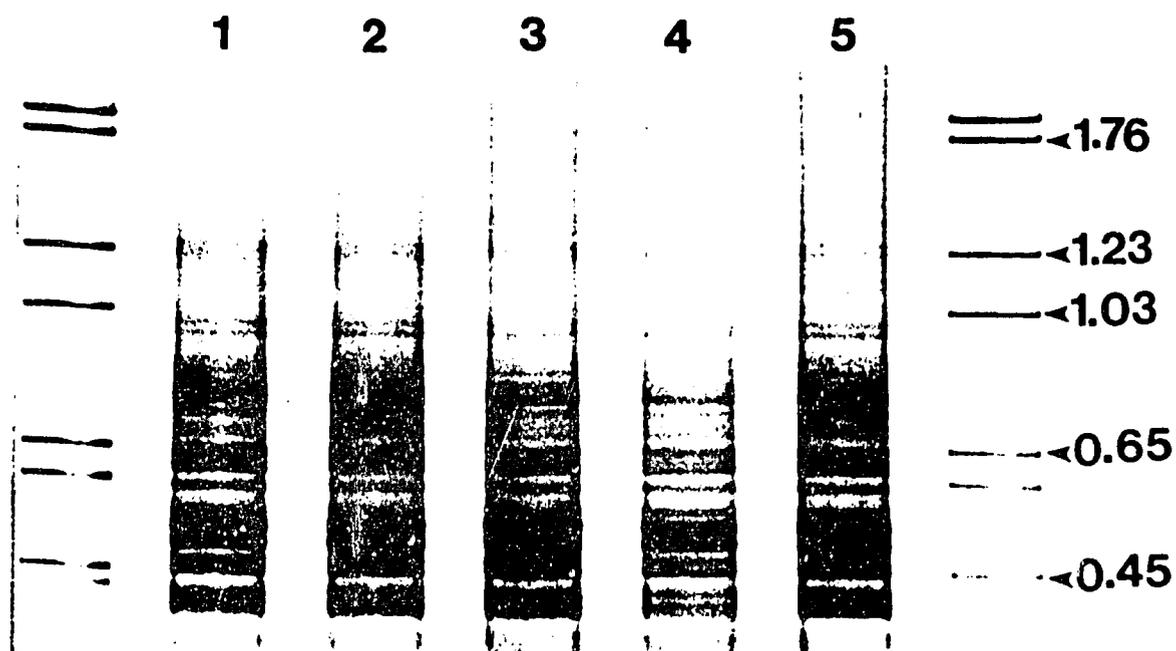


Figure 2: Reproducibility of the sweetpotato DAF profiles. Lanes 1 to 5 represent products from independent amplifications using the template DNA from sweetpotato accession 531143 (6.9 ng/ μ l), primer GGAGACCC (8.7 μ M), Stoffel fragment (0.2 units/ μ l) and 5 mM MgCl₂.

The reproducibility of the DAF profiles was tested by analyzing DNA banding patterns from five independent DNA samples isolated from a single sweetpotato genotype. Amplification was performed using the template DNA from the accession PI531143 and the primer GGAGACCC. The DNA fingerprints from all five amplifications were uniform and similar for all major bands and most of the minor bands although intensity of bands between lanes differed (Fig. 2). This experiment was repeated and no variation in bands were detected between DNA fingerprints from repeat runs. When variation in amplification patterns due to the use of two thermal cyclers was also tested (Hybald and FTC-100; MJ Research Inc., Watertown, MA), amplification profiles from both thermal cyclers were similar. Thus the thermal cyclers may not contribute to the variation in DNA banding patterns. The PCR cycling duration employed in our study is based on Yu and Pauls (1992) and is very rapid—especially the denaturation at 96° C for 5 sec. Because of the reproducible nature of the patterns observed in our study, we have not tested other incubation regimes. Throughout our study, we have consistently observed that minor or faint bands, primarily in the very high (>1500 bp) or very low (< 300 bp) molecular weight regions, are most vulnerable to artifactual variations. Thus, to ensure reproducibility, we suggest that only clear bands in the molecular weight range 300 bp to 1500 bp be scored.

DAF profiles of eight sweetpotato genotypes obtained using a single primer GTAACGCC are shown in Figure 3. Most of the amplified DNA fragments were between 300 bp and 1200 bp in size (Fig. 5). One band (marked with an arrow; Fig. 3) was monomorphic and may represent either species- or genus-specific sequence. Typically, about six to eight intense, well-resolved major bands and about ten to fifteen minor bands were observed (Figure 3). Scoring only the major bands permitted all eight accessions to be differentiated from one another. Thus, DAF conditions defined here result in the production of informative fingerprint profiles of sweetpotato genotypes. This approach may be useful in sweetpotato germplasm studies.

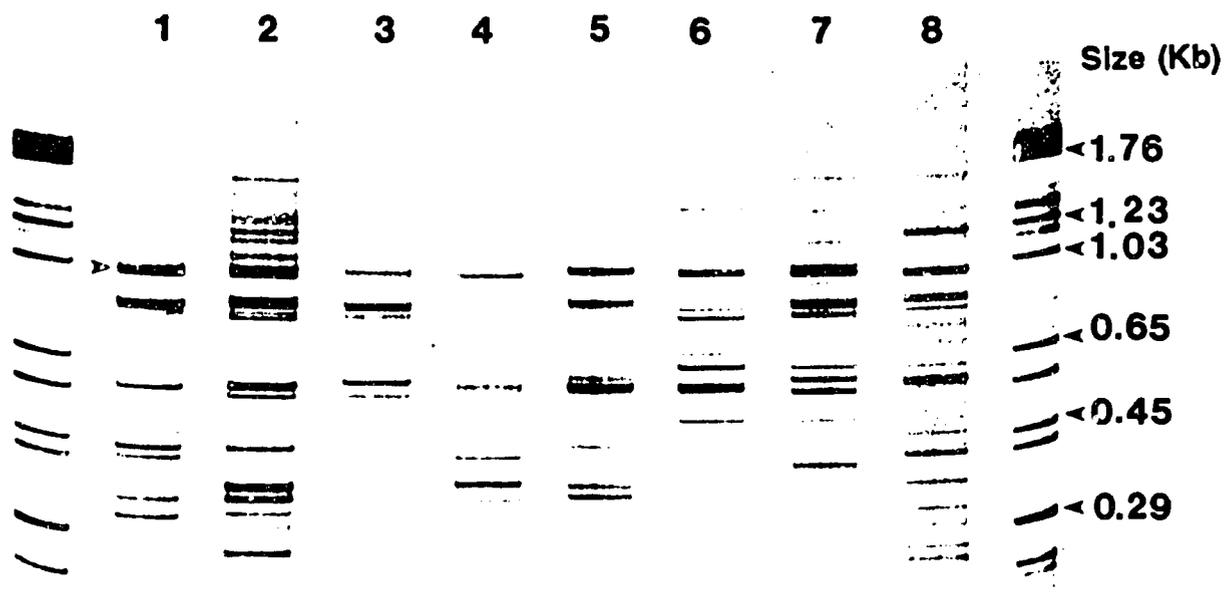


Figure 3: DAF profiles of eight sweetpotato genotypes. Lanes 1 to 8 show DNA amplification fingerprint profiles of genotypes 28001, 28002, 28006, 28008, 28012, 28017, 25718, and 25720 (11.2 ng/ μ l) respectively. Reaction mixes contained primer GTAACGCC (7.7 μ M), Stoffel fragment (0.2 units/ μ l) and MgCl₂ (5 mM). Arrow represents major bands that were monomorphic across genotypes.

Objective 2: Comparison Of Gel Matrices For Electrophoresis Of PCR Amplified Sweetpotato DNA Fingerprint Profiles

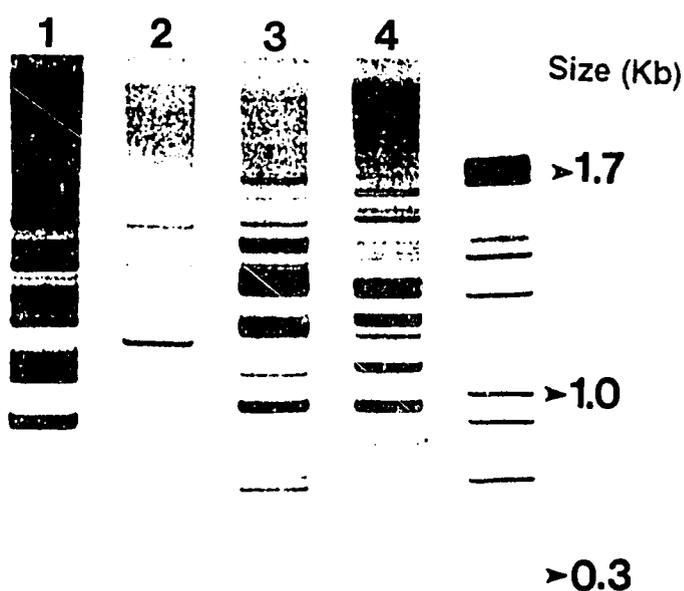
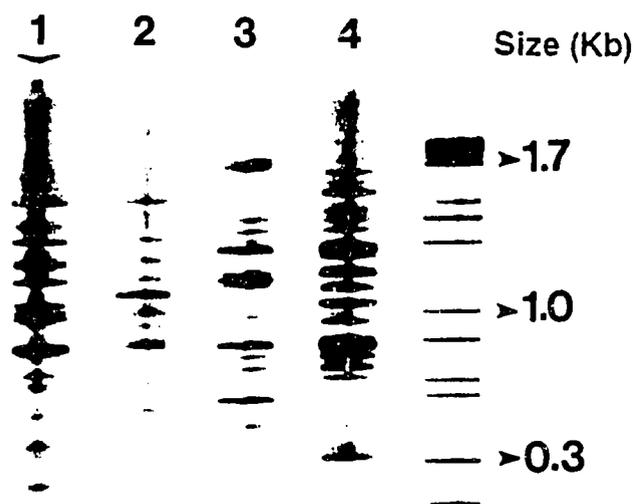
Agarose is the preferred matrix for resolving DNA fragments resulting from polymerase chain reaction (PCR) amplifications using arbitrary primers for the detection of Random Amplified Polymorphic DNA (RAPD) markers. However, when multiple DNA fragments of similar sizes are generated, as often is the case in DNA Amplification Fingerprinting (DAF), agarose may be inefficient in adequately resolving DNA fragments. DAF differs from traditional PCR in that it employs 8-mer primers, uses less stringent annealing conditions, and results in the amplification of multiple bands with varying intensity. Well-resolved DNA fragments facilitate accurate comparisons of banding profiles between lanes. The original protocol for DAF describes the use of polyacrylamide for separation of DNA fragments and employs silver staining procedure to visualize the DNA.⁽²⁾ We compared the gel matrices - agaroses, polyacrylamide and vinyl-polymer of polyacrylamide for their ability to efficiently resolve the products of DAF.

