BIOTECHNOLOGY IN CASSAVA GERMPLASM CONSERVATION AND BREEDING IN INDIA

Santha V. Pillai, S. Sundaresan and M. N. Sheela[1]

ABSTRACT

Biotechnological tools such as tissue culture and DNA analysis are increasingly being utilized for germplasm management and breeding in crop plants. Germplasm is the basic material for plant breeding, and as such, management of genetic resources in the most efficient way is pivotal for crop improvement. Realizing the importance of this, the collection of cassava germplasm started in India about 50 years back, and more than 1600 accessions have been collected. These are being maintained in a field gene bank with annual replanting. Part of the germplasm is also being maintained in *in vitro* form.

The large number of accessions is a problem in many old collections, and it is more so in a vegetatively propagated annual crop like cassava. Attempts were made to minimize the number in a scientific way without affecting the genetic variability. Many of the accessions were found to be apparent duplicates. This was confirmed in a systematic way by identifying the duplicates based on standard morphological characters, isozyme patterns and DNA polymorphism.

The existing germplasm has been characterized for the 11 morphological characters suggested by the Cassava Germplasm Network. The data have been analyzed for similarity and about 138 apparent duplicate sets were identified. Morphological duplicates were analyzed for esterase isozyme polymorphism and about 60% were found to be duplicates at the isozyme level. These isozyme duplicates were again analyzed for their similarity at the DNA level using RAPD polymorphism. About 50% were found to be duplicates at the DNA level. Such duplicate accessions could be eliminated from the field germplasm bank and preserved only in *in vitro* or cryo form. This information, along with that on economic characters and passport data was utilized for identification of a tentative core collection consisting of 15% of the accessions. Isozyme and DNA analyses were also used for basic genetic studies, such as assessing the variability and genetic diversity available in the population. Based on this, clusters were identified and crosses were made between distant clusters to produce a wide spectrum of variability.

Attempts were also made to identify molecular markers for economic characters, which will be useful for Marker Assisted Selection (MAS). Duplicates, which differed in one or two economic characters, were found to be good material for marker analysis. Research is under way to use DNA analysis for phylogenetic studies. Future strategies involve the use of proven SSR markers for Marker Assisted Selection of disease resistant varieties, and utilization of commercially available genes for quality improvement in cassava.

INTRODUCTION

Germplasm is the raw material for crop improvement. Realizing the importance of germplasm, cassava breeders in India started the systematic collection of germplasm in the 1940s. Cassava is an introduced crop in India, and it is believed to have reached Indian shore during the 17th century through Portuguese sailors. Even though it was introduced as a botanic specimen in the Calcutta Botanic Garden during 1794 (Tan, 1994), it was not until 1840 that improved varieties were officially introduced for cultivation in southern India, especially in the state of Kerala. Research on cassava started in India about one century later. At the time, breeders could collect as many as 75 different cultivars from Kerala (Joseph et *al.*, 1992). Even though the number of varieties introduced in the beginning was

[1] Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram 695 017, Kerala, India.

limited, a large number of recombinant varieties were selected and cultivated by farmers. These selections were mostly for cooking quality, in contrast to the bitter nature of the introduced varieties. Later exotic varieties were introduced through governmental agencies, as well as through CIAT (Colombia) and IITA (Nigeria).

In addition to the conventional way of conservation, cataloguing and evaluation of germplasm, biotechnological tools such as tissue culture and molecular characterization are increasingly being used for germplasm management and utilization (Taksley and Orton, 1983; Ocampo *et al.,* 1993; Carvalho *et al.,* 1998). A brief account of the biotechnological approaches for management of cassava germplasm, as initiated in India, is given in the paper.

