

Variation of quality traits in cassava roots evaluated in landraces and improved clones

A.L. Chávez¹, T. Sánchez¹, G. Jaramillo¹, J.M. Bedoya¹, J. Echeverry¹, E.A. Bolaños¹, H. Ceballos^{1,2,*} & C.A. Iglesias^{1,3}

¹International Center for Tropical Agriculture (CIAT). Apartado Aéreo 6713. Cali, Colombia, U.S.A.; ²Universidad Nacional de Colombia. Carrera 32, Chapinero vía Candelaria. Palmira, Colombia, U.S.A.; ³Present address: Weaver Popcorn Company, 1000 N 325W, New Richmond, IN 47967, USA; (*author for correspondence: e-mail: h.cebillos@cgiar.org)

Received 4 June 2004; accepted 1 March 2005

Key words: carotene, crude protein, minerals, postharvest physiological deterioration

Summary

About 70 million people obtain more than 500 cal per day from cassava roots. The crop is fundamental as food security of poor rural communities, but little is known about variability of root nutritional and quality traits. Roots from 2457 genotypes comprising landraces and improved clones, were screened for their nutritional (cyanogenic potential, carotene, minerals, and sugars contents) and agronomic (dry matter content, color intensity, and postharvest physiological deterioration) traits. The objective was to assess the range of variation for the traits evaluated to define future research strategies. Results are mostly based on unreplicated measurements. Carotene contents in the roots ranged from 0.102 to 1.040 mg/100 g fresh tissue and correlated positively with color intensity ($\rho = 0.860$) and cyanogenic potential ($\rho = 0.305$). Average levels of Fe and Zn were 17.1 and 7.5 mg/kg, respectively. Many clones derived from Meso-America showed high protein levels in the roots, probably as a result of the introgression from wild relatives only found in that region. The observed values for carotene, proteins and minerals contents suggest the potential for improving the nutritive value of cassava.

Abbreviations: PPD, postharvest physiological deterioration; HCN, cyanogenic potential; VAD, Vitamin A deficiency

Introduction

Cassava (*Manihot esculenta* Cranz) is a perennial crop native to tropical America, (Allen, 1994; Olsen & Schaal, 2001). About 70 million people obtain more than 500 cal per day from cassava (Cock, 1985; Kawano et al., 1998). Cassava offers the advantage of a flexible harvesting date, allowing farmers to keep the roots in the ground until needed (Iglesias et al., 1997). The crop produces reasonably well under critical conditions of climate and soil and is recognized as a famine reserve due to its tolerance to drought or infertile soils, and its ability to recover from disease and pest attacks. The area of cassava under unfavorable environments has been continuously increasing (El-Sharkawy, 1993).

The starchy root is its best-known and used product worldwide, however, the fresh foliage is also consumed in several regions of the world for animal and/or human consumption.

A limiting characteristic for the human or animal consumption of cassava roots is their cyanogenic glucosides content (Kakes, 1990). However, cyanide (HCN) is largely removed by the traditional processing methods of grating, fermenting, boiling and/or drying. Cassava roots are also particularly low in protein and are valued only as a source of energy in human and animal diets (Buitrago, 1990; Babu & Chatterjee, 1999). An additional constraint for cassava is the short shelf life of its roots. Postharvest physiological deterioration (PPD) often begins rapidly within 24 h (Beeching

et al., 1998). Because of PPD, cassava roots need to be consumed shortly after harvesting (Van Oirschot et al., 2000). The short postharvest storage life of cassava is a characteristic that limits the marketability of the roots.

Vitamin A is an essential micronutrient for the normal functioning of the visual and immune systems, growth and development, maintenance of epithelial cellular integrity and for reproduction (ACC/SCN, 2000; Combs, 1998). Improving the vitamin A status of children reduces mortality rates by 23% to 30% (ACC/SCN, 1992; Beaton et al., 1993; West, 2001). It is estimated that 75 to 251 million children have sub-clinical symptoms (WHO/UNICEF, 1995; MI/UNICEF/Tulane University, 1998) of vitamin A deficiency (VAD). In addition to the direct effect of VAD, there is growing evidence of vitamin A having synergistic effects with iron and zinc bio-availability (Graham & Rosser, 2000). Carotene from vegetables contribute two-thirds of dietary vitamin A, worldwide, and more than 80% in the developing world (Combs, 1998).