Amplifications were performed in 25 μ l reaction mixes containing template DNA (175 ng), single primer (5' TGTCCTCG or 5' ACGGGTGC; 6.0 μ M), MgCl₂ (5 mM), dNTPs (200 μ M each), five units of truncated *AmpliTaq* DNA polymerase (the Stoffel fragment; Perkin-Elmer Cetus) with 1x reaction buffer supplied by Perkin-Elmer Cetus.⁽²⁾ Reaction mixtures were overlaid with two drops of mineral oil, and heated to 96°C for 10 min, and then subjected to 35 cycles of 96°C (5 sec), 45°C (20 sec) and 72°C (30 sec), with a final extension step (72°C for 10 min) using a Hybaid thermal cycler as described earlier.

Reaction product aliquots from the same PCR reaction were analyzed by electrophoresis on polyacrylamide, vinyl-polymer of polyacrylamide (PCR Purity Plus[®], #550, AT Biochem, Malvern, PA) or agarose. A vertical electrophoresis system (Mini-Protean II cell; Bio Rad, Richmond, CA) was employed to run both regular and vinyl-polymer polyacrylamide gels. Polyacrylamide gels were prepared as described by Ceatano-Anollés et al (1991), and vinyl-polymer of polyacrylamide (0.7 mm) were prepared according to manufacturer's recommendations. Among many commercially available agarose types, Metaphor agarose (FMC Bio Products, Rockland, ME) provides a superior matrix for resolving PCR products and thus was chosen for this comparative study

Polyacrylamide

PCR Purity Plus

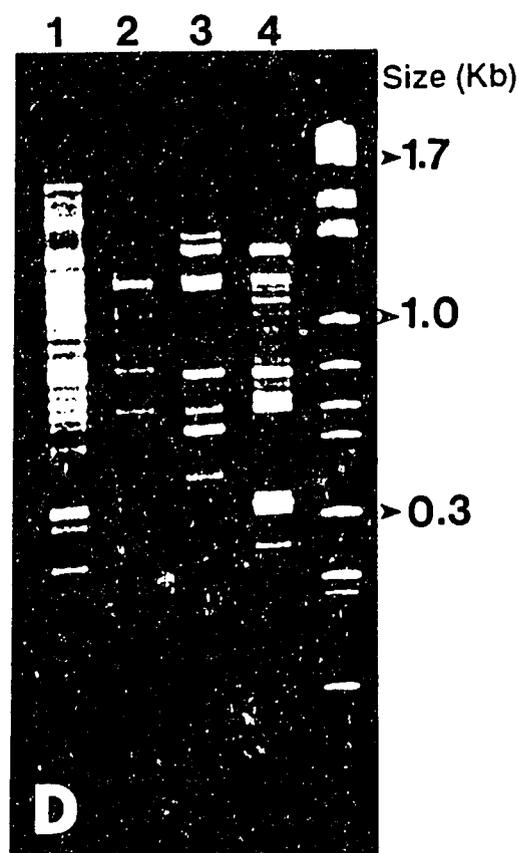
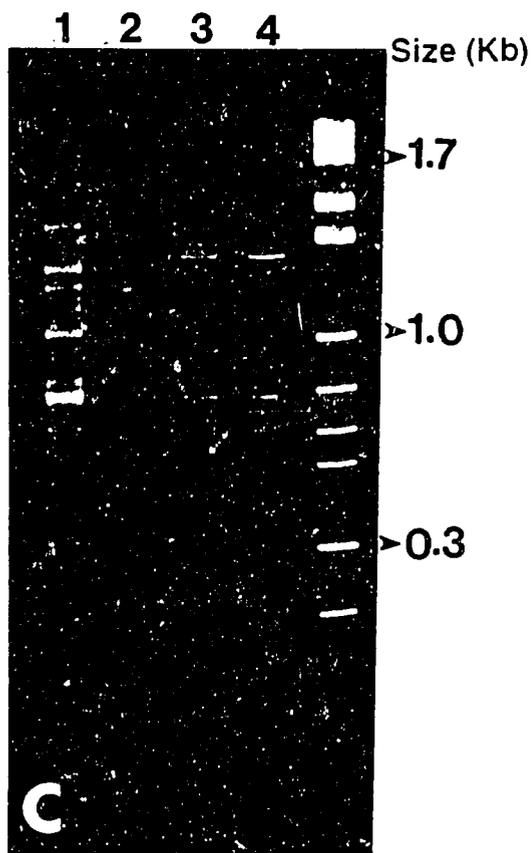


A

B

NuSeive 3:1

Metaphor



C

D

Figure 4. DNA amplification fingerprint products of plant DNA separated on three gel matrices: A = vinyl-polymer of polyacrylamide (PCR Purity Plus), B = polyacrylamide, C = agarose (Metaphor). Lanes 1 and 2 show PCR products using template DNA of *Ipomoea batatas* W221 and *Ipomoea trifida* (2X) using primer 5' TGTCTCG for amplification. Lanes 3 and 4 show PCR products obtained from the same template DNA with primer 5' ACGGGTGC. DNA was visualized in A and B by silver staining and in C with ethidium bromide fluorescence on UV light. Arrow shows the presence of a pair polymorphic bands that are distinct in A but not in B and C.

(*unpublished*). Agarose was tested at 3%, and electrophoresis was performed horizontally (11 X 14 cm) (Hoeffer; Horizon 11X14). Gel and electrophoresis buffers were TBE (100 mM Tris.HCl, 83 mM boric acid, 1 mM Na₂ EDTA, pH 8.3). Following amplification, 2 μ L of the PCR reaction mix was combined with 1 μ L of loading buffer (#556, AT Biochem), and loaded into the wells of the gel. Electrophoresis was conducted at 15 V/cm (polyacrylamide gel), 25 V/cm (vinyl-polymer of polyacrylamide; PCR Purity Plus) or 6.42 V/cm (agarose). DNA bands on polyacrylamide and vinyl-polymer of polyacrylamide gels were visualized using the silver-staining procedure described by Bassam *et al* (1991) with an addition of an oxidization step for five minutes using the Bio-Rad oxidizer (#161-0444), prior to silver staining. Agarose gel was stained with ethidium bromide (0.5 mg/L for 15 min), destained with distilled water (20 min) and bands visualized on a UV transilluminator. Gel images were photographed using a hand-held Polaroid camera with 667 film.

DNA products electrophoresed on vinyl-polymer of polyacrylamide were more clearly identifiable, well separated from each other and thus better resolved than those on either polyacrylamide or agarose gels (Fig. 4A-C). On the polyacrylamide gel, the primary or dark bands often coalesced with the secondary, fainter bands— especially those between 1.0 to 1.7 kb (Fig. 4B). In contrast, bands on the vinyl-polymer of polyacrylamide gels were much sharper, tighter and with uniform thickness; the primary and the secondary bands could be distinguished with relative ease (Fig. 4A). The polyacrylamide gel, however, does offer a good separation of bands of very low molecular weight (< 0.5 kb). DNA fingerprints from primary (or dark bands) on the agarose gel were well resolved but secondary bands do not appear very clear on the agarose gel when compared to the regular or vinyl-polymer polyacrylamide gels (Fig. 4). The background fluorescence of the ethidium bromide-stained agarose gel markedly reduced the clarity of the DNA fragments especially those of faint or secondary bands (Fig. 4C).

The improved resolution of bands run on the vinyl-polymer of polyacrylamide was especially apparent with PCR fragments of near-equal molecular weights. For example, two DNA bands of approx. 1.2 kb appear distinctly separate (lane 4; marked with an arrow) on the vinyl-polymer of polyacrylamide (Fig. 4A) while they appear as one band in polyacrylamide gel

(Fig. 4B); both of these bands were hardly visible in the agarose gel (Fig. 4C). Thus, the use of vinyl-polymer of polyacrylamide enables better identification of polymorphic bands than either polyacrylamide or agarose gels and thus may be especially useful in DNA fingerprint studies.

The electrophoresis on the vinyl-polymer of polyacrylamide gel was more rapid as it was more tolerant of higher running temperatures and this permitted the use of higher voltages. This gel is less brittle and thus easier to handle when compared to the polyacrylamide gel. The vinyl-polymer of polyacrylamide gel swells during staining and this makes it easier to discern bands and to score polymorphisms. Gel backing films such as Gelbond (FMC Bio Products) can be used to support the gel after staining, and backed gels can be stored for long periods without any distortion of lanes.