Status of Cassava Germplasm

The Central Tuber Crops Research Institute (CTCRI), in Thiruvananthapuram, Kerala, is the main Center for conservation of cassava germplasm in India. Some of the State Agricultural Universities are maintaining a limited number of accessions as a working collection. At present, CTCRI is holding about 1,635 accessions of cassava (*Manihot esculenta* Crantz), which consists of 785 exotic and 850 indigenous collections. The indigenous collections include land races and breeding lines. The exotic groups include collections from different cassava growing countries like Nigeria, Madagascar, Thailand, Ghana, Colombia, Uganda, Sri Lanka and Malaysia. There are nine other species of cassava namely *Manihot anomala, M. caerulescence, M. epruinosa, M. flabelifolia, M. glaziovii, M. dichotoma, M. maracasensis* and *M. peruviana.*

The available germplasm has been profitably utilized for the selection and release of three short-duration varieties and eight high-yielding hybrids. The wild species are being utilized in interspecific crosses for the incorporation of mosaic resistance to cultivated varieties.

Collection of Germplasm

The National Bureau of Plant Genetic Resources (NBPGR) in New Delhi is the nodal agency for collection of germplasm in India. Land races are mostly collected through germplasm collection trips, jointly organized with NBPGR. Exotic accessions are also procured through NBPGR. Sexual seed of various crosses have been obtained from CIAT/Bangkok.

Conservation of Germplasm

The existing germplasm has been characterized based on International Plant Genetic Resources Institute (IPGRI) descriptors and these are documented in a computerized database. This data has been utilized for identification of genetic stocks for economic characters (Santha Pillai *et al.,* 2000).

Identification of morphological duplicates

Occurrence of duplicate accessions is a problem in large collections of germplasm. This adds to the cost of field maintenance, without contributing to the variability. Scientific means of identification of duplicates, and their elimination have been thought off by several workers. As per the decision taken in the First Meeting of the International Network for Cassava Genetic Resources (Ekanayake, 1992), the existing germplasm was screened for 11 morphological characters (**Table 1**). The data was analyzed to identify those accessions,

which were identical in all 11 characters. By this exercise, 138 sets of morphological duplicates were identified (**Table 2**). It was found that the number of duplicates was greater in the indigenous group. It may be due to periodic collection of germplasm from the same area or spreading of good varieties to far off places. It is also observed that the same cultivars are know by different names in different places (Santha Pillai *et al.,* 1999).

Table 1. Key morphological characters of cassava.

Shoot characters	Root characters
Stem epidermis color	Root surface color
Stem periderm color	Root cortex color
Apical leaf color	Root flesh color
Shape of central leaf lobe	Root neck (peduncle length)
Color of petiole	
Apical pubescence	
Flowering	

Table 2. Frequency of morphological duplicates.

	Exotic	Indigenous
No. of accessions screened	727	786
No. of duplicate groups	46	92
Frequency of duplicates in each group	2(45)	2(67)
	3(1)	3(14)
		4(7)
		5(2)
		6(2)
		7(1)
Total no. of duplicates	47	140
Percentage of duplicates	7	18

BIOTECHNOLOGICAL APPROACHES FOR CONFIRMATION OF DUPLICATES

Isozyme Analysis

Studies at CIAT/Colombia showed that esterase isozyme produced large polymorphism in cassava, and it can be used as a varietal marker for routine identification of cassava cultivars (Ramirez *et al.,* 1987). Accordingly, the morphological duplicates identified in the collection, were analyzed for esterase isozyme polymorphism. Those, which showed 100% similarity in banding pattern, were rated as isozyme duplicates. In addition to visual scoring, it was confirmed by analyzing the molecular weight of the bands using the software 'AAB 1D Advanced'. Out of the 138 sets of morphological duplicates analyzed for esterase enzyme, 85 showed similarity in banding pattern (Sumarani *et al.,* 2002; Harisankar *et al.,* 2002). Details are given in **Table 3**.

Table 3. Morphological duplicates and isozyme duplicates in cassava germplasm.

