CASSAVA GERMPLASM CONSERVATION AND CROP IMPROVEMENT IN THAILAND

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ABSTRACT

Creation of broad genetic variability in the cassava population through collection and introduction is essential for the successful recombination of certain desirable traits, in order to produce superior cassava cultivars for release to the farmers. Cassava germplasm in Thailand has been introduced mainly from Latin America via CIAT since 1975. The Thai cassava germplasm collection also includes earlier introductions from the Virgin Islands and Indonesia. Formerly, the cassava germplasm collection was maintained only in the field. Some cultivars were lost due to stress environments, i.e. drought or excessive rain, as well as by insect and disease attack. An alternative conservation method to solve these problems is to maintain the collection also in the laboratory as *in-vitro* cultures. Rayong Field Crops Research Center (RFCRC) established a tissue culture laboratory for this purpose in 1993. Recently, CIAT collaborated with the Department of Agriculture (DOA) of Thailand to send a duplicate of the CIAT cassava core collection, containing about 630 accessions, to Thailand. The purpose of this collaboration was to keep those genetic resources *in-vitro* at another safe site away from CIAT, as well as to evaluate in the future these genetic resources for traits that may be useful in future breeding efforts. At the present, RFCRC has received 601 accessions in the form of *in-vitro* plantlets. At least five plants of each accession are kept *in-vitro* for conservation. These genetic resources are also multiplied in order to evaluate them in the field for specific traits under Thai conditions.

Most achievements of the Thai cassava breeding program were reported in the sixth Regional Cassava Workshop held in Vietnam in February, 2000. During the past 20 years seven cultivars have been officially released, five from DOA and two from Kasetsart University. These new cultivars are characterized by high yield capacity, high harvest index, high root starch content and early harvestability; they are all suitable for planting in the northeastern region of Thailand. From 1995 to 2000 some hybrids from crosses made in 1992 and 1993 were identified that were slightly superior to these cultivars in terms of root yield capacity and starch content. One of the 1992 hybrids, identified as CMR35-48-196, performed well under late rainy season planting conditions. The other three lines, i.e. CMR35-21-199, CMR35-22-196 and CMR35-64-1 yield fairly high and have high starch contents. They are being evaluated for ethanol yield at different ages. CMR36-55-166 and CMR36-30-329 are two of the 1993 hybrids which have high potential in various locations. All of these lines are now being further tested in the farmers' field for possible future release.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is grown in over 90 countries and provides a livelihood for half a billion people in the developing world. It is one of the most important calorie-producing crops in the tropics. It is efficient in carbohydrate production, adapted to a wide range of environments, and tolerant to drought and acidic soils (Jones, 1959; Rogers and Appan, 1970; Kawano, 1978; Cock, 1982). Thailand was the first country to exploit the industrial prospects of cassava on a large scale. Since the 1970s it has exported enormous quantities of dried cassava chips and pellets to the countries of the European Union, which

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use these as a carbohydrate source in animal feed. More recently it has been used increasingly for animal feed and industrial starch, and is becoming an important source of cash income for a large number of small farmers (Lynam, 1986; Bottema and Henry,1992).

The cassava breeding programs of the Department of Agriculture (DOA) and Kasetsart University (KU) were started in the early 1970s and 1980s, respectively. Due to a shortage of breeding materials, the researchers started by collecting local cultivars and conducted yield trials to select the best among those materials. One local variety appeared very promising and was released to farmers in 1975 under the name "Rayong 1". Since then, it has become a check variety in all yield trials and at the same time became on very important parent of many F_1 hybrids that have been evaluated and selected in Thailand as well as in many other cassava producing countries in Asia.

Hybridization started in 1975 with a very narrow genetic base until the early 1980s when the collaboration with CIAT was established. CIAT supported the Thai cassava breeding program by supplying thousands of F_1 seeds annually, and sometimes transferring elite clones from other countries. The broadening of cassava's genetic base through germplasm exchange became the major factor for the success of varietal improvement in Thailand. Most of the improved varieties that have been released contain some exotic genes from germplasm supplied by CIAT, but in different proportions. Rayong 3 and Rayong 2 are one hundred percent exotic, since they were selected directly from CIAT's F_1 hybrid seeds under Thailand conditions. Rayong 60, Rayong 90 and Rayong 5 are fifty percent exotic, since they were products of crosses between Thai and introduced parental materials. Kasetsart 50 and Rayong 72 have some exotic genes from one of their parents, i.e. Rayong 90 and Rayong 5, respectively (**Table 1**).

