### CASSAVA GERMPLASM CONSERVATION AND IMPROVEMENT IN INDIA

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#### ABSTRACT

Genetic diversity of cassava (*Manihot esculenta* Crantz), obtained by open or controlled pollination and selection, as well as from germplasm introductions from abroad, is being conserved, both in the field and in an *in vitro* gene bank. Nine hundred and eighty five germplasm accessions are being conserved under slow-growth conditions using an osmotic retardant medium. The major thrust given in the varietal improvement program are on Indian cassava mosaic disease (ICMD) resistance, improvement of dry matter and starch content, early bulking and culinary quality. Collaborative activities with international organisations like CIAT, and EMBRAPA (Brazil) and with the national All-India Coordinated Research Programme (AICRP) on Tuber Crops have been beneficial.

Field evaluation of breeding lines received as *in vitro* cultures from CIAT has resulted in the identification of one line, MNga 1, showing resistance to ICMD. For the past eight years this line has shown only very mild symptoms of ICMD in the field. The resistant character was confirmed by a grafting test. This variety had a root yield of 34 t/ha, had 43% dry matter and 33% starch content, while showing normal flowering and seed set. This variety has now been crossed with both released and popular local varieties in order to incorporate the ICMD-resistant gene.

Interspecific hybridization of cassava with other *Manihot* species, such as *M. caerulescence*, *M.tristis*, *M. glaziovii*, *M. epruinosa*, *M. esculenta* var *flabellifolia*, *M. esculenta* var *peruviana* have been carried out at CTCRI for the past ten years with the objective of transferring ICMD resistance. The interspecific hybrid of cassava with *M. caerulescence* has shown stable resistance, which was confirmed through wedge grafting. The ICMD-free backcross breeding lines produced root yields ranging from 0.5 to 8.5 kg/plant, and had starch contents ranging from 19.2 to 36.6%. Efforts to improve the culinary quality and root shape continue. A new cycle of interspecific crossing has now started using *M. tristis*, *M. caerulescence* (both cultivated and wild), *M. catingae*, *M. dichotoma*, *M. pseudoglaziovii* and *M. epruinosa* as parents.

Starch and dry matter content have also been improved through triploidy breeding, which resulted in the release of the first triploid cassava variety, Sree Harsha, which is presently undergoing testing in industrial areas of Tamil Nadu. Triploids with early bulking and good cooking quality (at 7-month harvest) have been selected from an intervarietal cross of the local variety Ambakadan with H-2304 (4x); hyperploids of varieties with good culinary quality (M4, Sree Jaya, Sree Vijaya) have also been developed. Inbreeding was found to be very effective in breaking up of populations into widely divergent but reasonably uniform groups. Crossing of such early generation inbred lines with high-yielding hybrids like Sree Visakham has resulted in the production of superior top-cross hybrids. Sree Rekha and Sree Prabha with yields of 48.4 and 42.3 t/ha, respectively, with good culinary quality, and high starch content (28 and 27%) were released in Kerala State. They are now being tested and popularized in the other states.

True cassava seed (TCS) technology was developed to enhance the rapid spread of crops to far-away and non-traditional tribal areas, overcoming the problem of shortage of planting material. Crosses made between the male-sterile line Ambakadan and selected male parents resulted in progeny with nearly uniform root yields and starch contents comparable with those of H-165 and H-226. First clonals (C-1 clones) of Ambakadan hybrid generations were found to be suitable for the starch industry. The All-India Coordinated Research Programme (AICRP) on Tuber Crops has been instrumental in the collection and conservation of cassava germplasm at its various Centers in India, as well as the widespread testing of the new varieties in diverse locations.

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### **INTRODUCTION**

Cassava is grown in India under different agro-climatic conditions and in different soil types, and for various end-uses, such as human food, animal feed and as industrial raw material. In most of the states it is grown as a rain-fed crop but in some districts of Tamil Nadu it is raised as an irrigated crop, giving very high yields. In Kerala, where the crop was first introduced in India, it is grown on open slopes or in coconut-based cropping systems in the uplands. But lately it has shifted to lowland rice-based cropping systems as the upland areas were being occupied by cash crops like rubber. This diversity in growing conditions of the crop has influenced the selection criteria in the varietal improvement program. Progress made in the varietal improvement program for the past 25-30 years in India has been summarized and presented in an earlier report (Abrahamet et *al.*, 2001). Conservation of germplasm through *in vitro* methods and the various breeding approaches presently being followed to meet the demands of the farmers and industry in the country are the subject matter of this presentation.