	Exotic	Indigenous	**Total**
No. of morphological duplicate groups	46	92	**138**
No. of isozyme duplicates	28	57	**85**
Percentage of isozyme duplicates	60	63	**62**

DNA Analysis of Isozyme Duplicates

DNA analysis, based on RAPD (Random Amplified Polymorphic DNA) has been used elsewhere for studies of variability and identification of duplicates in cassava (Marmey *et al.,* 1994; Carvalho *et al.,* 1998). Accordingly, 85 sets of isozyme duplicates were analyzed for RAPD banding pattern following Dellaporta *et al.* (1983). Random primers from OPERON Q- series consisting of 20 primers were used for polymerization. Thirty-six groups including multiple sets were found to be duplicates at the DNA level as well (Santha Pillai *et al.,* 2002). RAPD banding pattern in a sample of five sets based on one primer is shown in **Photo 1** and that for 3 primers is shown in **Photo 2**. In addition to the visual scoring, the genetic relation was analyzed numerically based on base pair and drawing dendrograms using the software "AAB 1D Advanced" (**Figure 1**). The duplicate accessions from each set can be eliminated from the field gene bank and maintained only in tissue culture or under cryopreservation.

IDENTIFICATION OF A CORE COLLECTION

This information on duplicates was used as an additional tool for identification of a core collection of cassava germplasm consisting of about 15% of the total germplasm (**Table 4**). The core collection consists of released varieties, geographic representatives, wild varieties and genetic stocks for various economic characters, after elimination of the duplicates (Santha Pillai *et al.,* 2002). The core collection is being analysed for DNA variability.

STUDIES ON MOLECULAR GENETICS AND BREEDING

Analysis of Genetic Variablility and Genetic Diversity

Molecular markers like isozyme and RAPD can be utilized for studies of genetic variability and genetic diversity in the population. To start with, esterase isozyme markers were used to study the variability in 113 elite breeding lines. The genetic distance between accessions was analysed by constructing dendrograms and identifying clusters (**Figure 2**). The population fell into 12 clusters (Santha Pillai and Sudaresan, 1998). Hybridization involving genetically distant genotypes is expected to show wide variability. Crosses within clusters and between clusters were made and their F1 performance is being studied.

Use of Molecular Markers to Confirm True Hybrids

Confirmation of hybrid nature of the F1 is very important in genetic studies. In heterozygous crops, it is very difficult to get morphological markers and in such cases DNA analysis can be used as an additional tool. RAPD pattern of a male parent, female parent, and the F1 based on 12 primers was analyzed. It was found that all the bands in the hybrid came from either the male or the female parent, and there was no additional band. This was confirmed by comparison of base pairs as well (**Table 5**).