Table 1. Background and outstanding characters of eight released Thai varieties.

Variety	Year released	Parents	Background and outstanding characters
Rayong 1	1975	Unknown	Selected from local cultivar. Well adapted to low inputs. Excellent agronomic traits. Moderately resistant to major pests and diseases.
Rayong 3	1983	(F) MMex 55 (M) MVen 307	Selected from CIAT's F_1 seeds. High dry matter content. Rather low HCN.
Rayong 2	1984	(F) MCol 113 (M) MCol 22	Selected from CIAT's F_1 seeds. High carotene and vitamin A; low HCN. Recommended for human consumption.
Rayong 60	1987	(F) MCol 1684 (M) Rayong 1	Selection from DOA F_1 seeds. High fresh root yield. Early harvestability.
Rayong 90	1991	(F) CMC 76 (M) V 43	Selection from DOA F_1 seeds. High dry matter content. Relatively high yield.
Rayong 5	1994	(F) 27-77-10 (M) Rayong 3	Selection from DOA F_1 seeds. High yield. High dry matter content.
Rayong 72	1999	(F) Rayong 1 (M) Rayong 5	Selection from DOA F_1 seeds. Very high fresh root yield. Low HCN.
Kasetsart 50	1992	(F) Rayong 1 (M) Rayong 90	Selection from KU F_1 seeds. High yield. High dry matter content. Wide adaptability.

CASSAVA GENETIC RESOURCES CONSERVATION AND CHARACTERIZATION

The CIAT cassava germplasm collection has over of 6,000 clones, i.e. landraces, mainly from Latin America, but also about 300 from Asia, as well as some elite clones selected by CIAT and the International Institute of Tropical Agriculture (IITA) in Nigeria. A subset of these accessions, called "the core collection", has been assembled at CIAT to represent the genetic diversity of the complete germplasm collection in a more manageable size (Hershey *et al*., 1994). Evaluation of a core group, which is a fraction of the total collection (5 to 10 percent) but representive of the total genetic diversity (Brown, 1989) can provide overall indicators of genetic diversity at a fraction of the cost. Such a cassava core collection, consisting of 630 accessions from the original germplasm bank, was recently established at CIAT and is now being evaluated for a range of traits, including cyanogenic potential and starch content, photosynthetic rate and nutrient use efficiency (Hershey *et al*., 1994).

The genetic resources held in trust in the genebanks of CIAT in Cali, Colombia, have been assembled with the participation of the countries providing the material, on the understanding that it will be made available to the research community world-wide. For this reason, and because the use of plant genetic resources is central to any crop improvement program, CIAT have followed a policy of allowing unrestricted access to the plant genetic resources in their collections. The materials in the CIAT cassava collection is partially duplicated in various national, regional and international research institutes

The core collection can be evaluated across different ecosystems in order to determine the genotype by environmental effects for important traits. Thailand is a suitable place for the safe duplication of this core collection. It is also an opportunity for Asian cassava breeders to use a wider genetic diversity in their breeding programs. (Wongtiem *et al*., 2002).

The main objectives for transferring the core cassava collection to Thailand are as follows:

1. To establish another safe site and maintain a duplicate of these cassava genetic resources
2. To further evaluate for desirable traits in Thailand
3. To enhance the safe exchange of cassava germplasm between Asian countries

Materials and Methods

In 2001, CIAT and the Department of Agriculture of Thailand agreed to establish a duplicate of the CIAT cassava core collection, presently held in trust for FAO at CIAT headquarters in Colombia, for safe keeping and utilization in Thailand. Thus, from 2002 , the Rayong Field Crops Research Center (RFCRC) has received about 20 boxes of test tubes with *in vitro* cassava plants, with two tubes (each with one plant) of each clone. RFCRC has now received almost the total core collection of 601 accessions, which comprises most of the genetic variability of the crop.