Our studies showed that vinyl-polymer of polyacrylamide provides an improved resolution of PCR amplification products for DNA fingerprinting. This matrix permits rapid electrophoresis of DNA fragments and facilitates better detection of polymorphic DNA bands. The paper describing this result has been published in *PCR Methods and Applications*.

Objective 3: Develop a Computer Scanning Process to Digitize Gel Images of DNA Profiles:

Gel and blot images are the primary means of documenting and presenting research results of most molecular biology studies including DNA fingerprinting. Traditionally, gel pictures are documented using an instant camera such as a Polaroid which produces either 7.3 x 9.5 cm or 8.9 X 11.4 cm prints. To add text such as lane numbers and molecular weight data to the photo, it is often necessary to photograph this picture with a regular 35 mm SLR camera to obtain an enlarged image. Text is then added by either dry letter transfer or with the help of a graphic specialist. The labeled picture is again photographed to get multiple prints for publication. The procedure is inherently cumbersome, expensive and time-consuming. The electronic photo-documentation systems such as the video-digitizing equipment are now commercially available that enable documentation of DNA bands from the ethidium-bromide stained gels with UV fluorescence. However, these devices are very expensive and thus are not cost-effective for use with silver-stained gels.

We showed that direct scanning of the silver-stained gel in a standard office scanner offers an improved, inexpensive and convenient approach for the documentation, analysis, sharing and archival of DNA fingerprint images.

Standard methods were employed for PCR amplification of sweetpotato DNA using a single octamer primer. As described earlier, amplified products were electrophoresed on PCR Purity Plus, silver-stained, backed with Gelbond film, and air dried. Gels were scanned directly using a HP ScanJet IIc flat bed scanner (300 dpi) (Hewlett Packard, Palo Alto, CA) into a Macintosh IIx computer (Apple Co, Cupertino, CA), and image acquired using DeskScan software (Hewlett-Packard) with the 'black and white photo' option. Digitized gel images were imported as TIFF files into Aldus Freehand software (Aldus Inc., Seattle, WA), labeled (when necessary), and saved as EPS files. Final images were forwarded through Professional Output Manager software (Visual Business Systems, Atlanta, GA) to a Montage photorecording device (Presentation Technologies, Sunnyvale, CA) and captured on Kodak TMax-100 print film (35 mm). We have also used a Howtek scanner (300 dpi) (Howtek Inc., Hudson, NH), a Macintosh IIfx computer, Adobe Photoshop software (Adobe Systems, Mountain View, CA) and Matrix PCR camera (Matrix Instruments, Orangeburg, NH) with similar results. A 35 mm SLR with TMax-100 film was used to photograph the gels (Fig. 1).

DNA fingerprint profiles as observed on the photographic reproduction of the gel is shown Figure 5. Figures 6 and 7 show the digitized image of the same gel obtained after computer scanning. The gel shown in Figure 6 was scanned using the default settings of the ScanJet scanner. The gel in Figure 7 was scanned using a brightness setting of 153 and a contrast setting of 167. Scanning generally resulted in excellent reproduction of the banding patterns, and images were comparable to those obtained by conventional photography (Figs. 5, 6 and 7). Scanning also provides greater opportunity for the investigator to control brightness and contrast factors. For instance, the brightness and contrast settings employed to generate Figure 6 results in the selective enhancement of major bands and the elimination of minor or faint bands. However, the same gel scanned with increased brightness and contrast, results in greater detail and resolution of all detectable bands (Figure 7).

Computer digitizing of gel images offers several advantages when compared to instant or contact X-ray photography. Digitized images of the gel are easily enlarged and printed using a laser printer. It is easier to score polymorphic bands on large scanned images than on the smaller Polaroid, contact X-ray or thermal prints. Use of high-resolution (> 600 dpi) scanners results in the production of superior grade 'publication-quality' pictures, and half-tone images can be produced by exporting the image to a photographic recording systems such as Montage. Alternatively, darkroom work can be totally eliminated by using a dye-sublimation-printer to directly produce gray-tone glossy prints with high resolution, although such devices are expensive. Archival or exchange of data is facilitated since gel images can be stored as electronic files on floppy or hard disks or in optical cartridges. Digitized gel images imported into the paint-draw programs such as Freehand readily lend themselves as templates for text addition, identification of molecular weight markers and placement of arrows to show polymorphic or monomorphic bands. Improvement in DNA fingerprint image resolution, brightness and contrast can also be easily achieved during scanning by using software and filters. The Adobe Photoshop and other software have interpolation and tonal correction features to improve image resolution and image quality. Gel images can be readily imported into word processing files for the preparation of reports and publications. Digitized gel images from computer screens can also be projected directly during presentations using an LCD projection device. Gel pictures can be exchanged between collaborating labs as compressed files through modem and phone lines or through Internet. As all the operations are conducted in-house, the approach provides considerable flexibility and expediency to the investigator.

In addition to the above mentioned logistical merits, the major utility of computerized scanning is in data analysis. The computer scanned image provides a digital output which is directly amenable for further analysis such as densitometry, computation of band-sharing and other analyses relevant to genetic polymorphism studies. There are many computer software programs available which read digitized image files and calibrate them to molecular weight standards (e.g. NIH Image; NCSA GelReader2), locate fingerprint matches (e.g. Pro-RFLP; DNA ProScan, Nashville, TN) or compute the degree of similarity.