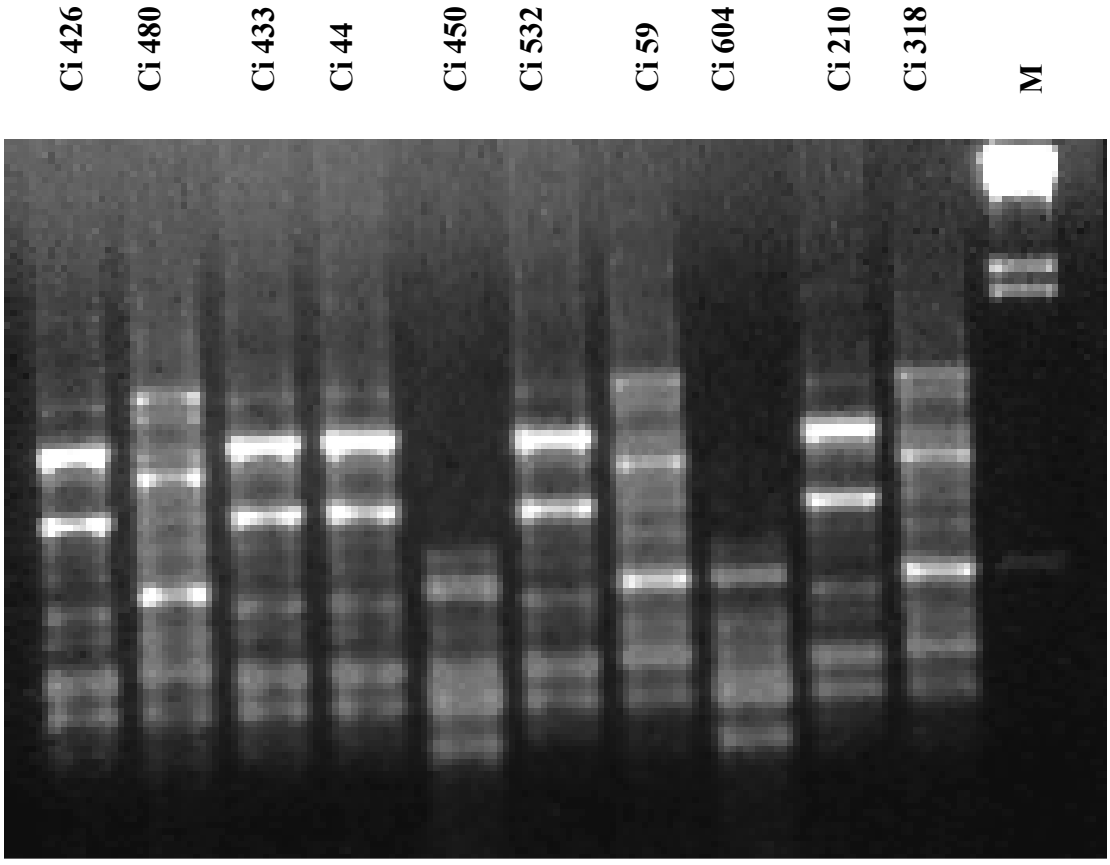

Photo 1. DNA (RAPD) banding pattern in a sample of five duplicate sets based on one primer: OPQ-5.

DNA Fingerprinting of Released Varieties and Elite Breeding Lines

RAPD markers give distinct banding patterns for different varieties, based on one primer or other and it can be used as an additional means for identification. RAPD patterns of 11 released varieties and some of the elite breeding lines based on 10 primers have been produced and documented. RAPD banding pattern of an elite variety based on 10 primers is shown in **Photo 3**.

Molecular Markers for Economic Characters

Identification of molecular markers for economic characters will be useful for Marker Assisted Selection (MAS). Usually this is done in inbreds or 'near isogenic lines'. Duplicate sets which differ in a limited number of DNA bands and a limited number of economic characters can be used to identify tentative markers for the character. By this method a tentative marker for mosaic resistance was identified from a duplicate set. However, this needs confirmation by conventional means. Similarly, a high starch triploid

showed intensity of bands for a particular primer and this again can be used as a tentative marker for starch.