In Vitro Conservation

After arrival in Thailand, these plants have been subcultured and all accessions are being preserved at RFCRC. For the *in vitro* collection, the tissue culture plants are maintained under slow-growth conditions i.e. at 23 ± 1ºC constant temperature, with 1000-3000 lux illumination for 16 hours a day provided by cool white fluorescent lamps, and at 70-90% relative humidity. Ten plants of each clone are routinely maintained containing a modified Morishige and Skoog medium developed at CIAT. In addition, some of the plants are transferred to the greenhouse and then to the field for further evaluation.

Details about all the steps in this process are as follows:

1. After arrival, let plants recover in the original test tubes with adequate light for 2-3 weeks.
2. Subculture each of the two plants of each accession to 4-5 test tubes.
3. Keep in the laboratory for 2-3 months and subculture again for further multiplication.
4. Ten plants of each clone are routinely maintained for conservation in the *in vitro* germplasm bank.
5. The rest of the plants are subcultured again until a total of 50 plants are obtained.
6. These 50 plants are kept at room temperature for 1 week and then transferred to the partially shaded greenhouse.
7. Carefully transfer plants from the test tubes to black plastic bags filled with ground coconut husk to maintain good moisture in the rooting zone.
8. Keep plants under plastic cover for 4-5 days and open the plastic by about 20% each 4-5 days.
9. After the plastic has been completely removed, keep in shaded greenhouse for 2 weeks.
10. Transplant to bigger plastic bags containing soil+coconut husk+fertilizer; keep in greenhouse for one month.
11. Transfer plants to a shaded spot outside and keep for another one month.
12. Tranfer plants to the field and water once a day during the dry season.

Field Genebank

The plants are grown initially in the field in single rows at 1 m between plants and between rows for evaluation under normal growing conditions, while root and leaf samples are taken for conservation and characterization. These evaluations also serve to multiply the planting material needed for future evaluations in larger plots.

Germplasm Characterization and Evaluation

In order for the germplasm collections at Rayong Fields Crops Research Center to be a truly useful genetic resource, researchers and other users need reliable data about key traits of particular materials. To provide such information, cassava researchers at the Center routinely characterize and evaluate germplasm from the cassava collection in the field. A total of 266 accessions in the original germplasm collection (**Table 2**) have been evaluated in the field for yield and some other traits, such as morphological and physiological characters, starch content, as well as DNA finger printing using molecular markers. The same 266 accessions have also been evaluated for disease (mainly CBB) and pest (red spider mite, mealy bug and white fly) resistance, and root quality characteristics such as starch, protein, fiber, mineral nutrients, cyanide and amylose contents, as well as the physico-chemical characteristics of the starch using a Brabender visco-amylograph. In the future, the same traits will also be evaluated for the newly received accessions of the core collection and the data will be shared with CIAT for the cassava germplasm database.

Table 2. Cassava germplasm collection at Rayong Field Crops Research Center (RFCRC) in 2000.

Source	No of accessions	Scientific name
Thai local varieties.	10	*Manihot esculenta* Crantz
Other *Manihot* species	1	*M. glaziovii*
Imported varieties:		
-from Virgin Island	17	*M. esculenta*
-from Indonesia	5	*M. esculenta*
-from CIAT	48	*M. esculenta*
Selected breeding lines from RFCRC	185	*M. esculenta*

RESULTS

Thailand has very limited cassava genetic diversity as indicated by a very small number of local varieties (**Table 2**). In order to improve those local varieties and to widen the genetic base, the country has introduced many varieties from abroad, mainly from Latin America through CIAT since 1975, but also earlier introductions from the Virgin Islands and from Indonesia (**Table 2**). In addition, CIAT has provided every year since the early 1980s thousands of sexual seeds produced by the Cassava Breeding Program at CIAT in Colombia. This has greatly increased the genetic diversity of cassava in the country. After passing through many stages of selection some of the selected breeding lines were released as improved varieties, or they were further crossed with local material to increase their adaptation to local conditions (**Table 1**). Finally, starting in 2002 , Rayong Field Crops Research Center received *in vitro* plants with two tissue culture tubes of each accession of CIAT's core cassava collection. It has now received nearly the total core collection of 601 accessions (**Table 3**), which comprises most of the genetic variability of the crop.

Results of some of the characterizations for a few well-known varieties in the original germplasm collection are shown in **Table 4**.