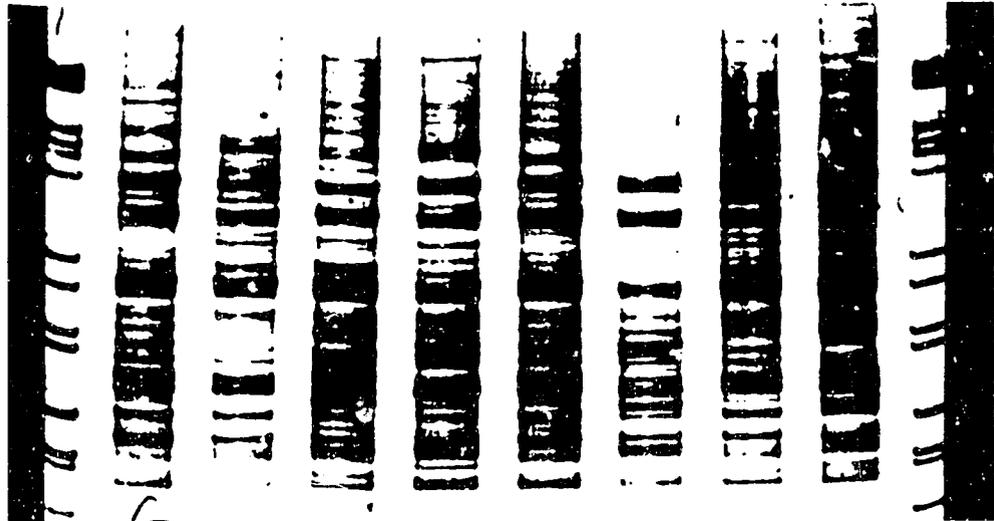


FIGURE 6

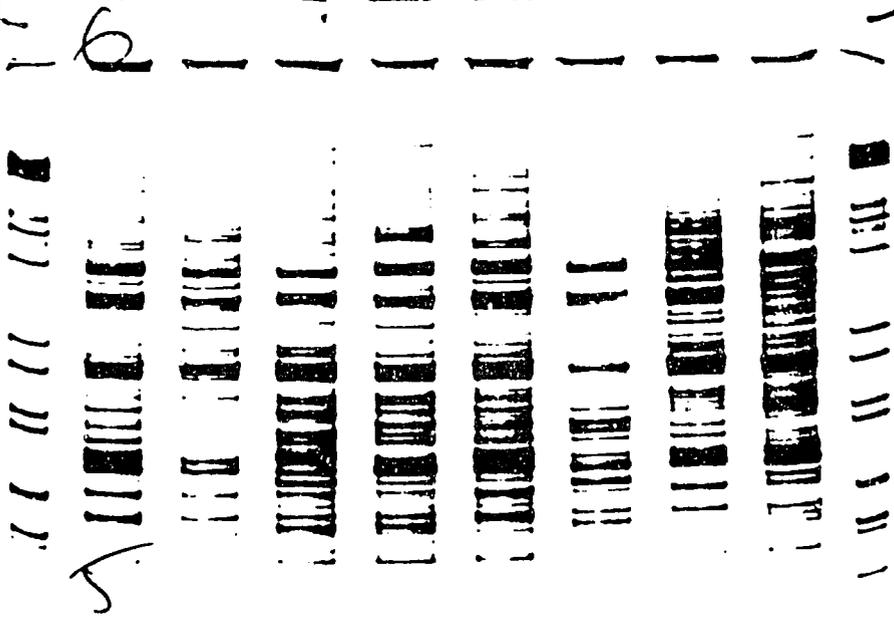


FIGURE 5

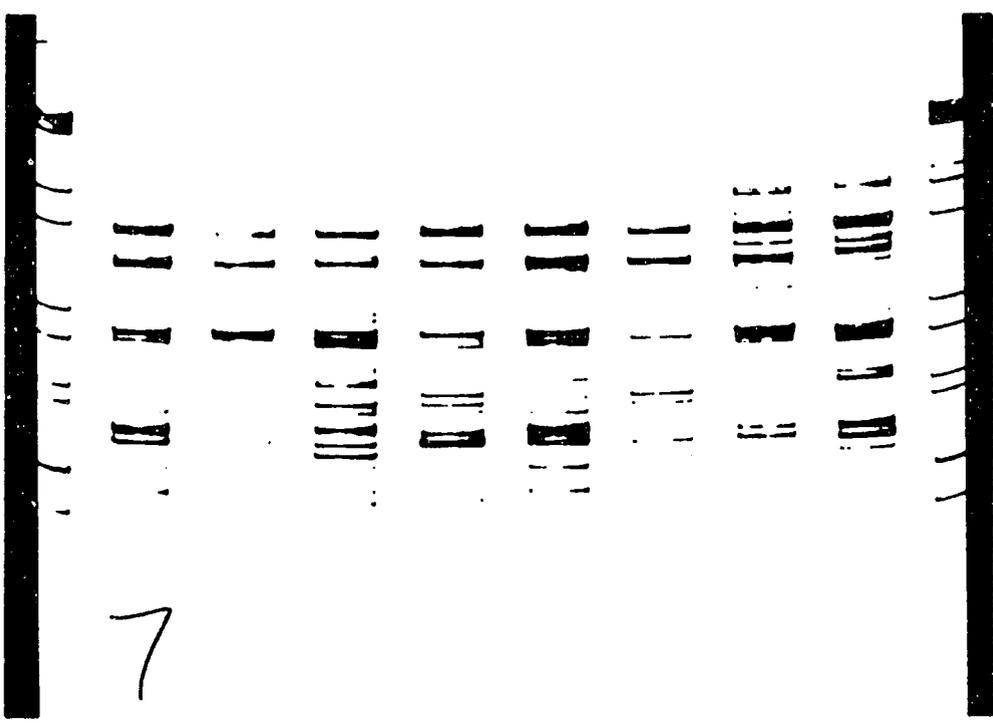


FIGURE 7

Figure 5: Photographic reproduction of the 35 mm picture of the DNA amplification fingerprint (DAF) profiles of sweetpotato genotypes resolved on PCR Purity Plus and visualized by silver staining. Gels were subsequently backed with Gelbond film, and air dried prior to scanning, or photography.

Figure 6: Photograph of a computer scanned image of the same gel shown in Fig. 1 using the default settings of the ScanJet scanner.

Figure 7: Computer scan Photograph of the same gel shown in Figs. 6 and 7 scanned with an increase in contrast and brightness settings to provide a greater detail of DNA bands.

Our results and those obtained by others on scanning DNA fingerprints cumulatively illustrate the power and versatility of computer processing of images as a useful tool for the documentation and presentation of molecular biological research results. As a cautionary note, it should be emphasized that digitized gel images, because they are easy to alter, are also vulnerable to data manipulation. The ethical issues related to potential data tampering with digital gel images have been raised recently.

NIH Image is a free software available on-line from America Online, CompuServe or on Internet through anonymous FTP at [zipper.nimh.nih.gov](ftp://zipper.nimh.nih.gov) [128.231.98.32] in the `/pub/Image` directory. The NCSA GelReader2 is also available on Internet through anonymous FTP at [fly.bio.indiana.edu](ftp://fly.bio.indiana.edu) [129.79.224.25] in the `/molbio/mac/gelreader` directory.

Objective 4: Analyze Genetic Diversity In A Sweetpotato Germplasm Collection Using DNA Amplification Fingerprinting; Estimate Genetic Relationships

There are thousands of sweetpotato genotypes cultivated around the world. In Papua New Guinea alone it is estimated that there are about 5000 cultivars (Takagi 1988). Sweetpotato exhibits great diversity for morphological and phenotypic traits such as growth habit, leaf shape, and storage root flesh and skin color (Woolfe 1992). Importance of sweetpotato and the high level of genetic diversity in this crop is reflected in the fact that nearly 8,000 accessions of sweetpotato are maintained at various gene banks around the world (Kuo 1991). Although this may represent only a fraction of the existing diversity, the germplasm collections form a valuable resource on which sweetpotato improvement programs are firmly based (Huaman *et al.* 1988; Jarret 1989). There are as yet undetermined number of duplications within and between the germplasm collections. Thus, the actual number of unique clones may be much smaller than the number of accessions currently held in gene banks (Kuo 1991). Much of the cultivated sweetpotato germplasm is maintained *in vitro* at the International Potato Center (CIP, Lima, Peru) and the USDA/ARS collection in Griffin, GA, USA (Huaman *et al.* 1991; Jarret 1989).

To facilitate efficient germplasm collection and management practices, there is a continual need for a greater understanding of the extent of genetic diversity within the germplasm collections and the nature of genetic relationships among the accessions. Information on genetic identity and relationships of genotypes is crucial to the development of 'core collections' and to tailor germplasm exploration to focus on those areas with maximal genetic diversity (Wilde *et al.* 1992).

DNA markers provide powerful and reliable tools to discern variation within crop germplasm and to study evolutionary relationships (Gepts 1993). Polymerase chain reaction-(PCR) based approaches using arbitrary primers are being increasingly employed in genetic research due to their speed and simplicity (Williams *et al.* 1990; Welsh *et al.* 1990; Caetano-Anollés *et al.* 1991). The DNA amplification fingerprinting (DAF) is especially useful in germplasm studies as it provides more informative DNA profiles than the random amplified polymorphic DNA (RAPD) protocol (Caetano-Anollés *et al.* 1991; Williams *et al.* 1990). We employed DAF to assess the genetic relationships among a random selection of sweetpotato germplasm accessions from around the world including a few select U.S. cultivars, and also made a critical examination of 30 U. S. cultivars and their progenitors.

The conditions for DNA isolation, PCR, gel electrophoresis, and silver staining were as described earlier. Informative primers were identified by screening 28 octamer oligonucleotides of arbitrary sequence individually on eight sweetpotato accessions. Seven of the 28 primers were ultimately selected for further use based on their ability to detect high levels of polymorphism and the production of clear bands: CGCACACC, GTAACGCC, GAACGGGT, GCGGACAG, GGAGACCC, GTGGAGCT and CCTGAGT (He *et al.*, unpublished). These seven primers were used to examine the genetic diversity in the sweetpotato germplasm .

Data analysis: Only intensely staining, clear bands within the size range of 300 - 1500 bp were scored. Bands in this size range were selected because they were reproducible and without artifactual variations based on our earlier studies. Gels were scored for the presence (1) or absence (0) of major bands using enlarged pictures of gels obtained through an image analyzer. NTSYS-pc software (version

1.7) was utilized to compute Jaccard's coefficients of similarity, to construct phenograms using the unweighted pair group method with arithmetic averages (UPGMA) and to develop a triangular matrix of percent similarity. A pairwise similarity matrix was developed for all 73 accessions (data not presented). Principal coordinate analysis (PCA) was conducted using the SAHN CLUSTERING, DOUBLE CENTER and EIGENVECTOR options of NTSYS-pc.

Using the seven primers listed in materials and methods, fingerprint profiles were developed for all 73 sweetpotato PIs and 30 U. S. cultivars. Typically, with most primers, there were 15-25 bands in each lane across genotypes. An average of 16.7 such bands were polymorphic. Thus, sweetpotato genotypes in the collection exhibited a high level of genetic variation. Genotype-specific profiles were obtained for all 73 sweetpotato PIs tested. A phenogram derived from Jaccard's similarity coefficients is presented in Fig. 8. In general, considerable genetic dissimilarities were observed among the PIs, and at least ten clusters were identified (Fig. 8). Within each cluster, genotypes tended to group based according to their geographic origin. For example, two clusters had a high representation of PIs from the South Pacific region including Papua New Guinea, Western Samoa and the Solomon Islands, while one cluster contained accessions from Taiwan, Korea and China. Sweetpotato accessions from South and Central America generally clustered together. Thirteen of the fifteen U.S. cultivars were represented in a single cluster; cultivars Tinian and HiDry being exceptions. Accessions from Papua New Guinea were exceptionally diverse and were dispersed across many clusters (Fig. 8). Accessions 508531 and 508530 had a similarity coefficient of 90% and thus appear to be closely related. Cultivars Jewel and Carver showed the closest similarity among all the accessions tested (92%).

The cultivar HiDry (U.S.), and accessions 518472 (Guatemala) and Q 28012 (PNG) clustered apart from all other cultivated sweetpotato accessions. The two wild species included for comparative purposes, *4X I. batatas* and *I. triloba* were clearly distinct from all cultivated hexaploid sweetpotato accessions, although the *4X I. batatas* was closer to the cultivated accessions than *I. triloba*.