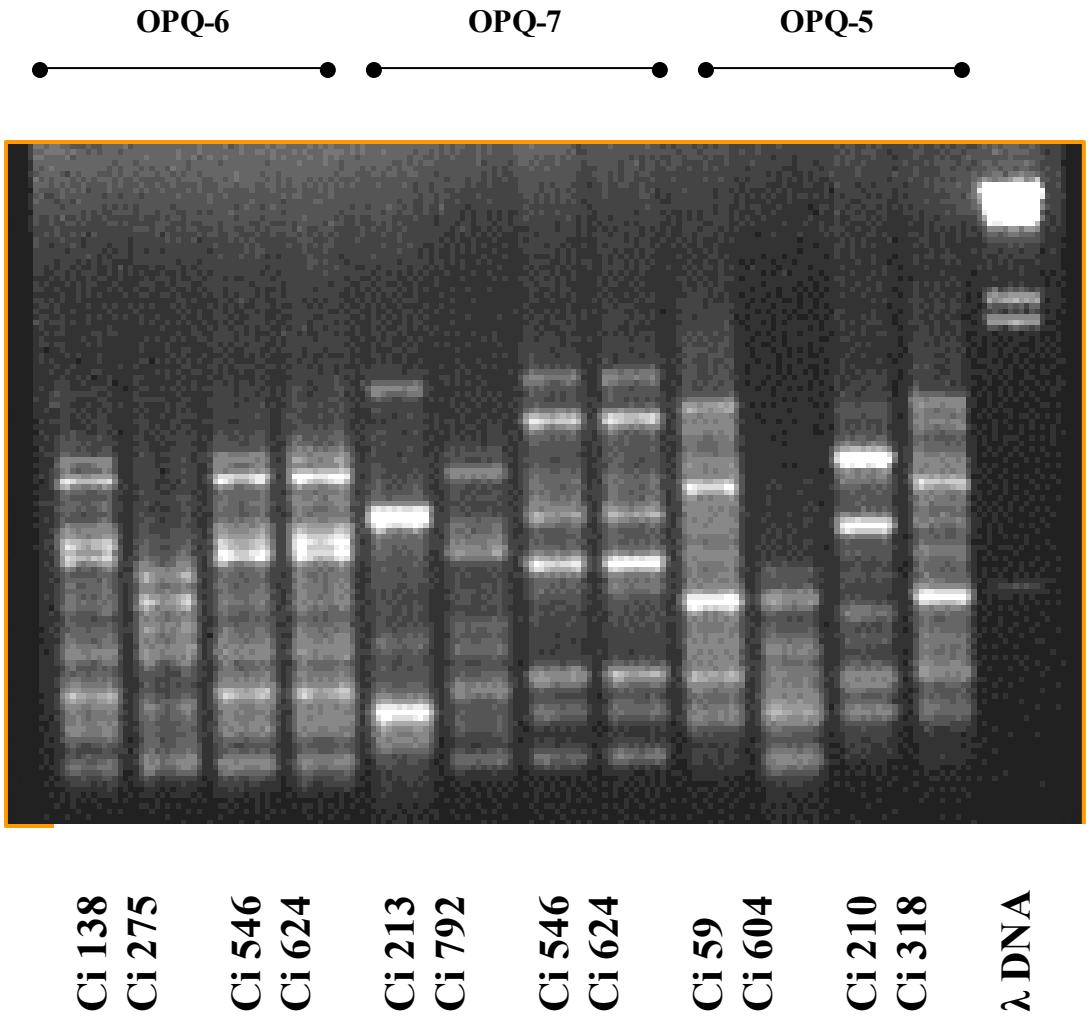

Photo 2. RAPD banding pattern in two duplicate sets based on three primers.

Phylogenetic Studies

The wild species available at the Institute have been analyzed for RAPD patterns and the data is being compared with cultivated varieties to find the genetic relationship and to identify the progenitors.

FUTURE STRATEGIES

Research is being conducted to confirm the tentative markers identified. It is proposed to try the SSR marker, already available for African Cassava Mosaic Disease, for

Marker Assisted Selection. It is also planned to identify molecular markers for drought tolerance using drought tolerant accessions identified from the cassava germplasm collection. The high carotene accessions available in the germplasm will be utilized for identification of gene sequence for β-carotene. Germplasm will be screened for unconventional characters like industrial quality of starch, keeping quality etc., and these again will be utilized for identification of markers and sequencing of the genes. It is also planned to use the 'available genes' for production of transgenics having higher nutritional status, especially protein and iron.