#### CASSAVA VARIETAL IMPROVEMENT

The major thrusts given in the varietal improvement program are on ICMD resistance, improvement of yield, high dry matter and starch content, early bulking and culinary quality. Tolerance to shade and drought also have been given importance. Value addition by way of reducing anti-nutrient factors like cyanogens, and increasing the nutrient contents like carotene and protein are the other improvement aspects being pursued. The programs undertaken and achievements made through different breeding techniques, like germplasm introduction, evaluation, direct utilization, hybridization and polyploidy breeding, have helped in strengthening the genetic base of the crop, which have enhanced its extending into non-traditional areas as well. Assistance provided by CIAT, Cali, Colombia, and EMBPRAPA, Brazil, in germplasm introduction, and by the All-India Coordinated Research Programme on Tuber Crops in extensive testing of the varieties, has contributed to the progress made in this program. The FPR program being conducted by the Division of Social Science of CTCRI has also contributed to the more widespread use of the improved varieties.

## I. Cassava Germplasm Introduction and Conservation

The Central Tuber Crops Research Institute (CTCRI) in Thiruvananthapuram has a cassava collection of 1,633 accessions, of which 849 are indigenous and 784 exotic; these are mainly conserved in field-grown gene banks. Work on *in vitro* gene banks was started with the objective of eliminating Indian Cassava Mosaic Disease (ICMD) infection from the germplasm collection through meristem culture, and to conserve the accessions under medium term storage. This project was in operation from 1998 to 2001 with funding from the Indian Council of Agricultural Research AP (Agricultural Produce) fund scheme.

Meristem culture using Murashige and Skoog (1962) medium with  $0.1\mu$ M of NAA, BAP and GA<sub>3</sub> was found effective for the initiation of cultures of diverse genotypes. The cultures were micro-propagated on the same basal medium. Nodal cultures from these *in vitro* plantlets were used for raising slow-growth cultures kept for medium-term storage (MTS). Slow growth was induced with osmotic retardants like sorbitol or mannitol. Studies were conducted on the effect of media constituents like sucrose, vitamins and

growth regulators in controlling *in vitro* growth (Unnikrishnan and Sheela, 2000). Genotypic response variation was observed among the accessions in meristem culture, micro–propagation and in slow growth. Addition of  $AgNO_3$  (0.001µM) and activated charcoal (0.1%) were found to help in reducing leaf shedding and preventing root browning in cultures stored for longer periods. Subculture cycles could be extended to 10 to 12 months (Unnikrishnan *et al.*, 2001). Nine hundred and eighty five accessions were stored in IVAG (*In vitro* Active Gene Bank) including indigenous (442), exotic (387), triploids and other breeding lines (156). CTCRI also has acquired facilities for initiating cryopreservation of germplasm with a view to build up IVBG (*In vitro* Base Gene Bank) in cassava.

### **Germplasm Introduction**

During the last ten years CIAT in Colombia has supplied several cassava germplasm accessions and breeding lines as *in vitro* cultures and as true seeds. Open pollinated seeds of different *Manihot* species were also received from EMBRAPA, Brazil.

*In vitro* cultures of 20 breeding lines were received from CIAT in 1994 (**Table 1**). Most of the cultures were received in overgrown or infected stage due to delays in transit as well as handling problems. Only six cultures could be salvaged, micro-propagated and field-transferred. All the lines showed CMD infection except MNga 1.

Table 1. Cassava germplasm introduction in *in vitro* culture from CIAT-Colombiain 1994.

CM 3064-4	CM 3435-5	CG 1141-1	CM 3401-2
CM 3555-6	CM 3306-4	CM 5253-1	CM 3997-1
MBra 191	CM 523-7	CM 494-2	SG 804-5
CM 4484-2	CM 3311-3	CM 2177-2	MNga 1
CM 4365-1	CM 4729-4	CM 3380-7	MNga 2

Seeds of wild *Manihot* species were received from EMBRAPA, Brazil and from Dr. Najeeb Nassar, University of Brasilia, Brazil. The following species were received and planted at CTCRI:

*Manihot tristis	*M. caerulescence
*M. epruinosa	*M. dichotoma
*M. catingae	*M. pseudoglaziovii

### 2. Evaluation and Utilization

#### Indigenous germplasm

Evaluation of indigenous collections for yield, early bulking, dry matter, low cyanogen and high starch content has resulted in the release of short-duration, early-bulking varieties like Sree Prakash (S-856), Sree Jaya (CI-649) and Sree Vijaya (CI-731) for direct utilization (**Table 2**) (Unnikrishnan *et al.*, 2000). These varieties have become popular in Kerala as table varieties, and in Andhra Pradesh and Tamil Nadu for industrial use. They are being utilized in the genetic improvement program for incorporation of better root

characters and compact, late branching plant type. All these varieties possess excellent culinary qualities.