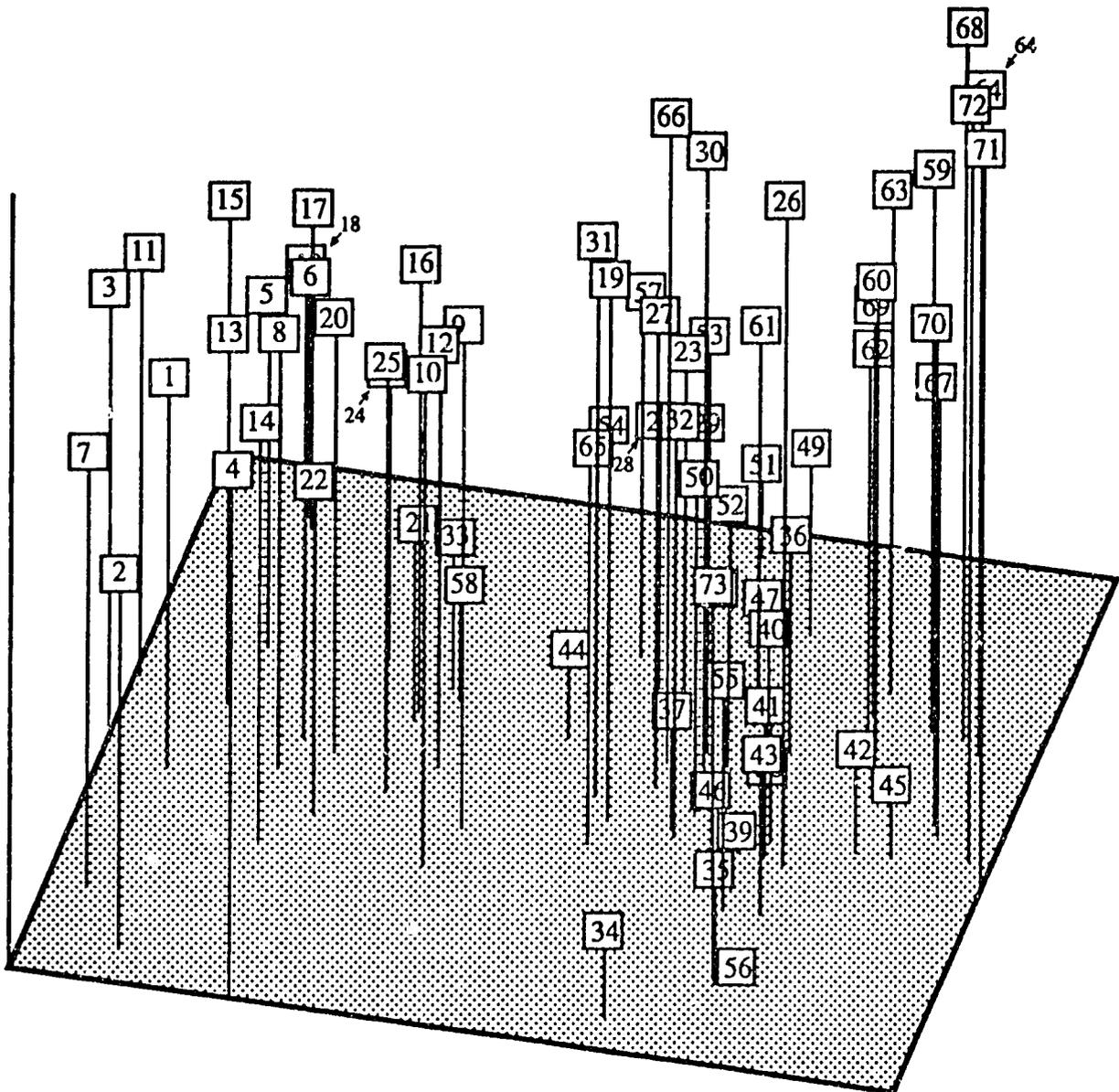


Figure 9: Principal coordinate analysis of 73 sweetpotato accessions based on Jacquard's coefficient derived from the DNA fingerprinting analysis data. Numbers refer to those in Figure 8.

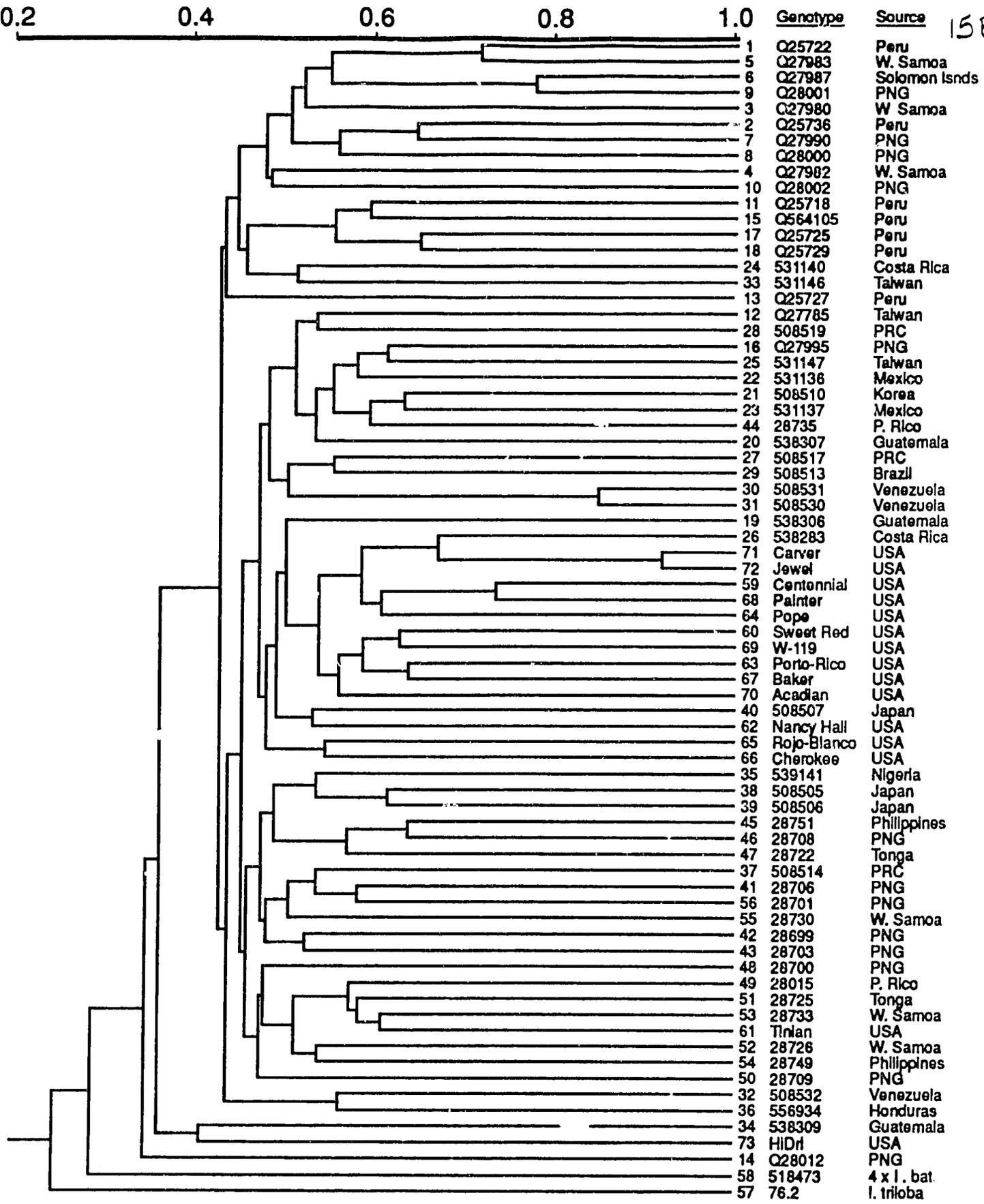


Figure 8: Phenogram of 73 sweetpotato accessions based on Jaccard's coefficient derived from the DNA fingerprinting analysis data. Right column refers to the country from which accessions were received. PNG= Papua New Guinea, PRC = People's Republic of China, and P. Rico = Puerto Rico.

The principal coordinate analysis (PCA) results illustrates the three-dimensional relationships among the 73 sweetpotato accessions examined (Fig. 9). The PCA generally support the clustering patterns of the phenogram (Fig 8). Three principal groups were observed (Fig. 9). One group (I) was dominated by accessions from Western Samoa, the Solomon Islands, Papua New Guinea while group II contained predominantly accessions from Central and South America. The third group (III) consisted exclusively of U.S. cultivars (Fig. 9). A few accessions (nos. 42 and 56 from Papua New Guinea, 45 from Philippines, 44 from Puerto Rico and 34 from Guatemala) were removed from the others on the third axis.

In a separate study, 30 cultivars of sweetpotato from United States were examined for their genetic relationships with each other. Most of the US cultivars tested in the DAF study using seven primers appear closely related to each other in the cladogram based on the Jaccard's coefficient (Fig. 10). The pairwise coefficient of similarity matrix between each of the 30 cultivars provides quantitative information on differences and similarities (Table 2). Cherokee and Porto Rico formed a cluster; the former is an offspring of the latter. The cultivar Centennial was related at 60% similarity to major US cultivars such as Jewel and Rojo Blanco. Excel and its open pollinated off-spring of Regal showed 71% similarity with each other. Copper Resisto, a mutant of Resisto, clustered together at 84% similarity. Similarly, Goldmar a mutant of Redmar clustered very close at 85% similarity. Jewel, the most leading sweetpotato cultivar in US, is an offspring of Nugget and Centennial, clustered very closely to Nugget (83%). Cultivar Carver, a selection from a cross Centennial X Jewel, showed 75% similarity with Jewel and 63% similarity to Centennial. Rojo Blanco which is a selection from Rose Centennial X White Triumph, fell in the same cluster as its parents with high similarities. Cultivar Scarlet which is a mutant of Jewel, appeared in the same cluster as Jewel but only with 68% similarity. Creole, a very old cultivar, was the most distinct with relatively little relationship to other more modern cultivars.

All the U.S. cultivars tested could be fingerprinted using the DAF approach as individual specific profiles could be identified. To facilitate such profiling, we followed the 'Bandmap' approach of Wilde et al. (1992) where the presence or absence of DNA fragments are presented in graphical fashion. The bandmap of 30 U.S. cultivars tested obtained from using the primer GGAGACCC is presented in Figure 11. There were nearly 21 fragments of varying length

observed per lane, and only for such fragments were monomorphic across all cultivars examined (arrow; Figure 11). The uniqueness of fingerprint profiles for 29 of the 30 cultivars were seen. Only Redmar and Goldmar appeared identical; Goldmar is a mutant of Redmar and thus this similarity was not surprising. However, these two cultivars were discriminated using other primers.

