Realizing the promises for high-value-cassava: root quality traits

H. Ceballos¹, D. Dufour^{1,2}, T. Sánchez¹, N. Morante¹, F. Calle¹, C. Hershey¹ and J. Tohme¹

¹ CIAT, Cali, Colombia; ²CIRAD, Montpellier, France

Introduction

The Global Cassava Initiative promoted by FAO and IFAD identified in 2000 that weak markets for cassava and its products was one of several reasons why the crop failed to meet its potential economic and social impact. Since then the CGIAR Centers working with cassava (IITA and CIAT) along with National Agriculture Research Centers gradually incorporated into their research agenda more attention to quality issues that may lead to the production of high-value cassava clones. This work summarizes the achievements at CIAT.

Materials and Methods

Initial steps relied on the screening of accessions from the germplasm collection. Valuable financial support (e.g. National Starch Company) and encouragement was provided by the private sector ⁽¹⁾. However, in spite of huge efforts regenerating germplasm from their in *vitro* condition, to extract and characterize starch and other root-quality traits, no valuable product could be obtained.

The strategy therefore shifted to the systematic self-pollination of landraces and improved germplasm in search of recessive traits that could not be detected through the phenotypic screening of the original germplasm. Efficient protocols for the identification of roots with unusual (and potentially useful) characteristics also needed to be implemented or developed. In addition to the always appealing approach of genetic transformation which has been successfully use to introduce useful genetic variability for root quality traits the induction of mutations have also been successfully applied (specifically gamma rays)

Results

A decade ago CIAT began the search of what it called "High-Value Cassava". What was then only a promise has gradually became a reality. The identification of high-value cassava traits considerable basic research needed to be done to adapt or develop protocols for the efficient screening of large segregating populations.

Development, improvement or adaptation of protocols

- Appropriate sampling and processing of root tissue for reliable and efficient carotenoids quantification.
 (2,3) (Figure 1A)
- Application of NIRs for carotenoids, dry matter and cyanogenic potential in fresh cassava roots (See poster on session S10).
- Potential applications of Chromameter in pre-selec tion of high-carotene cassava roots (See poster on session S10). (Figure 1B)
- Nitrogen is **not** a reliable approach (as done for many crops) to select for high-protein cassava roots. (See post er on session S10).
- Sampling of root tissue for detailed biochemical or genetic studies related to post-harvest physiological deterioration (Figure 2).
- Confirmation of the effectiveness of the iodine test to identify amylose-free starch and development of molecular markers.⁽⁴⁾
- Ongoing research to develop a protocol for the induction of mutations and selection of herbicide tolerance in vitro.

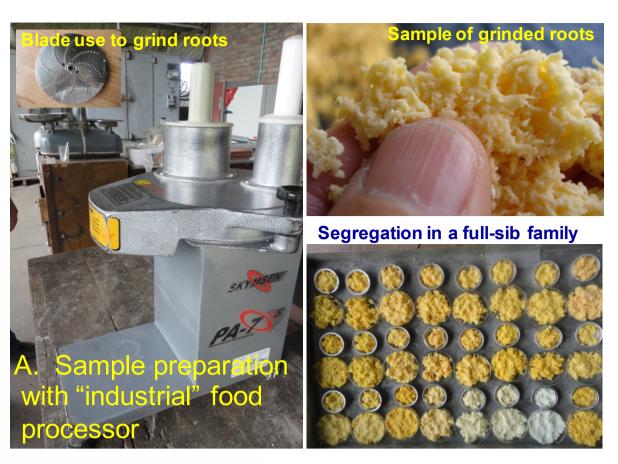




Figure 1. Illustration of the protocol currently used for quantification of carotenoids in fresh samples of cassava roots. **A.** An industrial food processor (stainless steel tools) is used to grind the roots (2-3 roots per genotype). Grinded roots are protected from light and processed quickly to prevent water loss. Two samples are used for two independent readings in the NIRS. Two samples are also taken to estimate dry matter content in the oven. **B.** About 100 grams are placed in plastic bags for the quantification with the chromameter. Three independent readings are made in different sectors of the bag.

