CIAT cassava *in vitro* collection cleaned against "seedborne" diseases of quarantine importance

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Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial crop plant that thrives in the tropics. Its roots stores starch, and they are considered as a source of energy in human food and raw material in animal foodstuff processing. It is vegetatively propagated using stem cuttings and as such can disseminate several systemic pathogens as virus, viroids, bacteria, fungi (Lozano et al, 1983; Lozano and Jayasingue,U., 1982).

Availability of germplasm is a basic requisite for the improvement of this crop across the tropics. To that end we need to move germplasm safely and conserve it according with FAO–IPGRI Genebanks Standards. In order to ensure that availability, the Genetic Resources Unit undertook in 1996 the checking of the entire *in vitro* collection for the absence of "seedborne" diseases of quarantine importance. The *in vitro* collection currently has 6,382 clones, of which 5,728 have been designated to the Food and Agriculture of the United Nations .

By using the *in vitro* technology, namely micropropagation, cassava germplasm is generally free of insects, fungi and bacteria. In addition, the techniques of thermotherapy followed by meristem tip culture have been used to eliminate viruses such as cassava common mosaic virus (CCMV), cassava X virus (CsXV), and the cassava frogskin disease agent (CFDSA). Methodologies of accurate diagnosis such as DAS ELISA and grafting have been used to monitor the effectiveness of those treatments (Roca et al. 1991).

Over the last five years a careful cleaning and indexing activity has been carried out in the Genetic Resources Unit, using the CIAT Cassava *in vitro* collection established before 1996. Each clone went through *in vitro* thermotherapy and meristem tip culture as a safe procedure to ensure the erradication of systemic pathogens. We then monitored each clone using accurate diagnostic methods. Results of the cleaning and indexing activities are hereafter presented.

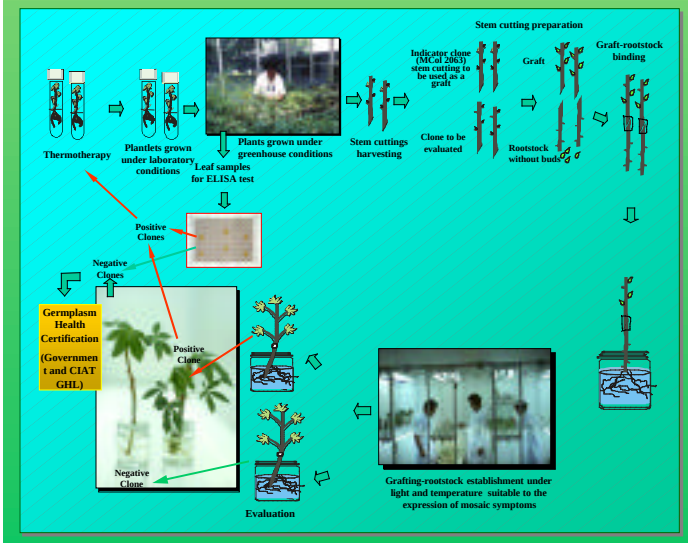
Materials and Methods

Shoot tips (0.5 cm) were cultured for 12 days in the propagation medium 4E (Roca et al. 1984) at a temperature of 35°C; 1,000 lux and 12-h photoperiod. They were after transferred under the same conditions once three cycles of thermotherapy were completed. After the establishment of explants each clone was micro propagated using stem segments, containing at least one node. Three to five plantlets were obtained per culture per month per meristem tip (CIAT, 1988). One of them was grown in order to transplant it under greenhouse conditions. The other ones were stored at 24^{0}C, at 1000 Lux , with a 12-h photoperiod in the URG tissue culture laboratory.