Accession No.	Variety name	Root yield (t/ha)	Cyanogen content (µg/g)	Starch content (%)	Time to maturity	Plant type
S-856	Sree Prakash	30-35	30-50	29-31	7-8 months	Late branching
CI-649	Sree Jaya	26-30	40-50	24-27	6-7 months	Late branching
CI-731	Sree Vijaya	25-28	40-60	27-30	6-7 months	Late branching

Table 2. Germplasm selections for direct utilization.

#### **Exotic Collections**

Exotic collections have not yet been used for direct utilization. They have been used as parents in the hybridization program and have contributed to the development of varieties like Sree Visakham, Sree Sahya and H-97. Most of the exotic collections were found susceptible to ICMD as was the case with the indigenous collections.

Rampant spread of the Indian cassava mosaic disease caused by ICMD has made it necessary to identify disease resistance in cassava varieties or in other *Manihot* species. Evaluation conducted during previous years has resulted in locating this in the accession MNga1 as well as in the species *Manihot caerulescence*, which has led to the resistance breeding work that is now in progress.

## MNga 1 as resistant source to ICMD

MNga 1 was one of the germplasm accessions received from CIAT-Colombia in 1994. It was received as *in vitro* cultures, which were micro-propagated, multiplied, hardened and field transferred. Field observations indicate that this line had only 0 to 1% ICMD infection when other lines showed severe infection (3 to 67%). This character was found to be stable in the observations conducted during the following years. The variety was found resistant on grafting it to a symptom-expressing-susceptible cassava variety used as root stock. MNga 1 was TMS-30001, one of the resistant lines against African cassava mosaic disease (ACMD) developed by IITA-Nigeria (Martin Fregene, CIAT, personal communication). This line showed root yields of 29 t/ha with 34% dry matter, 25.8% starch and a low cyanogen content of 38  $\mu$ g/g in the preliminary evaluation trials conducted during 1998-2001 (**Table 3**). Besides, it also had good culinary quality. The line is presently being tested in different locations in India (Kerala, Tamil Nadu, Andhra Pradesh, Chathhisgarh and Bihar) under the All-India Coordinated Research Programme (AICRP) for evaluating direct utilization as a resistant variety with normal yield and root characters.

MNga 1 is an erect branching variety with grey stem color, green petioles and dark green, thick leathery leaves. Roots are compact, smooth and of cylindrical shape, with light brown skin and white flesh. It shows normal flowering and seed set; it is now being used as a resistant parent in the resistance breeding program.

Sl.No.	Varieties	Root yield (t/ha)		
	-	1998-99	1999-2000	2000-01
1	CI- 848	32.71	38.1	36.62
2	CI- 849	-	-	30.45
3	CI-850	-	-	35.39
4	MNga 1	30.24	23.1	34.17
5	H-152/93		35.8	39.09
6	H-282/94	32.9	32.6	39.92
7	H-740/92	-	38.9	38.68
8	TCH-2	-	-	37.04
9	TCH-1	-	-	38.68
10	Sree Visakham	-	-	34.98
11	CO-3	-	-	38.27
12	H-296	30.45	27.9	-
13	H-60	26.1	-	-
14	H-135	31.0	-	-
15	H-156	24.89	-	-
16	H-290	30.86	-	-
17	H-120	-	27.0	-
18	H-346	-	9.1	-
19	Sree Prakash	28.8	-	-
20	Sree Jaya	30.24	-	-
21	Sree Visakhom	-	34.4	-
CD (5%)	)	NS	6.77	4.548

Table 3. Root yield of MNga 1 in 1998-2001 in field trials at the Central Tuber Crops
Research Institute in comparison with other selections.

Note: MNga 1 mean root yield = 29.17 t/ha

## **II.** Cassava Breeding

# **1. Breeding for ICMD resistance**

Breeding varieties with resistance to ICMD is being carried out through intervarietal and inter-specific hybridization.