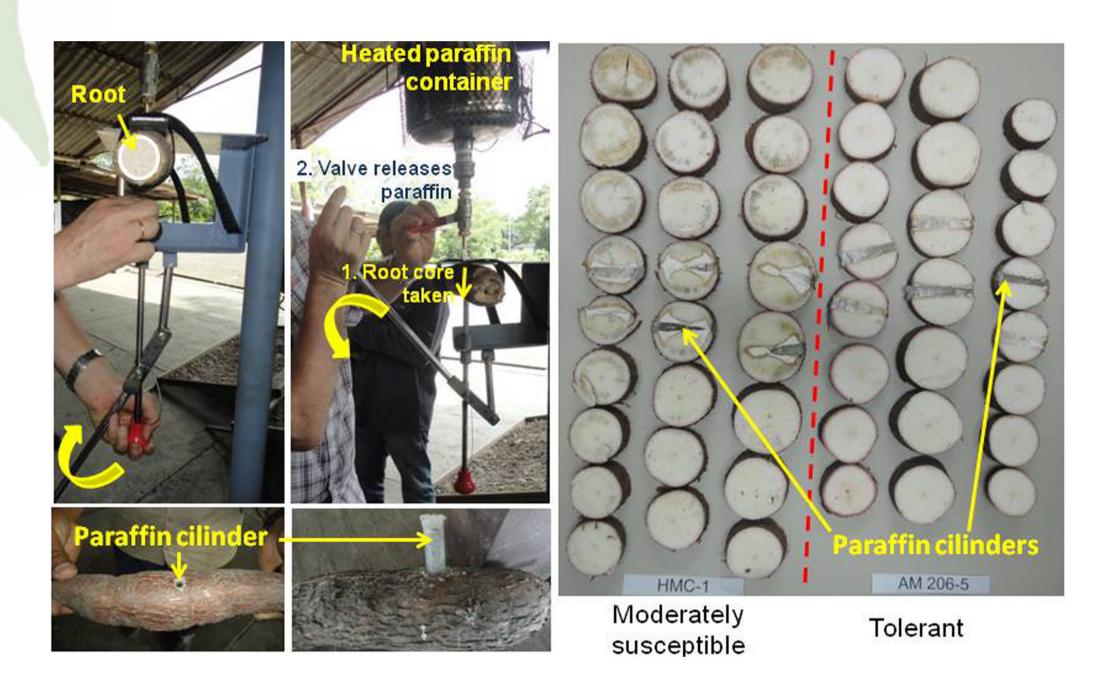


Figure 2. Illustration of the protocol under development for the extraction of a sample of root tissue without induction of PPD. As soon as the core of root tissue is removed the space is filled with paraffin to prevent direct access of oxygen to the parenchyma. In some cases there is a healing process around the paraffin cylinder. Contrast of reaction to PPD between a moderately susceptible and a tolerant genotype.

High-value traits identified or developed

- Amylose-free cassava starch. (5,6)
- Small starch granule mutation. (7, 8)
- High-amylose starch: up to 42% compared with the normal levels of around 20% (Figure 3)
- Continuous improvement of levels of carotenoids content (See poster on session S10).
- Sources of tolerance to PPD. (9)

In the last six years an array of high-value cassava traits could be identified or develop. Many of these traits are currently being introgressed into breeding populations to develop commercial varieties. Breeding work with the small granule mutation allowed the production of small-granule starches with normal ($\approx 20\%$) and duplicated (> 40%) levels of amylose. Future work will attempt increasing further amylose levels hoping to develop "resistant starches" which offer important health advantages. Attempts will also be made to produce high-amylose starches with normal granule size. Future work will concentrate in developing herbicide resistance through induced mutations *in vitro*. Also there is ongoing work to combine the waxy starch with the small-granule and the sugary mutations to develop new starch phenotypes and a better understanding of starch biosynthesis in cassava.