PROGRESS IN CASSAVA VARIETAL IMPROVEMENT

After having achieved a large increase in yield and yield components in Kasetsart 50, Rayong 5 and Rayong 72, as compared to Rayong 1, it seems to be more difficult for cassava breeders to produce still better varieties. Thousands of F_1 hybrids produced during the 1990s were unable to compete with these earlier varieties and were discarded every year. Only a few breeding lines resulting from F_1 hybrids crossed in 1992 and 1993 can outperform these three varieties in at least some useful traits:

Table 3. Number and origin of the accessions in the CIAT cassava core collection received at Rayong FCRC in 2001 and 2002.

Varietal prefix	Origin	No. of Accessions	Varietal prefix	Origin	No. of Accessions
MArg	Argentina	8	MNga	Nigeria	3
MBol	Bolivia	3	MPan	Panama	9
MBra	Brazil	100	MPar	Paraquay	39
MChn	China	2	MPer	Peru	71
MCol	Colombia	139	MPhi	Philippines	2
MCr	CostaRica	20	MPtr	Puerto Rico	4
MCub	Cuba	18	MTai	Thailand	4
MDom	Dominican Rep.	3	MVen	Venezuala	52
MEcu	Ecuador	28	MUSA	USA	4
MFji	Fiji	2	HMC	ICA variety	1
MGua	Guayana	16	CG	CIAT breeding lines	12
MInd	Indonesia	7	CM	CIAT breeding lines	18
MMal	Malaysia	15	SG	CIAT breeding lines	2
MMex	Mexico	19			

Table 4. Root characteristics of important cassava varieties from Thailand, Indonesia, Philippines and Colombia present in the germplasm bank at Rayong FCRC in Thailand.

Variety/line	Paren-chyma color	Moisture content (%)[1)]	Starch content (%)[1)]	HCN content (%)[1)]	Fiber content (%)[2)]	Protein content (%)[2)]	Amylose content (%)[3)]
Hanatee	white	59.5	19.8	53	1.72	1.99	26.3
Rayong 1	while	60.7	22.8	56	1.86	1.15	22.3
Rayong 2	yellow	68.7	14.3	21	1.60	1.75	29.0
Rayong 3	white	54.0	26.5	32	1.92	1.85	26.6
Rayong 5	white	57.6	22.7	55	1.45	1.43	20.5
Rayong 60	creamy	63.0	19.9	175	1.57	1.78	24.8
Rayong 72	white	63.8	20.0	21	1.75	1.33	26.2
Rayong 90	white	60.0	24.1	85	1.47	2.06	25.6
KU 50	white	58.4	23.3	81	1.95	1.47	27.7
Sri Racha 1	creamy	61.0	23.7	64	1.86	1.43	25.3
Adira 4	white	63.4	21.1	62	2.26	1.05	25.2
Golden Yellow	yellow	68.2	12.5	26	2.31	-	25.2
MBra 12	white	69.2	16.8	43	2.07	1.63	28.1
MCol 22	yellow	63.2	20.5	30	1.58	1.73	28.9
MCol 1684	yellow	61.6	20.9	89	1.79	0.94	24.3
MMex 59	white	63.1	17.5	39	2.66	1.09	24.0
CM 523-7	white	57.4	26.3	25	2.23	0.84	26.4

[1)] on fresh weight basis
[2)] on dry weight basis
[3)]as percent of dry starch
Source: *Jinnajar Hansetasuk et al., 2006.*

1. CMR35-48-196

This is a very promising 1992 hybrid from the cross of a high-yield female parent, CMR30-71-25, and a high-starch male parent, OMR29-20-118. It was selected under late rainy season planting conditions. The average yield in 16 locations was higher than those of Kasetsart 50, Rayong 5 and Rayong 72 (**Table 5**). This clone will be tested in farmers' fields during the next 2-3 years, and it is expected that this line will be released as a variety, specifically for late rainy season planting.

Table 5. Average yields of CMR35-48-196, Kasetsart 50, Rayong 5 and Rayong 72 in seven Regional Trials and nine On-farm Trials during October 2000/01 and October 2001/02.

Variety/line	Fresh root yield (t/ha)	Starch content (%)	Starch yield (t/ha)	Relative to Rayong 5 (%)
CMR35-48-196	34.68	27.6	9.80	124
Kasetsart 50	32.30	27.5	8.88	112
Rayong 5	31.23	25.1	7.92	100
Rayong 72	31.18	25.0	7.97	101

Source: *Rayong Field Crops Research Center, Annual Report 2002.*
Rayong Field Crops Research Center, Annual Report 2003.