So far, the genetic variability in cassava roots for carotene and mineral contents has not been extensively screened. Iglesias et al. (1997) evaluated carotene contents in roots from 632 clones. This research is a continuation of that pioneering work, not only increasing the number of clones screened but also incorporating mineral analyses, agronomic traits and their relationships.

Materials and methods

A total of 2457 cassava clones have been evaluated and a description of the origins of this germplasm is provided in Table 1. There were two types of clones, those produced from breeding projects at International Center for Tropical Agriculture (CIAT, Colombia), International Institute of Tropical Agriculture (IITA, Nigeria) or Rayong Experimental Station in Thailand, and clones from landraces from the germplasm collection held at CIAT.

Because of limitation in the number of samples that can be analyzed at any given time and the impossibility of storing the roots, the evaluations were carried out through a period of four years since 1998 through 2001. Plants maintained at the *in vitro* germplasm collection were hardened in greenhouse conditions and, after 2 months, transplanted to the field. Evaluations were unreplicated, because of the lack of planting material and the time required to multiply it. Tissue samples from no less than three roots per accession were taken

Table 1. Summary of the origin of the cassava clones evaluated in one or more of the different analyses described in this article

Origin	No.
CIAT's clones	337
IITA's clones	4
Argentina	13
Bolivia	3
Brazil	585
China	4
Colombia	720
Costa Rica	32
Cuba	45
Dominican Rep.	8
Ecuador	77
Fiji Islands	2
Guatemala	31
Indonesia	19
Malaysia	31
Mexico	49
Nigeria	4
Panama	23
Paraguay	77
Peru	217
Philippines	7
Puerto Rico	10
Thailand	9
USA	7
Venezuela	122
Other	21
Total	2457

10–11 months after transplanting. All plants evaluated were grown at CIAT station in Palmira (Valle del Cauca Department, Colombia).

Carotene concentration

The extraction procedure outlined by Safo-Katanga et al. (1984) was modified by extracting root parenchyma with petroleum ether, as described and utilized by Iglesias et al., 1997. The modified protocol included several extractions with petroleum ether (35–65 °C). Approximately 5 g of tissue was obtained from representative and randomly selected roots from plants of each clone. The use of alternative solvents has been suggested more recently (Rodriguez-Amaya, 2001) and incorporated in more recent quantifications, which are not reported in this article. The quantification was

done by visible spectrophotometry using a Shimadzu UV-VIS 160A recording spectrophotometer. Detection was done at = 455 nm (Rodriguez Amaya 1989; 1990; Scott & Hart, 1993).

Postharvest physiological deterioration (PPD)

Five commercially sized roots (minimum length 18 cm) were randomly chosen. Roots were analyzed using the method of Wheatley et al. (1985), with one modification: prepared roots were stored under ambient conditions for 7 days instead of 3 days. The proximal and distal root ends were cut off and the distal end was covered with clingfilm. After 1 week, seven transversal slices, 2 cm thick were cut along the root, starting from the proximal end. A score of 1–10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1 = 10%, 2 = 20%, etc). The mean score of PPD for each root was calculated by averaging the score across the seven slices.

Minerals concentrations

The sampling procedure was the same as for the evaluation of carotene content. Roots were dried, ground to powder and sent to the Analytical Laboratory of University of Adelaide where the samples were analyzed by inductively coupled plasma atomic emission spectrometry. All sample processing was carried out to avoid as much as possible contamination from soil, which has mineral concentrations higher than that of vegetal tissues. Protein content was estimated by multiplying *N* concentrations by a constant of 6.25, although Hock-Hin and Van-Den, reported in 1996 that in the case of cassava this figure is probably ranging from 4.75 to 5.87. The original conversion factor has been maintained to facilitate the comparisons with previous reports. *N* quantification was based on dried root flour.

Therefore, HCN had already been released before the quantification and no nitrogen from cyanogenic compounds should have remained.

Dry matter content

Dry matter content was estimated using the well-known specific gravity methodology (Kawano et al., 1987). Approximately 5 kg of roots were weighted in a hanging scale (WA). The same sample was weighted with the roots submerged in water (WW). Dry matter content was estimated with the following formula:

$$\text{Dry matter content (\%)} = \left(\frac{\text{WA}}{\text{WA} - \text{WW}} \times 158.3 \right) - 142$$

where WA is the weight in the air and WW the weight in water.

