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TRUE CASSAVA SEED: RESEARCH FOR A PRODUCTION ALTERNATIVE

Carlos A. Iglesias

Abstract

The overall objective of the proposed strategy is to develop True Cassava Seed (TCS) as an alternative commercial production system to traditional vegetative reproduction of cassava. The specific objectives are: to generate experimental results determining the potential for TCS as a viable production system; to study different alternatives to overcoming the presently recognized constraints for TCS; and to formulate an interdisciplinary research approach supporting the new cropping system.

The components of this strategy are: a) Socioeconomic studies to define potential acceptability and impact in different regions, farming systems, markets, and economic strata of farmers. b) Research on alternatives for genetic structure of cassava varieties produced via TCS. Apomixis induction, double-haploid production, and selection for TCS related traits will be studied. c) Development of TCS crop management alternatives that enhance crop establishment and production. d) Supporting research in the areas of seed production, conservation, germination, and phytosanitary management.

Highest benefits would accrue to farmers in regions where vegetative propagation of cassava is especially problematic. This includes much of Africa, where African Cassava Mosaic Virus is a major yield constraint, and is passed from one cycle to the next by planting cuttings; and the drier cassava producing regions, where stem storage is problematic due to long storage periods. High potential is also likely in more intensively managed systems, as are commonly found in Asia. Due to the interinstitutional nature of the research, this project would strengthen linkages among a wide range of institutions in both the developed and developing world. The research will also provide spin-off results applicable in improvement of cassava for traditional vegetative reproduction.

**True Cassava Seed: Research for a Production Alternative<sup>1</sup>**

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 Clair H. Hershey

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INDEX DESCRIPTORS: Cassava, True Cassava Seed, reproductive aspects, genetic improvement.

## True Cassava Seed: Research for a Production Alternative

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### INTRODUCTION

The development of a true cassava seed (TCS) technology offers promising alternatives for overcoming some of the more serious constraints of cassava production. However, to develop TCS propagation as a commercial production system will require a long term multidisciplinary research effort. The International Center for Tropical Agriculture (CIAT) in Colombia is in a strategic position to coordinate this effort (Hershey, 1988).

The idea of TCS is being proposed based on several theoretical comparative advantages with respect to conventional vegetative propagation, and some empirical research results. The benefits, in view of the many constraints resulting from vegetative propagation, could be enormous. Changing the production system from stake propagation to TCS will represent the conception of a totally different cassava crop. It will require a very wide range of research activities, and the payoff will only begin to be tangible by the end of the first decade of research. A prioritization of research needs will allow investment on those key aspects that could determine the success of the technology, leaving research in technology adjustment for later stages.

## **BACKGROUND AND JUSTIFICATION**

Cassava breeders have observed for many years that true-seed derived plants can be highly productive under good management conditions in nurseries. Comparisons between stake and seed progenies derived from the same parental clone showed comparable potential for TCS in terms of root production, advantages in relation to plant type, and disadvantages in terms of early crop development and root variability (Bolaños, 1987). The theoretical balance between advantages and disadvantages of a TCS crop provides a basis for optimism about the potential of a TCS technology (Table 1). Valuable experience from true potato seed (Malagamba and Monares, 1988; Fallais, 1991) will be analyzed in order to visualize technical problems and constraints in the diffusion of the new technology. Some of the constraints to developing a TCS technology were previously seen as impractically difficult to overcome, or requiring an excessively long time frame. However, the use of wild species as a source of genetic variability for TCS-related traits together with new areas of advances in molecular biology give promise of overcoming these obstacles.

### **A) Propagation-related Constraints in Cassava**

Stake propagation confers the advantage of large carbohydrate reserves in planting material, resulting in high initial vigor and better crop establishment under stress conditions when compared to TCS. However, there are inherent constraints in vegetative

propagation that cannot be overcome by management. As a consequence, a wide range of propagation-related problems may influence the establishment, development, and final yield of the crop.

Cassava is potentially subject to infestation or infection by many pests and pathogens. Many of these can affect quantity and quality of planting material, and may be transmitted from one vegetative cycle to the next, often with cumulative effects (CIAT, 1987). While in vitro techniques have been developed to eliminate viruses and other pathogens from cassava clones, there is often a high probability of reinfection within a few years after a clone is moved to the field (Table 2).