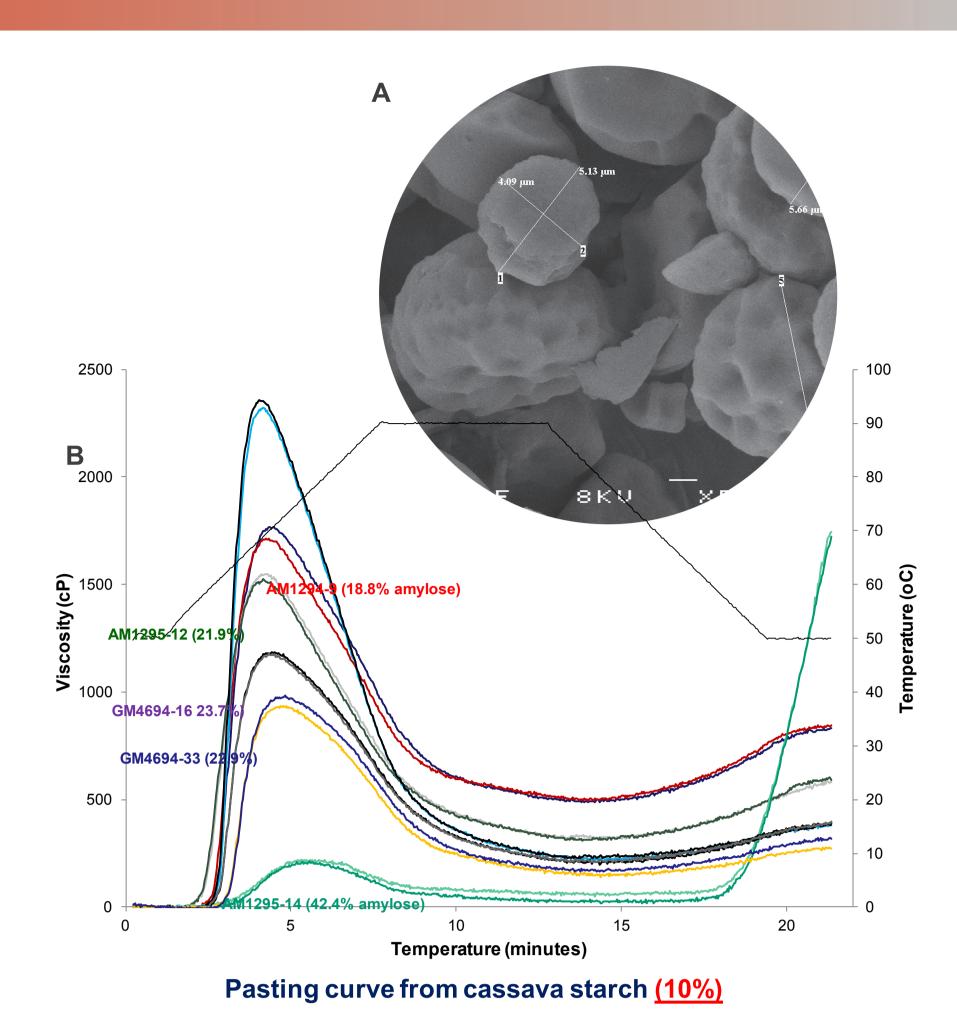


Figure 3. Illustrations of the small granule mutation. **A.** Data from the original genotype where the mutation was found. Granules are smaller and with more irregular surface. **B.** As a result of crosses and recombination several genotypes with small granule have been produced with very contrasting levels of amylose content. This figure presents the amylograms obtained from a Rapid Viscoanalyzer (RVA).

Conclusions

- Cassava offers a wealth of hidden high-value traits that need to be identified.
- The identification of high-value traits requires the system atic phenotypic screening of cassava germplasm.
- Many of the high-value traits already (or to be) indentified are recessive in nature, therefore a systematic self-pollination of germplasm is also required.
- Cassava roots do not mature the way cereals seeds do.
 Visual inspection of maize kernels can easily lead to the identification of useful mutations. In the case of cassava special protocols are required to identify high-value traits.
- Cassava is a "plastic" crop that can quickly respond for excellent genetic gains through conventional breeding.
- Since the root is not a propagation organ in cassava, mutations that could be lethal in cereals may be exploited in the case of cassava.

References

- 1. Sánchez et al. (2009). Screening of starch quality traits in cassava (Manihot esculenta Crantz). **Starch/Stärke**, 61:12-19.
- 2. Ortiz *et al.* (2011). Sampling strategies for proper quantification of carotenoids content in cassava breeding. **Journal of Plant Breeding and Crop Science**, 3(1):14-23.
- 3. Ceballos *et al.* (2012). Spatial distribution of dry matter in yellow fleshed cassava roots and its influence on carotenoids retention upon boiling. **Food Research International**, 45:52-59.
- 4. Aiemnaka *et al.* (2012). Molecular Characterization of a Spontaneous Waxy Starch Mutation in Cassava (*Manihot esculenta* Crantz). **Crop Science**. doi:10.2135/cropsci201201.0058. Published online 6 June 2012.
- 5. Ceballos *et al.* (2007). Discovery of an Amylose-free Starch mutant in cassava (*Manihot esculenta* Crantz). **Journal of Agricultural and Food Chemistry,** 55(18):7469-7476.
- 6. Sánchez *et al.* (2010). Pasting and gel stability of waxy and normal starches from cassava, potato, maize, and rice under thermal, chemical and mechanical stress. **Journal of Agricultural and Food Chemistry**, 58:5093-5099.
- 7. Ceballos *et al.* (2008). Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). **Journal of Agricultural and Food Chemistry**, 56(16):7215-7222.
- 8. Rolland-Sabaté *et al.* (2012). Structural characterization of cassava, maize and potato starches with low and high amylose contents. **Food Hydrocolloids**, 27:161-174.
- 9. Morante *et al.* (2010). Tolerance to post-harvest physiological deterioration in cassava roots. **Crop Science**, 50:1333-1338.









Since 1967