Root coloration and other measurements

A 1 to 9 scale for the visual estimation of root coloration was developed and printed for a uniform estimation of color intensity. The color of root parenchyma can vary from white, cream, yellow, and orange. Pinkish roots (score 9) have also been observed in cassava. Total and reducing sugars were estimated following the procedure outlined by Cronin & Smith, 1979. Cyanide potential (HCN) was quantified following the colorimetric procedure suggested by Essers et al. (1994).

Results

Table 2 presents a summary of measurements for dry matter, HCN, total carotene for roots and leaves, as well as color, PPD and sugars in the roots. Descriptive

Table 2. Descriptive parameters for root traits of industrial relevance in accessions from the Cassava Germplasm Bank and the Breeding Project at CIAT

Variable	Sample size (No.)	Minimum	Maximum	Average	Standard deviation
Root dry matter (%)	2022	10.72	57.23	34.27	6.95
HCN (ppm)	2022	13.9	2561.7	263.7	324.2
Carotene (mg/100 g FT)	1789	0.102	1.040	0.2457	0.1351
Root color (1 to 9)	788	1	8	2.26	1.46
PPD (%)	1374	0	100	24.47	19.63
Total sugars (%)	1755	0.2	15	2.876	2.028
Reducing sugars (%)	1755	0.0	12.9	0.753	0.957

statistics make use of all the data available for each variable. However, for the association between two traits, only data taken on the same roots for the traits whose association is analyzed were used.

The observed values for dry matter content and HCN in roots agree with those reported in the literature (Buitrago, 1990). The average for PPD was 24.47%, with individual values ranging from 0 to 100%. Distribution of PPD was asymmetrical with a longer tail to the right, and concentration of frequencies around the low-PPD values.

Carotenes in the roots and related traits

Carotene content in the roots ranged from 0.102 to 1.040 mg/100 g fresh tissue (FT), demonstrating the potential of cassava clones with yellow roots to contribute overcoming VAD in regions of the world where this malady is a chronic problem. There was a clear asymmetrical distribution for carotene in the roots, which concentrated frequencies in the lower values to

the left of the plot (Figure 1), and a long tale to the right (skewness value = 2.64). The visual scoring of root color, based on a sample of 788 clones also had a higher frequency of roots with light or white coloration, with fewer cases of roots with intense coloration (skewness = 1.73).

Table 3 presents the correlation coefficients among different root traits. There was a clear and positive association between carotene and HCN in the roots. This association, as suggested by Graham et al. (1999), is probably due to the fact that clones with yellow roots are commonly found in the Amazon basin, where highly cyanogenic (bitter) lines are also preferred. It is possible, however, to obtain clones with intense yellow coloration in the roots yet low levels of HCN. For instance, the elite clone CM 2772-3 developed for the Putumayo Department in Colombia's Amazon Basin, has yellow roots with a relatively high concentration of carotene (the average from two evaluations was 0.51 mg/100 g FT) and low level of HCN (93.5 mg kg⁻¹) from the same samples were carotene were measured).