2. CMR35-21-199, CMR35-22-196 and CMR35-64-1

Three promising clones were selected from 1992 hybrids due to their exceptionally high root starch contents (**Table 6**). They are currently being tested in farmers' fields under early rainy season planting conditions. The roots will also be evaluated for ethanol yield at the age of 8, 12 and 18 months after planting. Testing for ethanol yield of these three breeding lines as well as four check varieties, Kasetsart 50, Rayong 5, Rayong 72 and Rayong 90, will be conducted at the Thailand Institute of Science and Technological Research in Bangkok. The test will initially be carried out at the laboratory level using small amounts of dried chips as raw material. The laboratory test will later be repeated at a pilot project level with 2-3 highly promising varieties or lines using approximately 10 tonnes of fresh roots as raw material. This project is being conducted under the cooperation of Rayong Field Crops Research Center and the Thailand Institute of Science and Technological Research, and will need another two years to finish.

Table 6. Average yields of CMR35-21-199, CMR35-22-196, CMR35-64-1, Kasetsart 50, Rayong 5 and Rayong 72 in 38 trials conducted during 1995-2001.

Variety/line	Parents	Fresh root yield (t/ha)	Starch content (%)	Starch yield (t/ha)	Relative to Rayong 5 (%)
CMR35-21-199	(F) R5 x (M) KU50	32.0	23.5	7.75	105
CMR35-22-196	(F) R5 x (M) OMR29-20-118	29.9	25.8	7.85	107
CMR35-64-1	(F) CMR31-19-23 x (M) OMR29-20-118	30.9	24.4	7.76	105
Kasetsart 50	(F) R1 x (M) R90	29.7	22.9	6.96	95
Rayong 5	(F) 27-77-10 x (M) R3	30.1	21.9	7.35	100
Rayong 72	(F) R1 x (M) R5	34.2	21.1	6.74	92

Source: *Rayong Field Crops Research Center, Annual Report 2001.*

3. CMR36-30-329 and CMR36-55-166

Two promising clones selected from 1993 hybrids were tested in 26 trials during 1996-2001. CMR36-30-329 has a high starch content, but the adaptability was not as good as that of the released varieties resulting in a lower average yield over locations and years. CMR36-55-166 is somehow opposite having a relatively high yield but lower starch content (**Table 7**). These two lines will be tested again together with the 1992 hybrids during the next two years to determine their true potential.

Table 7. Average yields of CMR36-30-329, CMR36-55-166, Kasetsart 50 and Rayong 5 in 26 trials conducted during 1996-2001.

Variety/line	Parents	Fresh root yield (t/ha)	Starch content (%)	Starch yield (t/ha)	Relative to Rayong 5 (%)
CMR36-30-329	(F) R5 x (M) KU50	26.5	26.1	6.91	101
CMR36-55-166	(F) CMR30-71-25 x (M) R5	32.2	23.4	7.53	110
Kasetsart 50	(F) R1 x (M) R90	28.0	24.7	6.92	101
Rayong 5	(F) 27-77-10 x (M) R3	28.5	24.0	6.84	100

Source: *Rayong Field Crops Research Center, Annual Report 2001.*

FUTURE DIRECTION

In order to help cassava farmers in the country, cassava researchers from different institutes need to collaborate more closely, helping each other to do research based on their own strengths in terms of experience, resources and facilities. For example, the Department of Agriculture might concentrate on the identification and evaluation for agronomic traits of cassava germplasm, making crosses to improve the yield potential and root quality characteristics of new varieties to be released to the farmers. Researchers at DOA should work closely with the Department of Land Development and the Department of Agricultural Extention to develop new technologies suitable for different farmers in different agro-ecological zones. Kasetsart University and other educational institutes, which have excellent laboratories and staff, might concentrate on the identification and evaluation of the chemical composition and qualities of each part of the crop, and develop new value-added products and new biotechnology tools. The Thai Tapioca Development Institute (TTDI) and the Thai Tapioca Trade Association (TTTA) can contribute much to the expanding of domestic and international markets, initiate contract farming systems and supply farmers with new varieties and improved production technologies, to strengthen the ecological and economic sustainability of cassava production in Thailand.

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