# Inter-varietal hybridization

This program was started in 2001 after establishing MNga 1 as an ICMD resistant parent. Crosses were made with MNga 1, using four released varieties (Sree Jaya, Sree Vijaya, Sree Rekha and Sree Prabha) and to promising selections from indigenous germplasm (CI-732 and CI-848). 3,045 pollinations were made, which resulted in 1,243 seeds. Open pollinated seeds (3,340) of the parent varieties were also collected and sown. Germination was almost equal in cross-pollinated (64.2%) and open-pollinated (OP) seeds (61%). OP seeds of MNga 1 showed 32% germination while it was 66.8% (mean of 10) in the crosses made with MNga 1. The resulting seedlings were planted in the field for evaluation of disease resistance and yield characters. At the third month of growth

observations were made for ICMD incidence. Initial scoring showed that open-pollinated populations showed less ICMD incidence than the crosses. **Table 4** shows that the OP progeny of MNga 1 had the lowest percentage of disease incidence. Symptom-free plants isolated will be subjected to graft testing and serodiagnostic testing before selection of resistant genotypes.

Seedling family	Percentage of CMD incidence
MNga 1 OP	15.7
MNga 1 crossed	37.0 (mean of 6)
Sree Rekha OP	21.3
Sree Prabha OP	23.0
Sree Vijaya OP	34.3

 Table 4. ICMD incidence in seedling populations of crosses made with MNga 1 and OP populations

## Inter-specific hybridization

At CTCRI, *Manihot* species, such as *M. glaziovii*, *M. caerulescence*, *M. tristis*, *M. esculenta* var *flabellifolia*, and *M. esculenta* var *peruviana* were used in the interspecific breeding program with the objective to develop breeding lines resistant to Indian cassava mosaic disease (ICMD). Among the crosses, one inter-specific hybrid of cassava x *M. caerulescence* (CMC-1) exhibited disease resistance. The hybrid CMC-1 has remained symptom-free for the past twelve years. The resistance was tested through wedge grafting. The scion of the interspecific hybrid was grafted on highly infected root stock. The hybrid, CMC-1, was found to be immune without any symptoms while other field-tolerant interspecific hybrids developed very mild to severe symptoms. The hybrid is now being backcrossed with elite cassava cultivars having less cyanogens (40-60 $\mu$ g/g) for recovering quality attributes in roots.

The inter-specific hybrids had high cyanogen content (209.6  $\mu$ g/g) while the back cross hybrids had a lower range, from 86.5 to 220.0  $\mu$ g/g. The cyanogen content of the backcross progeny was further reduced to a range of 13.13 to 130.55  $\mu$ g/g in the fourth backcross generation (**Table 5**).

Clone	Starch content (%)	DM content (%)	Sugar content (%)	Cyanogen content (µg/g)
CTM 4	20.45	30.15	3.26	108.92
CTM 5	30.00	37.05	2.92	92.18
CTM 10	25.86	36.15	3.08	13.13
CTK 4	24.45	32.30	2.31	15.45
CTK 5	30.00	38.70	2.84	130.55
CTK 9	25.86	35.25	2.36	22.66
CFM 3	22.50	31.10	3.42	145.23
CFM 4	26.47	39.70	2.74	126.94
CFM 7	23.43	39.95	3.40	29.87
M4	31.25	41.10	2.52	32.70

#### Table 5. Biochemical traits of promising backcross lines.

The backcross lines showing field tolerance to ICMD coupled with reasonable culinary quality are now being evaluated under yield trials. The promising backcross hybrids had high yields, ranging from 28.5 t/ha to 40.12 t/ha. These hybrids are being backcrossed for further quality improvement.

### 2. Polyploidy breeding

Ploidy manipulation gives breeders great versatility for moving genes between ploidy levels, and from wild species to cultivated forms. Successful polyploidization is the first step in polyploidy breeding. For this, the crop must be low in chromosome number, cross pollinated and grown primarily from vegetative parts. Cassava has almost all the necessary features required for successful polyploidy breeding since sterility is not a decisive factor in the crop and seeds are not used for commercial cultivation.

The cultivated varieties of cassava are diploids with 2n = 36. Preliminary studies on induction of tetraploidy and production of triploids in cassava were reported earlier (Magoon *et al.*, 1969; Jos *et al.*, 1970). However, detailed studies on the usefulness of polyploidy, especially triploids in cassava improvement, was only made recently at the Central Tuber Crops Research Institute, India.

### Tetraploids in superior lines

Auto-tetraploidy was induced in eight agronomically superior varieties, i.e. S-300, H-1857, OP-4, Sree Sahya, Sree Visakham, M4, H-50 and S-1315. The induced tetraploids were more vigorous morphologically compared to diploids in having dark green, thick, broad leaves and strong stems. Flowering was delayed and pollen fertility was drastically reduced. However, the root yield of tetraploids was similar to that of diploids. Hence, triploids were produced from these tetraploids with a view to enhance the yield characters.