Plantlets under greenhouse conditions were grown at least for 2-3 months to obtain leaf tissue and stakes to do the indexing according established procedures (**Figure 1**). Two diagnosis techniques were used: DAS-ELISA for cassava common mosaic virus (CCMV) and cassava X virus (CsXV) using leaf tissues, and grafting for the causal agent of Frogskin disease (FDA). DAS ELISA test was done using young leaves from 2 weeks old plants. For the grafting we use the landrace Secundina or M Col 2063, a highly susceptible clone previously certified as viruses and virus-like agents free. Evaluations were made 20-45 days after the establishment of the grafting under illumination, photoperiod and temperature suitable to the expression of symptoms (2000-3000 lux, 12 h light/12h dark, 20-27 ^{0}C), looking for the reaction on the indicator clone's leaves.

When a test showed that a clone was affected by viruses (ELISA and/or Grafting Positive test) the Germplasm Health Laboratory sent the information to the Tissue Culture Laboratory for resuming the *in vitro* thermotherapy and re-do the established procedures (**Figure 1**).

Negative tested clones were health certified, consequently they were considered to available for distribution. We entered the pertinent information in a database.



Results and discussion

The cleaning and indexing activities carried out in the Genetic Resources Unit during the last five years have achieved the full health certification to 3589 clones, which are available for distribution (Table 1). This achievement has special significance for the safe movement of germplasm as to reduce risks of pathogen dissemination not only between continents or countries but even within national borders. The other 2,793 clones have been indexed only against some viruses.

Collections with a larger proportion of clones ready for distribution at present are those from Colombia, Brazil, Peru, Venezuela, Costa Rica, and Paraguay. Other collections have lower numbers of clones *in vitro* but in general those have been certified. Hybrid materials have certified clones available for distribution, especially the G Hybrids (CM2177-2 x Nigeria 2) used in research for cassava gene mapping (Table 1).

Analyzing the clones indexed against viruses or virus like-diseases as CCMV and CsXV (symptomless virus), one sees that a high number of them are ELISA negative. About the FSDA indexed by grafting, Table 1 shows that 3,732 clones are negative for the presence of that quarantine important disease. The apparently low number of CFSDA indexed clones could be explained by the efficiency of the diagnostic method used. Grafting is a biological technique that requires the production of suitable stem cuttings and a long time for successful uniting of rootstock and Secundina and for health evaluation.

The results obtained so far are a step ahead for the safe movement of cassava germplasm and the fulfillment of the FAO-IPGRI Genebank Standards, for current and future efforts in cassava improvement across the tropics.

Table 1. Indexing status of the Cassava Germplasm Collection in the Germplasm Genetic Resources Unit by October 2001.

Collection Source	Number of clones *in vitro*	Number of negative clones against viruses or virus-like diseases			Number of clones available for distribution
		CCMV	CsXV	FSDA	
Country's Collection					
Argentina	101	69	79	51	42
Bolivia	7	7	7	4	4
Brazil	1342	1248	1215	866	841
China	2	2	2	2	2
Colombia	2018	1878	1774	1290	1202
Costa Rica	148	145	124	123	106
Cuba	77	77	77	73	74
USA	9	9	9	7	7
Ecuador	116	110	106	90	82
Fiji	6	5	5	5	5
Guatemala	91	89	85	43	43
India	51	51	51	21	21
Malaysia	67	67	66	40	40
Mexico	102	97	94	71	67
Nigeria	19	19	19	12	12
Panama	43	38	35	32	29
Paraguay	210	199	197	105	100
Peru	406	384	372	296	282
Philippines	6	5	5	3	3
Puerto Rico	15	15	15	11	11
Dominican Republic	5	4	5	3	4
Salvador	8	4	4	5	3
Thailand	31	29	28	13	12
Venezuela	244	227	223	164	157
Hybrids					
CG	118	53	44	36	40
CM	448	161	152	133	180
HMC	4	3	4	2	1
SG	47	45	37	35	32
SM	79	63	59	54	57
KM	81	6	4	4	3
CT	1	1	1	-	-
G Hybrids (CM2177-2XNigeria 2)	147	142	140	138	137
Wild species	333	-	-	-	-
Total	6,382	5,252	5,038	3,732	3,589

CCMV = Cassava common mosaic virus ; CSXV = Cassava X virus; CFSD = Cassava frog skin disease

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