The principal coordinate chart of the 30 U. S. cultivars (Fig. 12) confirmed the trends observed from the phenogram (Fig. 10). Close relatives such as [Jewel-Nugget-Scarlet-Carver]; [Resisto-Copper Resisto]; [Regal-Excel]; [Redmar-Goldmar] clustered very closely. This further underscores the reliability of evolutionary information gleaned from the DAF data. Older cultivars such as Creole, Tinian, PortoRico and Bunch PortoRico were separated slightly from newer cultivars on the third PCA axis. Cultivars derived from W-lines such as Regal and Excel exhibited a relatively dissimilarity from other U. S. cultivars. These cultivars have been developed by Dr. Alfred Jones and colleagues at USDA/ARS, Charleston, SC [Jones, 1985 #1154] for disease and pest resistance using population improvement approach. The broader genetic base and divergence of these cultivars were clearly apparent in our study.

The DNA amplification fingerprinting technique described results in the detection of a large number of genetic polymorphisms among sweetpotato genotypes, indicating that DAF can be productively employed in efforts to examine the distribution and extent of genetic diversity in this crop. The overall genetic similarity among the sweetpotato genotypes in our study was considerably lower than that observed for other crops such as tomato, beans and papaya. The high level of genetic diversity in the sweetpotato gene pool may be due to this crop's allogamous nature, large genome size, allopolyploidy, high heterozygosity, or directed selection in sweetpotato for varied uses. Self-incompatibility and outcrossing nature of the crop favors high gene flow among genotypes while vegetative reproduction helps maintain diversity. Sweetpotato is a subsistence crop in many developing countries and genetic erosion due to the introduction of high-yielding varieties is not yet a major problem. This also may account for the high levels of genetic variability observed in our study.

Although certain clusters in the phenogram and PCA (Figs. 8 and 9) contained accessions from geographically proximal locations, it is difficult to

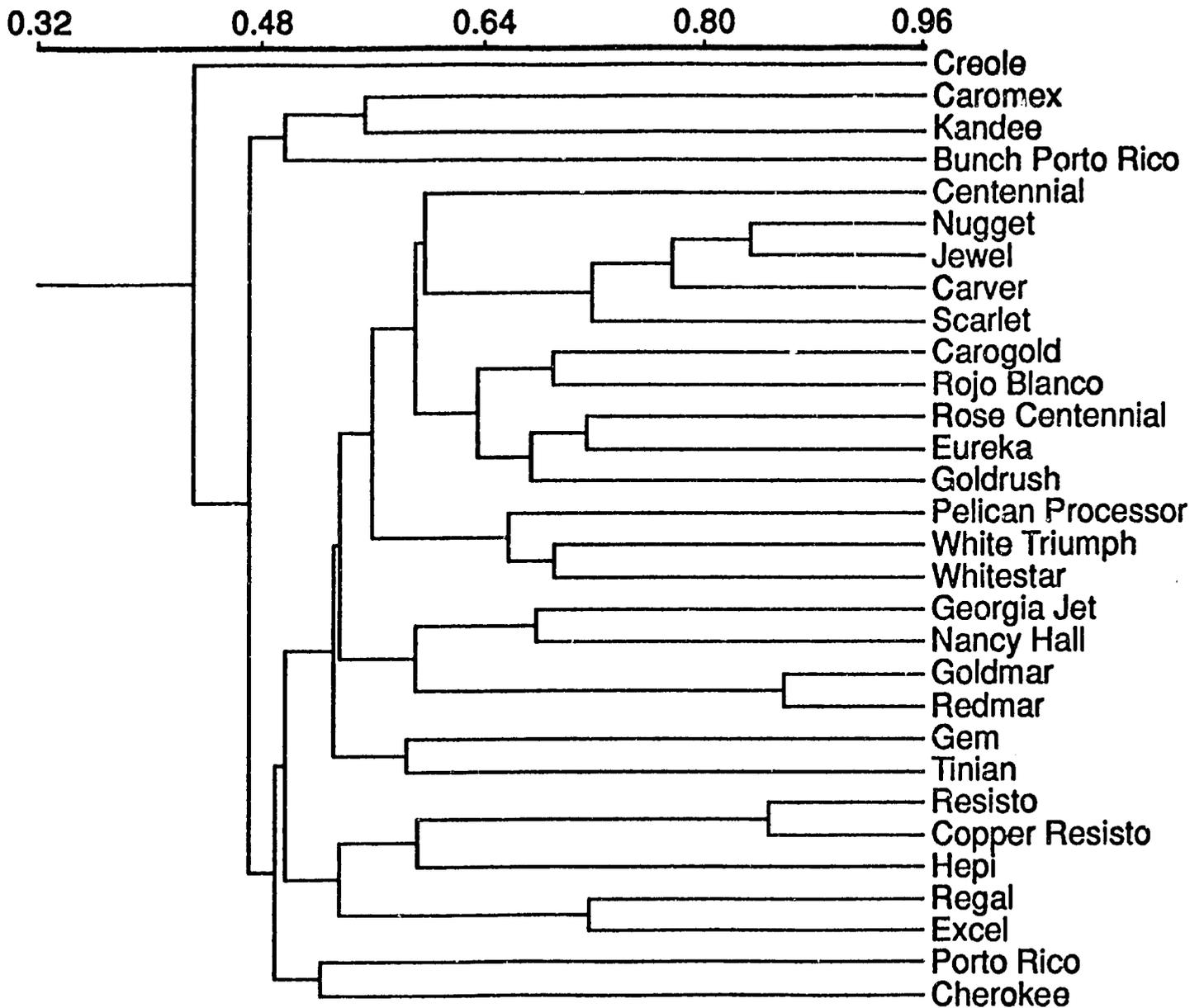


Figure 10: Phenogram of 30 sweetpotato cultivars from U. S. based on Jaccard's coefficient derived from the DNA fingerprinting analysis data.

make generalizations on the regional bias in the relationships except for those from U. S.. The geographic information available on these clones often represents only the geographic source from which material was received and not necessarily its place of origin or initial cultivation. Nevertheless, many accessions from the South Pacific and also Central American countries clustered together suggesting an evolutionary relatedness among those lines from the same region. The high level of DNA polymorphism between the geographic groups may be due to the independent introductions of sweetpotato from its origin in South America to the Old World along with its continued divergence in the introduced areas. Many accessions from Papua New Guinea were diffused across several clusters (Fig. 8). Sweetpotato is a staple crop in Papua New Guinea and Irian Jaya. This island is known to have the largest number of sweetpotato cultivars under cultivation and is considered to be a secondary center of diversity. Linguistic and archeological evidence suggests that sweetpotato had a pre-Magellan introduction into the Pacific region. Our sampling of accessions from Papua New Guinea was very small; however, this group exhibited a high level of genetic diversity suggesting a broad genetic base of sweetpotato in this country.

The cultivars Porto Rico and Centennial have been used as parents in the development of many U.S. cultivars and thus it is not surprising that most U.S. cultivars examined in our study cluster together. The high degree of genetic similarity observed between many of the cultivars underscores the narrow genetic base of the U.S. sweetpotato cultivars. The genetic vulnerability of the U.S. sweetpotato germplasm has been noted, as 79% of all U.S. cultivars are believed to have been derived from just three parents (NRC/NAS Report, 1993). 'Jewel', until recently the most popular sweetpotato cultivar with approximately 70% of U.S. sweetpotato acreage during 1970s and 1980s, shows 50% or more similarity with at least half of the other cultivars tested. Cultivars Carver and Jewel showed the highest similarity among all accessions. 'Carver' is a selection from a cross between Jewel and Centennial, but shows much higher than expected similarity to Jewel. Cultivar Tinian, which was distinct from most other U.S. cultivars is an introduction from the Tinian Island and thus this divergence is to be expected. The close proximity of U. S. cultivars to each other in the phenogram is in concordance with the fact that most of these cultivars share a common ancestry. Thus patterns of sweetpotato diversity detected by DAF