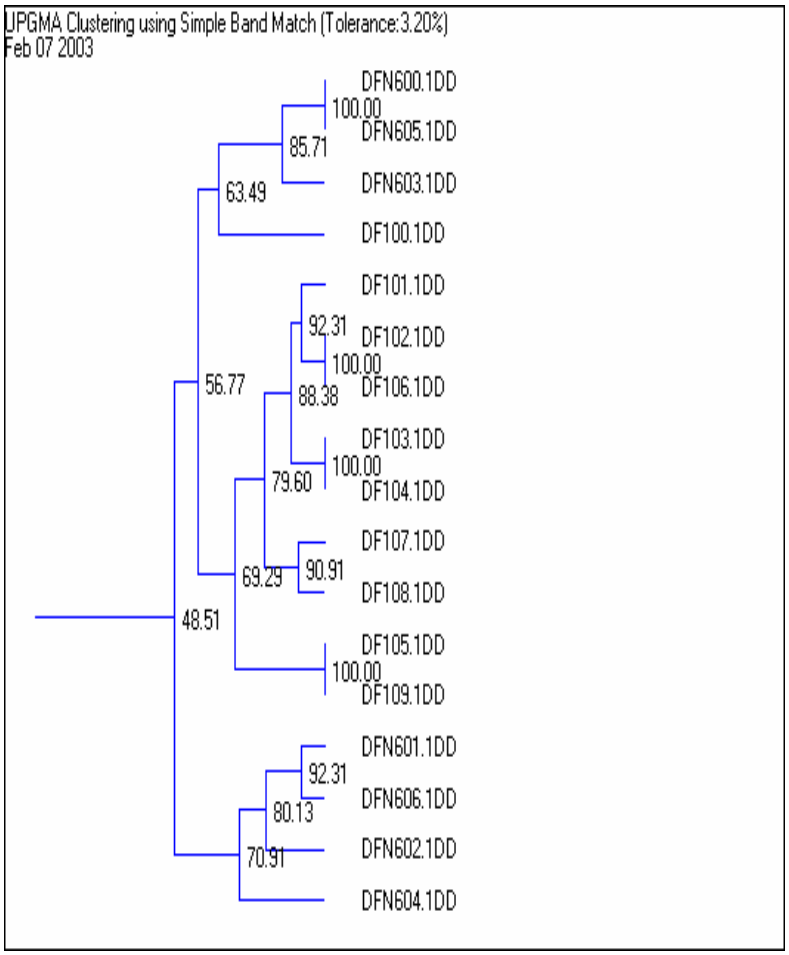

Figure 1. Genetic relation between DNA variants based on dendrogram.

Table 4. Tentative core collection of cassava germplasm at CTCRI, India.

Sl no.	Criteria	No. of accessions
1.	High yield (>5 kg/plant)	30
2.	High starch (>33%)	25
3.	Low cyanogens (<10 ppm)	25
4.	High carotene (>450 ppm)	35
5.	CMD symptom free	75
6.	Popular varieties	20
7.	Released varieties	18
8.	Geographic representatives	4
9.	Wild relatives	9
	Total	**241**

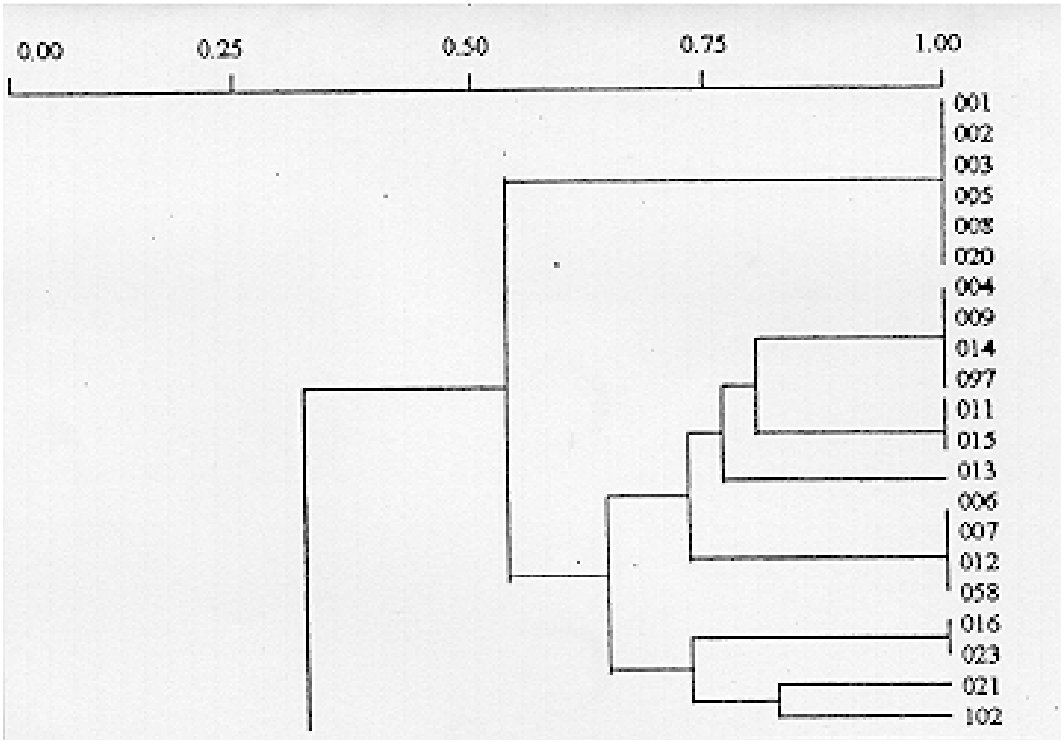

Figure 2. Genetic distance between isozyme variants based on dendrogram.