The ability of a cassava plant to produce good quality planting material depends to a significant degree upon the plant architecture. In general terms, later branching, more erect plant types will produce the most uniform and highest quality planting material. While this plant type is also generally considered favorable from other perspectives as well (e.g., physiological efficiency, suitability for intercropping) (Cock et al., 1979), it does place constraints on the breeder or physiologist to experiment with highly variant types which have desirable traits such as earliness or high yield potential.

Stake storage in cassava is a persistent problem, and especially so in areas with long dry or cold seasons, where keeping good quality

planting material from one cycle to the next is difficult. Loss of quality of planting material may be one of the most important intrinsic factors in limiting cassava yields worldwide.

The multiplication rate of cassava is very low compared to most seed-propagated crops. While this may be no limitation under conditions of stable planting area and good stake management practices, it can be a severe constraint when a new variety is introduced to a region, when the area is being increased, or when loss of planting material occurs from poor management or other reasons.

Preliminary studies have shown that dry matter (primarily starch) may accumulate in the planting stake at levels up to 10-15% of the total root production in cassava (Bolaños, 1987; Gijzen et al., 1990). This production is lost to commercial uses, and, at least in theory, could be directed to the roots, in a system in which a lignified stem is not used as the planting piece.

Early maturity is a major breeding objective in cassava. However, a constraint to achieving this is that, to be successful, a variety must not only produce good root yield, but also good quality and a reasonable number of planting stakes. Since well-lignified stems are required to produce high quality stakes, this places certain limits on how early a variety can be harvested, apart from root yield and quality considerations.

## **B) Potential for a True Seed Technology**

Developing a set of technologies for commercial cassava production through true seed has the potential to overcome all of the constraints listed above.

Only one cassava virus (Cassava Green Mottle Virus, a nepovirus) has been demonstrated to pass through true seed, and therefore each generation of propagation would serve as a filter for most viruses which might infect a given plantation. Since the effects of viruses often become important only as they accumulate over generations, this phenomena could be obviated. Some fungal and bacterial agents will pass through true seed, but simple and effective treatments are already developed for their eradication.

Cassava seed can be stored for one to two years under ambient conditions without decline in viability. Storage at low temperature and low humidity can prolong the viability to many years.

The constraints on plant architecture imposed by the need for lignified stems would not apply for a seed propagated crop. This flexibility would possibly allow the design of a plant with higher efficiency of photosynthate partitioning. Especially, it could open the way for selecting much earlier genotypes, without the need to adjust the growing cycle to the production of lignified stems



along with roots. A completely herbaceous plant type might even be envisioned.

Although true seed production varies widely with genotype and environmental factors, selection for good seed production could almost certainly raise by many fold the multiplication rate of the crop.

## **OBJECTIVES**

A TCS technology has not been seriously considered in the past because some of the constraints to its development were too difficult to attack with traditional breeding methods. New opportunities offered by biotechnology now make TCS a potentially viable alternative.

This project has the following objectives: a) to generate supporting information to confirm the potential for TCS as a production system; b) to formulate an interdisciplinary research approach in order to support the new cropping system; c) to study different alternatives for the bottlenecks recognized at the moment for TCS; and d) to generate, as a byproduct, basic information benefitting conventional breeding.

## **ONGOING RESEARCH AND FUTURE REQUIREMENTS**

In order to develop TCS as a viable alternative technology, four major thrusts are contemplated, in the areas of socioeconomics, crop management, breeding, and seed production and management.

### **A) Socioeconomics**

Probability of farmer and consumer acceptance of a TCS technology should constitute one of the first phase of research. From the farmer viewpoint, TCS would involve in essence the introduction of a new crop, since so many management factors would be different from a conventional vegetatively propagated cassava crop. From the viewpoint of processors and consumers, the principal variable would be possible increased variation in product uniformity. Ex ante studies would be very difficult due to the "newness" of the concept of TCS, and scientists inability to provide a technology on a trial basis until many of the genetic and management components have had further research. Nevertheless, socioeconomic studies to define probable acceptability in different regions, farming systems, markets, and economic strata of farmers, will be essential at the outset.