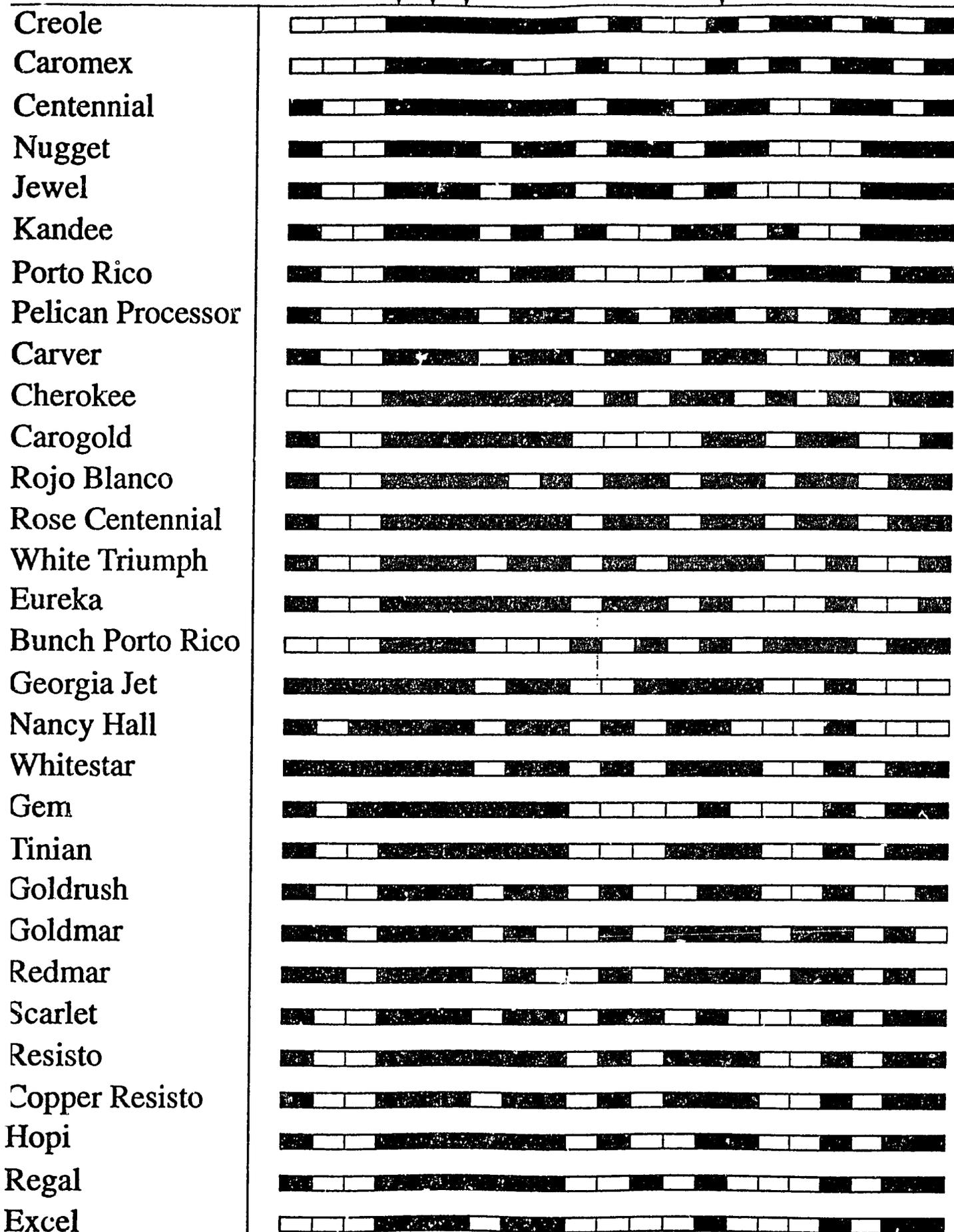


Figure 11: Bandmap of 30 sweetpotato cultivars from U. S. developed from DAF profiles generated by using the primer GGAGACCC. The dark color-filled rectangle represents a presence of the band while the hollow rectangle shows the absence of the band. The molecular weights are highest on the left. Arrows identify bands that were monomorphic across cultivars.

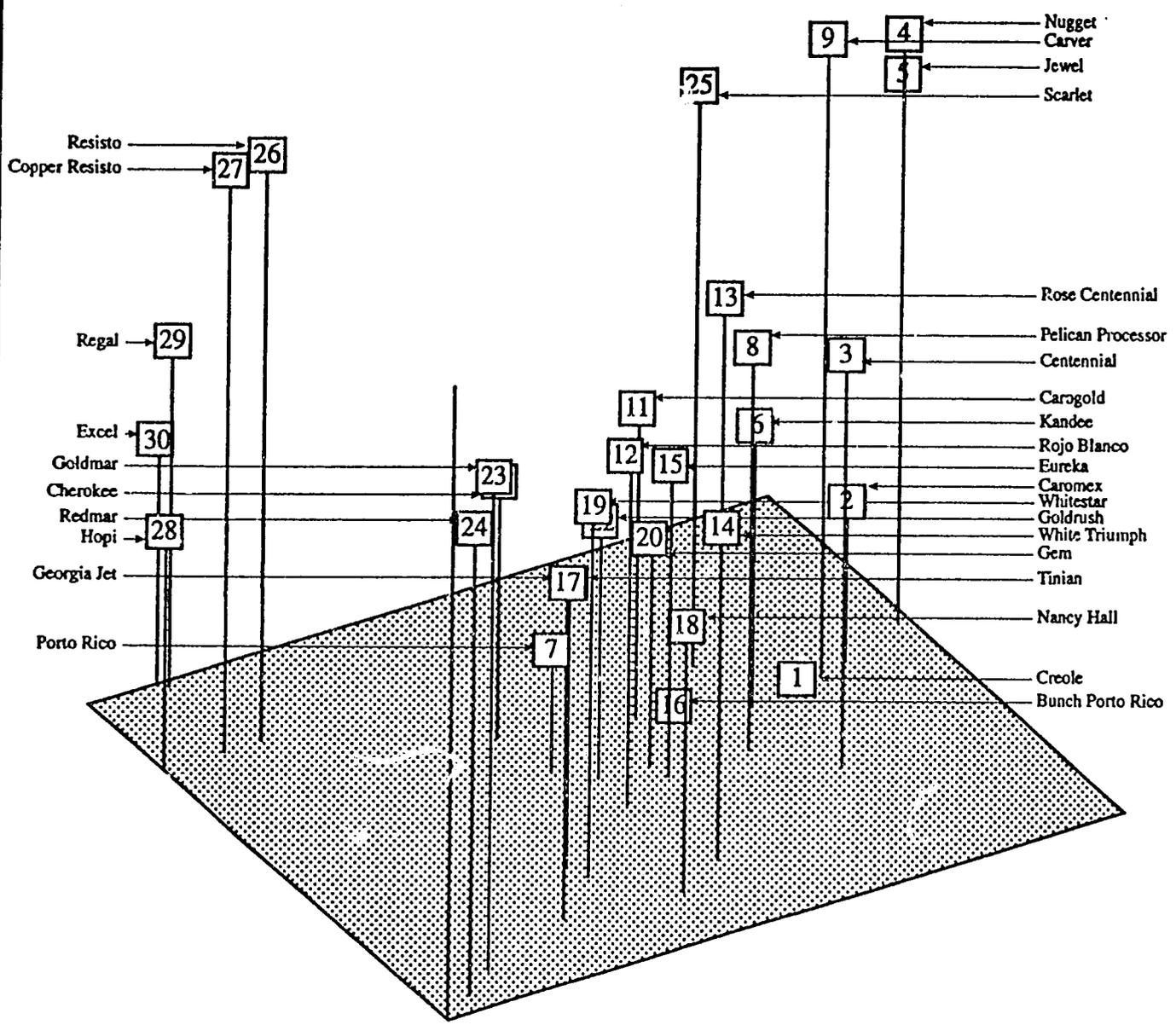


Figure 12: Principal coordinate analysis of 30 sweetpotato cultivars from U. S based on Jacquard's coefficient derived from the DNA fingerprinting analysis data.

$\alpha = 50$ $\beta = 50$ $\gamma = 50$

analysis appear to validate (or fairly reflect) the known relationships between sweetpotato genotypes.

The cultivar HiDry did not fall within any of the clusters containing the other U. S. cultivated clones, and was distinct from all the accessions examined (Fig. 8). This cultivar was specifically developed for high dry matter production for industrial uses and originated as an open-pollinated selection from a Japanese cultivar 'Minamiyutuka' (Jones 1985). This cultivar originated from a cross between a cultivated sweetpotato with a wild *Ipomoea* species (Sakamoto 1976). Thus cv. HiDry may be of different genotype composition relative to other cultivated sweetpotatoes due to the introgression of portions of the wild *Ipomoea* genome. DAF thus provides the means to examine the genetic base of sweetpotato and thus may be useful as a tool to monitor the genetic base of the crop and for identification of appropriate parents based on dissimilarity.

We have also conducted preliminary studies on the phylogenetics of *Ipomoea* species employing the DAF approach in collaboration with Dr. Janice Bohac (USDA/ARS, Charleston, SC) and Dr. Sriyani Rajapakse (Clemson University, SC). The results of this study were presented recently at the sweetpotato collaborators meeting.

In conclusion, our data suggest that the DNA amplification fingerprinting may be a significant tool to study sweetpotato genetic resources, identifying genotypes in germplasm collections, estimating genetic relationships among accessions and may also be useful in the improving the productivity and stability of crop's yield through identification of diverse parents.

Spin-Off Research Arising from the Current Project

A similar research employing DAF to study peanut (*Arachis hypogaea*) germplasm has been initiated in our lab. Over 100 accessions of the cultivated the peanut and twenty accessions representing wild *Arachis* species have been obtained from Dr. Roy Pittman (USDA). We have identified the optimum range of concentrations of template DNA, primer and AmpliTaq (Stoffel fragment) in PCR that results in clear DNA fingerprint profiles. A research proposal by Dr. Prakash to develop a DAF system for peanut and to link-up with ICRISAT (India) has been

funded by USDA/OICD. A proposal to characterize the peanut germplasm using DNA markers has been funded by USDA/CSRS under the capacity building grants program. The National Museum of Kenya has expressed an interest in the use of our DAF technology in their research of characterizing plant genetic resources including sweetpotato. Dr. Rashid Aman of NMK and Dr. Edward Carey of CIP are collaborating with Dr. Prakash in this regard. The EMBRAPA of Brazil has also expressed an interest in the use of this technology in their sweetpotato germplasm research. Dr. Patricia Ritschel, CENARGEN, EMBRAPA, Brasilia, Brazil has developed a proposal to CNPq, Brazil (with Dr. Prakash) to visit Tuskegee University for learning the techniques. Dr. Prakash has also developed a project proposal (to World Bank) with Prof. Rubaihayo of Makerere University (Uganda) to conduct a similar research on banana. Dr. Prakash was one of the team members of a successful proposal approved by McKnight Foundation for a planning grant. Dr. Prakash visited Uganda and helped develop a proposal on sweetpotato improvement with National Agricultural Research Organization of Uganda.