Table 5. Comparison of DNA base pairs in the hybrid and its parents.

Primer	Genotype	Number and molecular weight of bands							
		1	2	3	4	5	6	7	8
OPQ-10	A[1)]	5366	2414	1960	1485	1164	1049	882	716
	B	3300	1893	1591	1248	852			
	C	3300	1893	1591	1248	852			
OPQ-11	A	2252	1537	1248	1013	823	623	472	
	B	4357	2872	1893	1591	1385	1013	716	
	C	4357	2872	1893	1591	1385	1013	716	
OPQ-12	A	2332	1829	1248	1013	913	767	581	
	B	2029	1434	1086	741	581	472		
	C	2029	1434	1086	741	581	472		
OPQ-13	A	2175	1537	1124	767	602			
	B	2414	1338	1049	882	623			
	C	2414	1338	1049	882	623			

[1)] A = Female parent; B = Male parent; C = Hybrid

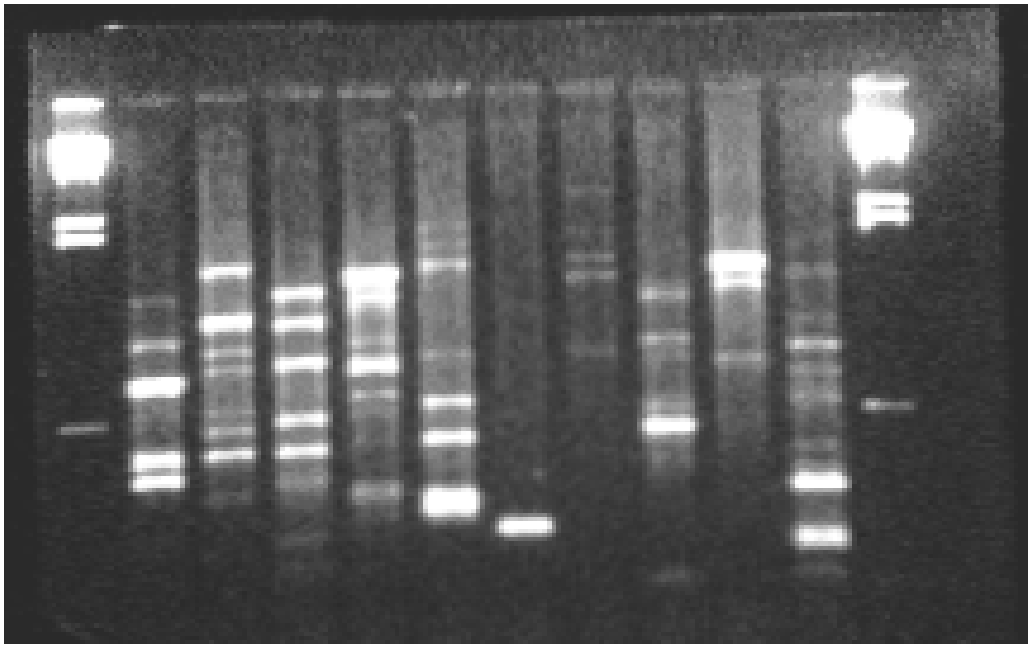

Photo 3. RAPD banding pattern of an elite variety based on 10 primers (Lane no 1 & 12 are DNA markers).

REFERENCES

Carvalho, L.J.C.B., B.A. Schaal and W.M.G. Fukuda. 1998. Morphological descriptors and Random Amplified Polymorphic DNA (RAPD) markers to assess the genetic diversity of cassava

(*Manihot esculenta* Crantz). *In*: L.J.C.B. Carvalho, A.M. Thro and A.D. Vilarinhos (Eds.). Cassava Biotechnology: IV International Scientific Meeting of CBN, held in Bahia, Brazil. Nov 3-7, 1998. pp. 51-64.

Dellaporta, S.L., J. Wood and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1: 19-21.

Ekanayake, I.J. 1992. Conclusions from the working group on cassava genetic resources databases and information systems. Report of the First International Network for Cassava Genetic Resources. CIAT, Cali, Colombia. Aug 18-23, 1992. pp.173-179.