### Production and evaluation of triploids

Triploids were produced in cassava by crossing diploids with induced tetraploids. Three hundred and fifty triploids were isolated from 18 different cross combinations. The plants were maintained in the field. In addition they were maintained in *in vitro* also. Triploids in general were intermediate in growth compared to diploids and tetraploids. The plants were tall with strong stem, late-branching with dark green thick broad leaves having acuminate leaf tips. The compact plant type and high harvest index allowed for closer spacing at 75 x 75 cm instead of the traditional 90 x 90 cm, resulting in higher yields. Systematic evaluation of the triploids identified several desirable clones. A selection with higher yield and high starch content was released under the name 'Sree Harsha', the first ever released triploid in cassava (Sreekumari *et al.*, 1999).

## Yield, dry matter and starch content of triploids

An outstanding general feature of triploids was that, although they were intermediate between diploids and tetraploids in a number of characters, root yields were superior to that of tetraploids and elite diploids. Eighty percent of the triploids had root yields that were higher than that of the better parent, indicating that triploidy enhances the possibility of recovering a high frequency of high-yielding progeny (Sreekumari et al., 2000).

In triploids the root dry matter (DM) content ranged from 38.0 to 50.0% and the starch content from 31.0-46.5%. In their corresponding parents, i.e. Ambakadan and Sree Sahya, the dry matter and starch contents ranged from 31.0-38.0% and 28.0-34.0%, respectively. This shows that triploids hold great promise for the starch industry, where the emphasis is on high dry matter and recoverable starch.

### Shade tolerance in triploids

When grown under partially shaded conditions the productivity of triploids was found to be reasonable, i.e. about  $1/3^{rd}$  of the normal yield as compared to that of diploids. In Kerala, cassava is grown under varying shade regimes in the homesteads and garden lands which form a sizeable area under cultivation. Preliminary evaluation of 20 triploids grown under shade revealed the better performance of two of them (**Table 6**).

Table 6. Performance of	' some triploids an	d diploids under sha	aded and open
conditions.			

Clones —	Root yie	Percentage	
	Open	Shade	shade/open
2-14 (3x)	37.0	13.6	36.7
76-9 (3x)	38.9	13.8	35.5
Sree Sahya (2x)	30.8	7.4	24.3
Sree Visakham (2x)	29.6	6.2	20.9
M4 (2x)	24.7	2.5	10.1

#### Early maturity in triploids

The high yielding triploids, i.e. 1-1, 2-14, 2-17, 4-2 and 5-3 were found to be early maturing. Root yields did not differ significantly from the  $7^{th}$  month onwards up to the  $10^{th}$  month, indicating the early bulking of the roots of these triploids (**Table 7**).

Clones		Mean root	yield (t/ha)	
Ciones	7 <sup>th</sup> month	8 <sup>th</sup> month	9 <sup>th</sup> month	10 <sup>th</sup> month
1-1 (3x)	28.90	29.10	30.60	30.80
2-14 (3x)	30.60	31.80	32.50	34.60
2-17 (3x)	29.50	28.60	27.60	29.10
4-2 (3x)	30.80	30.10	31.80	33.30
5-3 (3x)	30.90	31.00	31.00	34.30

Table 7. Evaluation of triploids at monthly intervals for root yield.

The possibility of developing clones with early bulking, high dry matter and starch characteristics, hitherto unreported in any elite diploid clones of cassava, and the easy mode of propagation and maintenance indicate the importance of triploidy breeding as a novel, additional tool in cassava improvement.

### 3. Heterosis breeding

In cassava, the basic aspects about the nature of the vigor expressed by the hybrids over the parents, i.e. hybrid vigor or heterosis, the magnitude of such vigor and the gene action involved in the expression of various characters were not known. Cassava being highly heterozygous is not ideally suited for genetic studies. A project on heterosis was started at CTCRI, with the objective of developing reasonably homozygous inbred lines of cassava, combining them into various hybrid combinations to exploit hybrid vigor and to study the nature and magnitude of heterosis using a 6x6 diallel cross of inbred lines of cassava to study these aspects in detail.

### Heterosis in cassava: nature and magnitude

The nature and magnitude of heterosis in cassava were assessed for 15 characters. (Easwari Amma and Sheela, 1993). Though hybrids displayed substantial differences in their heterotic response, heterosis over mid-parental and better-parental values were recorded for all the characters under evaluation (**Table 8**).