### **B) Crop Management**

Table 3 presents preliminary results showing root yield for TCS to be comparable to stake-derived crops under good management

conditions (Bolaños, 1987). Comparisons across similar genetic background and with stakes originated in areas with different disease and pest problems will have to be conducted.

Reduced early vigor and later branching of a TCS crop will impose particular restrictions in terms of cultural practices. Studies on plant density and distribution, weed control, fertilization, and cropping systems will be necessary for TCS to succeed. Potential major changes in plant type for TCS cultivars (e.g., more herbaceous types) will imply associated changes in cultural practices.

Due to relatively slow establishment of the crop and particular morphological traits of the seedlings, pest and disease management will deserve special research attention especially during the first 2-3 months of the crop.

Once the best technology for TCS crop establishment and management has been determined, feasibility studies must be conducted under diverse agroclimatic and socioeconomic situations in order to establish the conditions for farmers' acceptance.

### **C) Breeding**

A cassava production field consists of a homogeneous population of heterozygous plants (although some heterogeneity might be found in farmers' fields). Uniformity for root traits (color, shape,

quality, etc.) is especially important when the production is for fresh consumption. In order to have a comparative advantage, a TCS cultivar must present a good production potential and uniformity for most of the important traits, especially those related to the roots.

The current propagation system allows for the immediate fixation of a genotype. For TCS, three broad alternatives, and a range of variations within these, exist for the genetic structure of cassava varieties produced via true seed. The path(s) chosen will have major implications on the research directions in developing a true seed technology.

a) One option would be to produce open-pollinated varieties. Since cassava is outcrossing and highly heterozygous, open pollination would result in heterogeneous populations as the commercial planting material. Parents will have to be selected for relative uniformity of the most critical traits, such as root quality, for such varieties to be acceptable. Variations would more likely be acceptable in industrial markets, and less acceptable in human food markets. This alternative for genetic structure would, in theory, allow farmers to produce their own seed from one generation to the next.

This alternative will require building up closed populations by crossing a set of elite clones with complementary characteristics, and conducting several cycles of selection in order to obtain

adequate levels of uniformity for the most important traits and increased flowering capacity within the population. Developing these types of populations will require relatively little additional effort to the normal breeding activities. Establishing populations based on heterotic patterns could contribute to other alternatives of genetic structures to be discussed below.

Preliminary results (Table 4), have shown good genetic variability in terms of the capacity for an open-pollinated progeny to have comparable performance when planted either by stake or by TCS (CIAT, 1990a). Early and abundant development of foliage seems to be essential in order to compete with weeds and secure good productivity.

b) A second alternative would be to produce hybrids from partially or fully homozygous lines. No inbred lines of cassava yet exist, but research is advancing in the area of dihaploid production from anthers or megaspores. It can be expected that a great deal of inbred selection and testing for combining ability will have to precede hybrid production. Also, this route would necessarily entail commercial production of hybrid seed to be sold every year to farmers, and concomitantly the development of a seed industry. Genetic male sterility has already been identified in cassava, and could be a valuable asset in commercial seed production.

To obtain double-haploids has not been as easy as first thought. Promising results are being obtained in relation to cell division in microspore culture from certain genotypes (CIAT, 1990b).

Different alternatives in culture media need to be tried in order to succeed in haploid regeneration. Since the process of regeneration appears to be genotype-specific, a cyclic selection and recombination for that ability will enhance the level of double-haploid regeneration and will amplify the genetic base from which the lines are derived.

Selfing and recombination of those families that show the smaller effects of inbreeding depression will be required in order to ensure that vigorous, good seed-producing inbred lines could be obtained. Also, studies on male-sterility inheritance will need to accompany the development of inbred lines, in order to know the differences among the existing sources of male-sterility.

c) A third alternative, and if achievable the most promising, is the use of apomixis to reproduce uniform heterozygous clones via seed. Apomixis would allow farmers to save seed from their known clones, and possibly have a choice of vegetative or seed propagation.