Seminars and Conferences

Dr. Prakash was an invited plenary speaker at the Symposium of International Society of Tropical Root Crops at Salvador, Bahia (Brazil) in November 1994. He was also an invited speaker at the International Society of Tuber Crops Meeting in Trivandrum, India during November 6-9, 1993; a trip report has been sent earlier to AID. Dr. Prakash was also an invited speaker at Auburn University on January 7, 1994 where he also serves as an Associate Member of the Graduate Faculty. Dr. Prakash was the chairman of the session on sweetpotato biotechnology at the National Sweetpotato Collaborators Group Annual Meeting in Nashville (February 5-6, 1994) and again in New Orleans (January 28-29, 1995) where he also presented the summary of the research from this AID-HBCU Project which was met with considerable enthusiasm from sweetpotato scientists. Dr. Prakash also presented the results of research from this project at the 4th International Congress of Plant Molecular Biology at Amsterdam, Netherlands (June 19-24, 1994). Dr. Prakash also presented invited seminars at University of California, Davis (April 1994), Makerere University (Kampala, Uganda; May 1994), CENARGEN, EMBRAPA, Brazil (November 1994), Monsanto Co. (St. Louis; December 1994), ICRISAT, India

(December, 1994) and University of Agricultural Sciences (India; DEC 1994). Dr. Guohao He, postdoctoral research associate, presented a poster on our sweetpotato DAF research at the Gatlinburg Symposium on Plant Genome (June, 1993) and at the Second International Conference on Plant Genome at San Diego (January 23-27, 1994).

Several scientists visited our lab during the project and delivered seminars. Dr. Peter Greshoff, Professor, University of Tennessee who developed the original DAF technique visited our lab on February 10, 1993, presented a seminar and provided useful new information on genome analysis of crop plants. Other scientists who presented seminars were

Dr. Jeff Lowe (Agricultural Genetics Co. Ltd.)
 Dr. Joe Shaw, Dr. Sadik Tuzun and Dr. Henry Daniell (Auburn University)
 Dr. Zhijian Li (Louisiana State University)
 Dr. Marja Poteri (Finnish Forest Research Institute)
 Dr. Zhihong Yu (Cornell University)
 Dr. Rick Jansson (University of Florida)
 Dr. Hartenese Dodo (Penn State University)
 Dr. Alexy McKentley (EPCOT Center)
 Dr. Art Weissinger (NS State University)
 Dr. Ray Bressan (Purdue University)
 Dr. A. K. Singh (ICRISAT)
 Dr. G. M. Reddy, Institute of Molecular Biology, India
 Dr. Naresh Pancholl (University of Reading, England)
 Dr. S. Satish (Ohio State University)
 Dr. Ana Rosu (Institute of Agronomy, Romania)
 Dr. George Bruening (University of California, Davis)
 Dr. Kenneth Keegstra (Michigan State University)
 Dr. David Kristofferson (Intelligenetics Inc.)

Training of Developing Country Personnel and Transfer of Technology

Dr. K. P. Sibuga, Professor, Sokolne University of Agriculture (SUA), Tanzania is the developing country collaborator of this project. Tuskegee University has a linkage project with SUA funded by AID. Dr. Sibuga visited the Plant Molecular

and Cellular Genetics Laboratory for two weeks during December 1992 to learn more about our research first hand. Dr. Prakash and Dr. Sibuga developed a research preproposal "Biotechnological approaches to crop improvement in Tanzania" which was submitted to AID under the PSTC program. Dr. Sibuga has nominated her colleague Mr. M. K. Mushobozy to be trained in our lab under the current project in sweetpotato DAF techniques. But Mr. Mushebozy could not participate because of some personal reasons. Mr. Ernest Gwebu, a graduate student in our school is from Swaziland and Mr. Matand Kanyand of Zaire have now been well trained in the use of DNA marker techniques. Both will apply this technology in their research at Swaziland or Zaire upon return.

International Potato Center (CIP, Lima, Peru) holds the world's largest germplasm collection of sweetpotato germplasm and is actively involved in plant exploration. We have communicated the results of our research to Dr. Zosimo Huaman, Germplasm Scientist, CIP and forward the DAF protocol so that technique developed in our lab at Tuskegee can be effectively employed in the sweetpotato germplasm research at CIP. Further, many of the research proposals mentioned above, if successful constitute transfer of technology from our lab to many developing countries.

Publications Resulting From the Project

- He, G., C. S. PRAKASH, R. Jarret, S. Tuzun and J. Qiu. 1994. Comparison of gel matrices for resolving DNA amplification fingerprint profiles. *PCR Methods and Applications* 4: 50-51.
- PRAKASH, C. S. 1994. Sweetpotato Biotechnology: Progress and Potential. *Biotechnology and Development Monitor* no. 18, p 18-19, 22.
- PRAKASH, C. S. 1994. Biotechnological Approaches to Sweetpotato Improvement at Tuskegee University. *BioLink* 2 (1): 5-7.
- PRAKASH, C. S., He, G., and R. Jarret. 1995. Computer scanning to document silver stained gels for DNA amplification fingerprinting. (Submitted)
- He, G., C. S. PRAKASH, S. Tuzun, and J. Qiu. 1995. Optimizing conditions for DNA amplification fingerprinting of sweetpotato. (Submitted).
- He, G., C. S. PRAKASH, R. Jarret 1995. Analysis of genetic diversity in sweetpotato germplasm using DNA amplification fingerprinting. (Submitted).
- C. S. Prakash, He, G. and R. Jarret. 1995. Evolutionary relationships among US sweetpotato cultivars analyzed using DNA markers. (to be submitted soon).
- He, G., C. S. PRAKASH and K. Matand. 1995. Strategies for the identification of polymorphic DNA markers in cultivated peanut (to be submitted soon).

Daniell, H. D., Porrobo-Dessai, A., PRAKASH, C. S., and Moar, W. 1994. Engineering plants for stress tolerance via organelle genomes. In: *Biochemical and Cellular Mechanisms of Stress Tolerance in Plants*. (Ed. Joe H. Cherry) pp 589-604. NATO-ASI Biology Series. Vol. H 86. Springer-Verlag, NY.

Invited Papers At Meetings

PRAKASH, C. S., He, G., and R. Jarret 1995. Genetic relationships among U. S. sweetpotato cultivars analyzed by DNA amplification fingerprinting. Am. Soc. Hort. Science Southern Section/Sweetpotato Collaborators Annual meet. January 28-29, 1995. New Orleans.

Rajapakse, S., Bohac, J. PRAKASH, C. S. and He, G. 1995. Analysis of phylogeny of *Ipomoea* using DNA markers 1995. Am. Soc. Hort. Science Southern Section/Sweetpotato Collaborators Annual meet. January 28-29, 1995. New Orleans.

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Gosukonda, R. M., Q. Zheng, A. P. Dessai, G. He, M. Egnin, and C. S. PRAKASH. Genetic Engineering and DNA Fingerprinting Research in Sweetpotato. 16th Int. Cong. of Biochemistry and Molecular Biology. Satellite Symp 1 on Plant Biotechnology Applications. Sept 14-16, 1994. Hyderabad, India.

PRAKASH, C. S., Porrobo Dessai, A., G. Ramanamurthy, K. Dumenyo, G. He, and Q. Zheng and M. Egnin. 1993. Biotechnological approaches to the improvement of sweetpotato. The International Symposium on Tropical Tuber Crops (ISOTUC). November 6-9, 1993. Trivandrum, India.

PRAKASH, C. S., He, G., and R. Jarret 1994. Genetic relationships between sweetpotato genotypes analyzed by DNA amplification fingerprinting. 4th Int. Cong. Plant Molecular Biol. Amsterdam, Netherlands. June 19-24, 1994.

PRAKASH, C. S., He, G., and R. Jarret 1994. DNA sequence polymorphism based genetic diversity studies in sweetpotato germplasm (Abstr.). *HortScience* 29: 727

He, G., C. S. PRAKASH, R. Jarret 1994. DNA amplification fingerprinting to analyze genetic diversity in sweetpotato germplasm. Conf. on Plant Genome II. San Diego, CA. January 24-27, 1994.

He, G., PRAKASH, C. S., Jarret, R. L., Tuzun, S. and Qiu, J. 1993. DNA amplification fingerprinting of sweetpotato. Gatlinburg Symposium on Plant Genome Analysis, Knoxville, TN. June 9-12, 1993.

Concluding Remarks

The project on DNA fingerprinting of sweetpotato genetic resources funded by AID under the HBCU grants program was highly successful. Research performed under the project enabled us to define conditions for the production of reliable and reproducible DNA profiles of sweetpotato genotypes, identified informative oligonucleotide primers that detect high polymorphism, identify a method for digitizing fingerprints and finally to employ the DAF technique in studying the genetic diversity and genetic relationships among sweetpotato genotypes in the germplasm collection including U. S. cultivars. We are confident that results from our research will have a positive impact on the sweetpotato germplasm collection and improvement efforts worldwide. The project also laid a solid foundation in our lab for pursuing research on DNA markers using PCR, and we have successfully applied the technique recently in studies also on peanut and cowpea.

The AID-HBCU project also strengthened our capacity for training graduate and undergraduate students in biotechnology and to train scientists from developing countries. Finally it provided us with valuable resources to conduct quality research in a cutting-edge area of science with a mission of targeting such science to problems of developing countries.

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