	Me	Mean		Highly significant heterotic crosses (%)	
Characters	Parents	Hybrids	Mid-parent	Better parent	
Height at first branching (cm)	44.3	51.8	60.0	40.0	
Plant height (cm)	139.6	164.6	50.0	33.3	
Petiole length (cm)	17.1	20.0	70.0	50.0	
Length of middle leaflet (cm)	14.9	15.5	63.3	26.7	
Breadth of middle leaflet (cm)	3.9	4.3	73.3	46.7	
Spread of foliage (cm)	148.0	169.5	10.0	36.7	
Root yield (kg/plant)	0.9	1.6	96.7	93.3	
Number of roots	5.4	7.3	93.3	86.7	
Length of roots (cm)	20.1	25.8	96.7	70.0	
Girth of roots (cm)	13.0	13.8	66.7	50.0	
Mean weight of roots (g)	171.1	215.3	-	-	
Total biomass (kg/plant)	2.4	3.6	100.0	83.3	
Harvest index (%)	37.5	44.7	63.3	36.1	
Dry matter content (%)	30.2	38.3	100.0	100.0	
Cyanogen content (µg/g)	139.7	120.8	70.0	93.9	

### Table 8. Nature and magnitude of heterosis in a diallel cross of inbred lines of cassava.

Hybrid vigor for root yield over better-parental values ranged from 6.3 to 100% in 28 out of 30 cross combinations. The highest yielding hybrid, P3 x P5, excelled the midparental and better-parental values by 136.0 and 79.0%, respectively, and the released variety Sree Visakham by 27.0%. Heterosis for root yield in the material was generally associated with heterosis for yield components, i.e. number of roots, length of roots, girth of roots and mean weight of roots. All the crosses registered highly significant positive heterosis for total biomass and dry matter content over mid-parent. Negative heterosis of considerable magnitude was obtained for cyanogen content of roots. The high magnitude of desirable heterosis obtained for various traits indicate the possibility of identifying superior hybrids for yield, yield components and quality characters by combining advanced generations of inbred lines of cassava.

## 4. Top-crossing in cassava and release of 'Sree Rekha' and 'Sree Prabha'

In cassava, inbreeding has been found to be very effective in seperating the population into widely divergent but reasonably uniform groups. Crossing of such early generation inbred lines with the promising released variety Sree Visakham resulted in the production of superior top-cross hybrids (Easwari Amma *et al.*, 1993). A comparative evaluation of the performance of top-cross with that of the parents ( $S_0$ ) inbred lines, OP population and diallel cross hybrids revealed top-crossing as an efficient method to improve root yield, plant type and important quality traits in cassava (Easwari Amma *et al.*, 2000).

Twenty top-cross hybrids selected from a population of 750, were evaluated in advanced yield trials for three seasons. From four elite top-crosses earlier identified in 18 multi-location on-farm trials, two were finally selected (TCH-1 and TCH-2). These two superior top-cross hybrids had a mean root yield of 48.1 t/ha and 42.3 t/ha, respectively, and a yield potential as high as 80 t/ha. TCH-1 and TCH-2, which combine high yield, good culinary qualities and good plant type, were released by the Kerala State Variety Release Committee under the names 'Sree Rekha' and 'Sree Prabha' in December 2000 for general cultivation in Kerala. Sree Rekha has 37.7% dry matter, 28.2% starch and 49.9  $\mu$ g/g cyanogens content. The root flesh color of Sree Prabha is yellow due to the high content of carotene; and it has 36.6% dry matter, 26.8% starch and 50.8  $\mu$ g/g cyanogens. The development and release of the top-cross hybrids 'Sree Rekha' and 'Sree Prabha' in December 2000 for indicate that, in addition to being a viable method of testing combining ability of inbred lines, top-crossing could be utilized as a successful method of crop improvement of cassava.

# 5. Recombination breeding

Seedling progeny of cassava produces a lot of recombinants due to the heterozygous nature of the crop. Evaluation of the progenies of selected crosses and the half-sib open pollinated progenies for yield, dry matter, early bulking and culinary quality of roots is being carried out as a continuous program. Early bulking lines, i.e. CI-848 and H-28, produced yields of 41-45 t/ha at 7 months, as well as H-740, H-152 and H-282, producing 38-49 t/ha at 11 months, are undergoing evaluations in farmers' plots. Another set of 24 selections are in preliminary evaluation trials. The selections had 30-46% root dry matter contents.

## 6. Gene pool development for enhancement of nutritional qualities of roots

Increases in the  $\beta$ -carotene contents of roots could be achieved through poly-cross methods and collecting seeds from randomly-mated parents showing low levels of carotene (650 to 670 I U) grown in an isolated block. The carotene content could be enhanced to 1.38 mg/100 g (2200 I U) (Jos *et al.*, 1990). Thirty four lines isolated from later seedling generations are being maintained for utilizing this character directly or in future varietal improvement programs.