Induced apomixis represents the ideal system to search for, because it will allow taking advantage of already existing elite clones, and it will also facilitate germplasm interchange among countries due to quarantine rules being more permissive with seed than with vegetative interchange. Developments on the identification and isolation of genes responsible for apomixis in other species will

be followed in order to have the alternative of introducing the trait via genetic transformation to cassava.

d) An intermediate alternative, and probably the most reliable for the short term, is the use of two-clone progenies as TCS cultivars. That will require generation of information about combining ability for production traits and uniformity, among male-sterile and male-fertile clones.

#### **D) Seed Production and Management**

a) Research for flowering and seed traits

A TCS cycle must be completed in one year or less. From the experience generated in breeding programs, it is known to take more than one year from planting the parental clones to harvesting all the seed. Heritability and selection studies will have to be conducted in relation to earliness of flowering, seed production, seed shattering, and dormancy.

Increasing seed production will result in competition for energy between the roots and the seeds, considering that the seeds have high energy reserves (47% fat and 25% protein). This intra-plant competition will have to be studied in order to keep the internal balance of the plant between the productive (roots) and the reproductive (seeds) elements. Preliminary results (CIAI, 1990a) have shown that interrupting the plant phloem (girdling), can

greatly enhance flowering and seed production for certain genotypes (Table 5).

The effects of different environmental conditions on flowering and seed production need to be assessed in order to determine the best conditions for seed production. Crop management and hormone treatment to enhance seed production will also have to be considered as alternatives.

Studies on fruit harvesting time, taking into account seed shattering, maturity and production, need to be conducted in order to ensure the production of good quality and quantity of seeds.

#### b) Seed germination under suboptimal conditions

This aspect constitutes one of the bottlenecks for the success of TCS (Ellis et al., 1962). Overcoming low germination and reduced initial vigor under the most representative cassava growing environments will determine the establishment of the crop and its future performance. The approach to these problems will include the study of seed pre-treatments and the selection and recombination of genotypes with good levels of germination and initial vigor. Preliminary experiments (CIAT, 1990a) showed a relatively larger importance of genotype as compared to pre-treatments, for the ability to germinate under suboptimal temperatures (Table 6).



c) Seed conservation

The TCS system relies on the flexibility that the seed can provide in terms of storage and handling for long periods of time. The effect of different environmental factors (temperature, relative humidity), and storage pests and diseases, on seed longevity and quality need to be addressed.

d) Phytosanitary management of seed

There has been one report of a virus transmitted through seeds (CGMV), and another possibly transmitted (CALV) (Walter et al., 1989). A major effort is needed to confirm or refute seed transmission of the major cassava viruses, since this is one of the crucial points that gives advantage to TCS over stake propagation.

## CONCLUSIONS

Commercial cassava production from TCS is a promising alternative since it will reduce or eliminate several present constraints to production. The most important contributions of TCS will be the reduction in virus build up in vegetative material, and solving the problems of stake storage, low multiplication rate, and long growth cycle.

There are several genetic and management related constraints to developing TCS as a viable technology. It will take a multidisciplinary approach, involving basic studies in breeding, physiology, agronomy, molecular biology, etc., in order to overcome those limitations. Preliminary results have shown promise in the potential of TCS compared to traditional stake propagation. There is good reason to believe that implementing these new technological alternative in cassava, would not be very different from similar options in other major crops.

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Table 1. Comparison between TCS and traditional vegetative propagation in cassava.

Characteristics	Vegetative propagation	TCS
Pest and pathogen problems	Many	Few
Seed quality	Variable	Unknown
Seed Storage	Few months	Many years
Multiplication rate	1:10-20	1:100-1000
Plant architecture	Restricted	Unrestricted
Carbohydrate distribution	Accumulation in stems	Efficient
Plant maturity	Lignified stems required	Unrestricted
Ease of breeding	Rapid fixation of genotypes	More problematic
Early vigor and establishment	Good	Limiting

Table 2. First cycle yields of meristem culture cleaned and uncleaned clones in various sites.