Harisankar, P., Santha V. Pillai, G.O. Sumarani and S. Sundaresan. 2002. Isozyme analysis of cassava germplasm: Identification of duplicates in exotic collection. Plant Cell Biotechnology and Molecular Biology. (in press)

Hussain, A., H. Ramirez, W.M. Roca and W. Bushuk. 1986. Identification of cassava (*Manihot esculenta* Crantz) by electrophoretic patterns of esterase isozymes. Seed Sci. and Technol. 15:19-22.

Joseph, J., G. G. Nayar, P.G. Ragendran, J.S. Jos, C.S. Eswari Amma, K. Vasudevan, M. Unnikrishnan and S.K. Naskar. 1992. Recent progress in cassava varietal improvement in India. *In:* R.H. Howeler (Ed.). Cassava Breeding, Agronomy and Utilization Research in Asia. Proc. 3rd Regional Workshop, held in Malang, Indonesia. Oct 22-27, 1990. pp. 44-55.

Marmey, P., J.R. Beeching, S. Hamon and A. Charrier. 1994. Evaluation of cassava (*Manihot esculenta* Crantz) germplasm collections using RAPD markers. Euphytica 74: 203-309.

Ocampo, C., C. Hershey, C. Iglesias and M. Iwanaga. 1993. Esterase isozyme fingerprinting of the cassava germplasm collection held at CIAT. *In*: W.M. Roca and A.M. Thro (Eds.). Proc. 1st International Scientific Meeting of the Cassava Biotechnology Network (CBN). CIAT, Cali, Colombia. pp. 81-89.

Ramirez, H., A. Hussain, W.M. Roca and W. Bushuk. 1987. Isozyme electrophoregrams of 16 enzymes in five tissues of cassava (*Manihot esculenta* Crantz). Euphytica 36:39-48.

Santha V. Pillai and S. Sundaresan. 1998. Biotechnological approaches for characterisation of cassava germplasm. *In*: P.J. Larkin (Ed.). Proc. 4th Asia-Pacific Conference on Agricultural Biotechnology, held in Darwin, Australia. July 13-16, 1998. pp. 173-176.

Santha V. Pillai, S.G. Nair, K.R. Lakshmi and M.N. Sheela. 1999. Characterisation of genetic resources of cassava. *In*: C. Balagopalan, T.V.R. Nayar, S. Sundaresan, T. Premkumar and K.R. Lakshmi (Eds.). Tropical Tuber Crops in Food Security and Nutrition. Oxford & IBH Publishers, New Delhi. pp. 112-117.

Santha V. Pillai, P. Geethakrishnan Nair and R.R. Nair. 2000. Identification of genetic stocks from cassava germplasm. *In*: Proc. of Intern. Symp. on Tropical Tuber Crops (ISOTUC III), CTCRI, Trivandrum, Kerala, India. Jan 19-21, 2000. (in press)

Santha V. Pillai, P. Harisankar, G. O. Sumarani and S. Sundaresan. 2002. DNA (RAPD) analysis in cassava germplasm: identification of duplicates in exotic collection. Euphytica. (in press)

Santha V. Pillai, R.R. Nair, G.O. Sumarani and V. Ravi. 2002. Germplasm management in cassava with special emphasis on core collection. Paper accepted for presentation in the International Conference on Vegetables, to be held in Bangalore. Nov 11-14, 2002.

Sumarani, G.O., Santha V. Pillai, P. Harisankar and S. Sundaresan. 2002. Isozyme analysis of indigenous cassava germplasm for identification of duplicates. Genetic Resources and Crop Evolution. (in press)

Tan, S.L. 1994. Cassava genetic resources programmes in Asia. *In*: Intern. Network for Cassava Genetic Resources. Report of the 1st Meeting held in CIAT, Cali, Colombia. Aug 18-23, 1992. Intern. Crop Network Series No. 10. IPGRI, Rome, Italy. pp. 41-55.

Tanksley, S.D. and T.J. Orton. 1983. Isozymes in plant genetics and breeding. Elsevier Publications. New York, USA.