### III. True Cassava Seed

True cassava seed (TCS) technology was developed to enhance the rapid spread of crops to far-away non-traditional areas and for areas like Salem and Peddapuram for industrial use. It is mainly for overcoming the problem of lack of planting material, increasing the multiplication rate several times, keeping cassava mosaic disease (CMD) under check, longer viability, easy storage and transport of "seed".

Preliminary screening was done for isolating male-sterile lines with regular flowering and seed set. Ambakadan, an indigenous cultivar, was found to be a male sterile clone having good yield and culinary quality along with profuse flowering and seed set throughout the year. The open pollinated (OP) seeds from Ambakadan were used to study germination, seedling and first clone ( $C_1$ ) performance. Germination and seedling vigor were found to be high in seeds soaked for one day in 1% KNO<sub>3</sub> or 300 ppm GA<sub>3</sub> (**Figure 1**). Due to this treatment the time of transplanting of tap root-removed cassava seedlings, can be reduced from 45 to 30 days after seeding.



Figure 1. Germination of true cassava seeds at 17 days after sowing.

The OP seedling progenies had high root yields of 20-25 t/ha while the first clonal stage had yields of 30-35 t/ha. The performance of first clones ( $C_1$ ) was significantly superior to the seedlings. The dry matter and starch contents of seedlings were comparable to those of the clones (**Figure 2**). The cyanoglucoside content and culinary quality of seedlings and the first clones were both acceptable.

Crosses made between the male-sterile line Ambakadan and selected male parents resulted in progeny with nearly uniform root yields and starch contents, comparable with those of the popular varieties H-165 and H-226 when field trials were conducted in cassava areas of Salem and Peddapuram districts of Tamil Nadu. First clones of Ambakadan hybrid generation were found to be suitable for the starch industry.



Figure 2. Dry matter content in parents and seedlings of two clones at six and eight months after planting.

## IV. All-India Coordinated Research Project on Tuber Crops (AICRP)

The All-India Coordinated Research Project on Tuber Crops has been instrumental in the collection and conservation of cassava germplasm at its various centers in India. Five hundred and fortyfour accessions of cassava are being maintained at 12 different AICRP centers. Coimbatore Center maintains the highest number of 316 accessions of cassava (**Table 9**).

Dholi	22	Faizabad	12	
Rajendra Nagar	46	Ranchi	14	
Coimbatore	316	Jagadalpur	59	
Jorhat	26	Shillong	7	
Dapoli	22	Navasari	5	
Kalyani	9	Port Blair	6	
-		Total	544	

 Table 9. Cassava germplasm maintained at different AICRP (All India Coordinated Research Programme) Centers.

Cassava varieties developed at CTCRI and at State Agricultural Universities are being tested in AICRP Centers and based on their performance they may be released in different regions or at the country level. A total of eleven cassava varieties have been released through the AICRP on Tuber Crops (**Table 10**).

Name of Variety	Year of release	Average yield (t/ha)	Duration (months)
$H-226^{1}$	1971	30-35	10
$H-165^{1}$	1971	33-38	8-9
H-97	1971	25-35	10
Sree Sahya	1977	35-40	10-11
Sree Visakham	1977	35-38	10
Sree Harsha <sup>2)</sup>	1996	35-40	10
Sree Prakash <sup>3)</sup>	1987	30-35	7
Sree Jaya <sup>3)</sup>	1998	26-30	6-7
Sree Vijaya <sup>3)</sup>	1998	25-28	6-7
Sree Rekha	2000	45-48	10
Sree Prabha	2000	40-45	10