Site	Clone	Yield (t/ha)	
		Uncleaned	Cleaned
Quilcace	Quilcace	7.5	17.4
	Valluna	4.6	16.5
Media Luna	Secundina	17.6	32.8
Carimagua	Llanera	4.1	14.8
	M COL 1468	8.4	17.6
	M VEN 77	13.0	13.9
CIAT	Secundina	17.2	36.1
	M COL 1684	26.8	29.6
	M COL 72	16.0	19.4
	M COL 1468	17.2	26.8

SOURCE: CIAT, 1987.

Table 3. Yield comparison between clone CM 340-30 and its open pollinated progeny as seed propagated (cycle 1) and vegetatively propagated (cycle 2) plants.

Group	Cycle	Propagation	Root yield (t/ha)	Total plant yield (t/ha)	Harvest index
CM 340-30	1	Vegetative	41	91	0.48
Progeny	1	Seed	43	78	0.54
LSD(0.05)			8.7	--	0.07
CM 340-30	2	Vegetative	20	46	0.43
Progeny	2	Vegetative	22	45	0.49
LSD(0.05)			9.4	--	0.10

SOURCE: Bolaños, 1967

Table 4. Comparison in agronomic traits for different progenies and planting systems (stake vs TCS).

Progeny Code	Parental Clone	Propagation system	Root yield (t/ha)	Foliage yield (t/ha)	Harvest index	% Dry matter
SM 732	M MEX 1	Vegetative	26.4	33.2	0.44	30.2
SM 878	CM 507-37	Vegetative	27.2	27.1	0.50	30.6
SM 948	CG 5-79	Vegetative	25.2	31.5	0.45	31.8
SM 949	CG 5-95	Vegetative	33.2	41.3	0.45	34.0
SM 959	CM 976-15	Vegetative	21.1	22.8	0.48	30.4
		Mean	26.6	31.2	0.46	31.4
SM 732	M MEX 1	TCS	25.5	21.0	0.55	33.7
SM 878	CM 507-37	TCS	6.2	4.7	0.57	31.6
SM 948	CG 5-79	TCS	11.3	8.8	0.56	33.3
SM 949	CG 5-95	TCS	10.4	9.1	0.54	33.6
SM 959	CM 976-15	TCS	14.3	13.5	0.51	35.0
		Mean	13.5	11.4	0.55	33.4
LSD(0.05) Propag. system			1.25	1.67	0.03	1.30
LSD(0.05) Progeny/system			1.97	2.65	0.04	2.05

SOURCE: CIAT, 1990

Table 5. Girdling effects on two cassava clones.

Clone	Treatment	Seeds per plant	100-seed weight (gr)	Germ. %	Root weight (t/ha)	Foliage weight (t/ha)	Harvest index
CG 915-1							
	Girdled	44	13.8	88	11.1	15.2	0.42
	100% flowering <sup>1</sup>	32	12.0	75	35.8	24.3	0.60
	0% flowering	--	--	--	34.2	19.6	0.64
CM 3372-4							
	Girdled	390	12.6	94	8.1	13.4	0.38
	100% flowering	125	11.9	81	27.8	24.0	0.54
	0% flowering	--	--	--	28.9	16.1	0.64
LSD(0.05) Treatm.		57	0.4	6	6.7	4.1	0.10

<sup>1</sup>Flowers manually removed

SOURCE: CIAT, 1990



Table 6. Means for the combination of TCS populations and pre-germination temperatures, under two germination temperatures.

Germination temperature(°C)	TCS popn.	Pre-germination temperature (°C)	Germination <sup>1</sup> rate %	Maximum germination
25	1	0	3.4	15.5
25	1	24	2.5	18.2
25	1	48	3.6	15.5
25	1	72	2.0	10.2
Mean			2.9	14.9
25	2	0	2.5	13.9
25	2	24	3.7	10.6
25	2	48	3.4	10.2
25	2	72	2.5	18.0
Mean			3.0	18.2
35	1	0	10.4	80.2
35	1	24	14.8	88.0
35	1	48	11.5	86.8
35	1	72	10.1	81.0
Mean			11.7	84.0
35	2	0	4.3	61.4
35	2	24	5.6	62.1
35	2	48	6.7	59.8
35	2	72	4.3	49.1
Mean			5.2	58.1

<sup>1</sup>Percent per day until maximum germination is achieved

SOURCE: CIAT, 1990