Table 10. List of cassava varieties released by CTCRI

<sup>1)</sup>Widely cultivated varieties in industrial areas

<sup>2)</sup> Triploid high starch variety

<sup>3)</sup>Early maturing variety

## **FUTURE PROSPECTS**

In India, cassava is gaining importance as an industrial crop, from its previous status as a subsistence food crop. The private sector has realized the crop's potential as a starch resource over and above the existing cereals crops, due to the lower production cost and better yield stability. The recent increase in the area of cassava cultivation in Tamil Nadu and Andhra Pradhesh, as well as the involvement of the banking sector in promoting the crop in these states (Project UPTECH by State Bank of India) are all the direct results of this realization. This has led to the high demand for quality planting materials, especially of disease resistant, early-maturing, high-yielding, high-starch varieties. Kerala, the crop still holds its position as a human dietary ingredient in the native situation, as well as an export item (fresh roots) for over-seas Indian nationals. The culinary quality of roots is an important selection criterion in this market. Hence, efforts to develop good eating varieties that are equal or excel the popular varieties like M4 still continues, though this has been achieved to a great extent with the release of varieties like Sree Jaya, Sree Vijava and Sree Rekha. In India, cassava has been identified as an important crop in the poverty alleviation program by the Department of Biotechnology (DBT) after realizing its ability to give normal or near-normal yields under marginal conditions. A network research program involving CTCRI has been initiated by the National Centre for Plant Genome Research in New Delhi, for improving the nutritional quality of roots using transgenic technology with DBT funds. Cassava has thus gained importance in the industrial, consumer and social sectors.

The future genetic improvement program is directed to meet the market demands by increasing yield potential, developing disease (ICMD) resistant varieties with highstarch and better nutritional and culinary qualities. The True Seed Program is expected to satisfy the demand for planting materials, especially for extending the crop to remote areas. Developing varieties with reduced post-harvest spoilage, varieties with enhanced starch quality (modified amylose to amylopectin ratio) for industrial use are the future priorities. Collaborative efforts with CIAT-Colombia, EMBRAPA-Brazil and IITA-Nigeria in the past have been helpful in widening the genetic base of the crop. Future interactions with these institutions are expected to strengthen the germplasm conservation, especially in developments of IVAG and IVBG in cassava. Collective efforts to utilize various biotechnological tools in genetic improvement of the crop will be of great help in meeting the market demands for its sustainable production and utilization by industry and the common man of this country.

#### REFERENCES

- Abraham, K., S.G. Nair and S.K. Naskar. 2001. Cassava breeding and varietal dissemination in India – Major achievements during the past 25-30 years. *In*: R.H. Howeler and S.L. Tan (Eds.). Cassava's Potential in the 21<sup>st</sup> Century: Present Situation and Future Research and Development Needs. Proc. 6<sup>th</sup> Regional Workshop, held in Ho Chi Minh city, Vietnam. Feb. 21-25, 2000. pp. 174-184.
- Easwari Amma, C.S. and M.N. Sheela. 1993. Heterosis in cassava: nature and magnitude. *In:* Int. Symp. on Tropical Tuber Crops "Problems, Prospects and Future Strategies", held at Trivandrum, Kerala, India. Nov 6-9, 1993. pp. 88-94.
- Easwari Amma, C.S., G. Padmaja and P.K. Thankamma Pillai. 1993. Performances of top cross selections of cassava. J. Root Crops. 21(1) 24-29.
- Jos, J.S., M.L. Magoon and S.G. Nair. 1970. A cytological and morphological analysis of triploid cassava. Genet. Iberica 22:27-39.
- Jos, J.S., S.G. Nair, S.N. Moorthy and R.B. Nair. 1990. Carotene enhancement in cassava. J. Root Crops: ISRC Symposium Special Edition. pp. 5011.
- Magoon, M.L., J.S. Jos, K.N. Vasudevan and S.G. Nair. 1969. Cytomorphological studies on induced polyploids in cassava. Genet. Iberica 21:27-47.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
- Sreekumari, M.T., J.S. Jos and S.G. Nair. 1999. 'Sree Harsha' a superior triploid hybrid in cassava. Euphytica 106: 1-6.
- Sreekumar, M.T., K. Abraham and S.G. Nair. 2000. Triploidy breeding in cassava. Technical Bulletin Series 30. CTCRI, Trivandrum, Kerala, India. 4 p.
- Unnikrishnan, M. and M.N. Sheela. 2000. Studies on media, explants and incubation conditions for *in vitro* conservation of cassava germplasm. *In:* J. Luiz, G.B. Carvalho, A.M. Thro and A.D. Vilarinhos (Eds.). Cassava Biotechnology Network, Fourth Intern. Scientific Meeting of CBN, held in Brazil. pp. 425-430.
- Unnikrishnan, M., M.N. Sheela, V. Gayathri and J.R. Nair. 2001. Genotype response variation to micropropagation and effect of retardants on *in vitro* growth in cassava. CBN Fifth International Scientific Meeting of the Cassava Biotechnology Network, held in St. Louis, Missouri, USA. Nov 4-9, 2001. S7-33.
- Unnikrishnan, M., S.G. Nair, C.R. Mohankumar and M. Anantharaman. 2000. Early maturing cassava varieties. *In:* ISOTUC III International Symposium on Tropical Root and Tuber Crops, held in Thiruvananthapuram, Kerala, India. Jan 19-22, 2000. Abstract of papers. GR. 10-13.