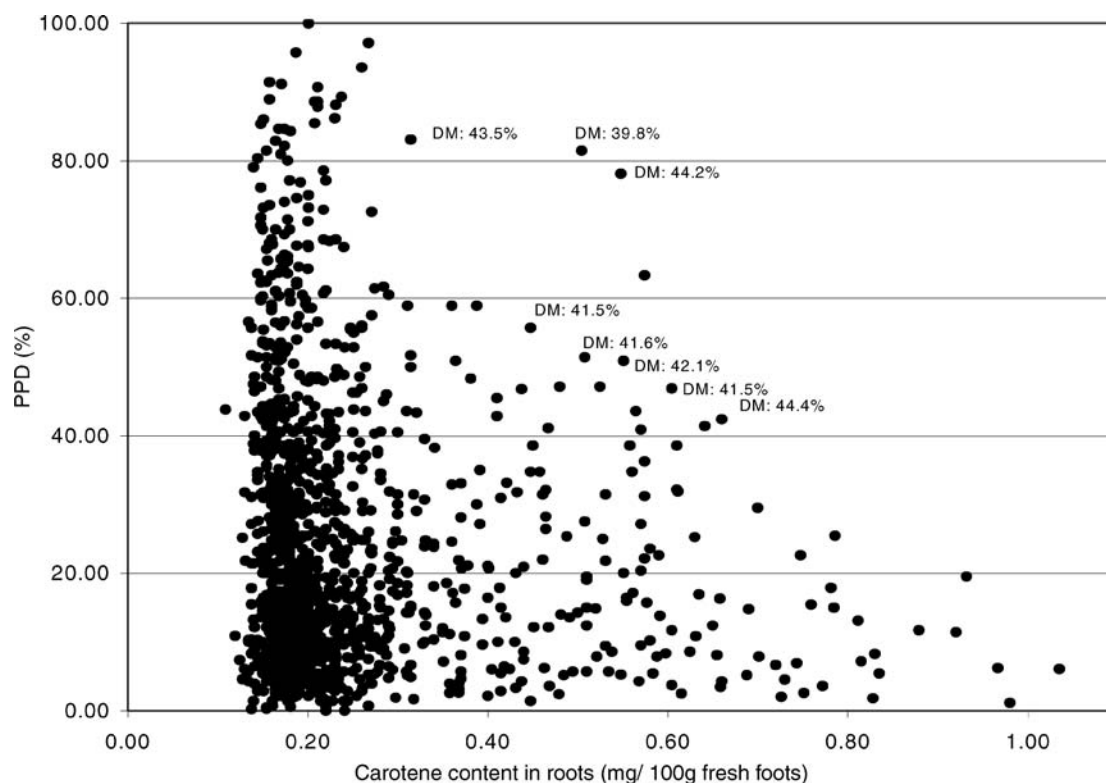


Figure 1. Relationship between carotene content (mg/100 g FT) and PPD (%) analyzed in a sample of 1315 cassava roots. Most data points in the upper periphery of the distribution came from root samples with dry matter content (%) considerably higher than the average for the sample analyzed.

Table 3. Simple phenotypic correlation among relevant root traits in cassava

	HCN (mg kg ⁻¹)	Carotene (mg/100 g FT)	PPD (%)	Total Sugars (%)	Red. Sugars (%)
Dry matter content (%)	-0.222** (1374)	-0.078** (1315)	0.348** (1374)	-0.364** (1374)	-0.349** (1374)
HCN (mg kg ⁻¹)	1.0	0.305** (1315)	-0.080** (1374)	0.167** (1374)	0.141** (1374)
Carotene (mg/100 g FT)		1.0	-0.123** (1315)	0.082** (1315)	0.052ns (1315)
PPD (%)			1.0	-0.114** (1374)	-0.120** (1374)
Total sugars (%)				1.0	0.595** (1374)
Red. sugars (%)					1.0

The size of the sample utilized to measure the correlations is given within parenthesis.

**Significant at $P < 0.01$; ns: nonsignificant.

Phenotypic correlation between total carotene content in the roots and root color score ($n = 788$) based on the visual scale was very high and positive ($\rho = 0.860$). This value (not in Table 3) demonstrates that the identification of cassava clones with high carotene density in the roots can be easily and effectively done through a visual evaluation of their parenchyma color. The higher the color intensity, the higher the amount of carotene present, supporting the reports by Iglesias et al. (1997) and Graham et al. (1999) but based on a considerably larger sample.

PPD and related traits

Because of their economic importance, the association between PPD and other traits was also analyzed. The correlation coefficient ($\rho = 0.348$) indicates that there is a positive association between dry matter content and PPD, further supporting previous reports (Jennings & Hershey, 1985; Van Oirschot et al., 2000). This is an unfortunate situation because, in general, breeding projects look for higher dry matter content, which leads to a faster or more serious PPD. HCN does not seem to have a strong effect on PPD, whereas carotene seemed to reduce and/or delay the onset of PPD ($\rho = -0.123$). Figure 1 illustrates the relationship between PPD and carotene, and suggests that with increased amounts of carotene there is an apparent reduction or delaying of PPD onset. The exceptions can be explained by samples having higher than average dry matter contents.

Evaluation of PPD is prone to large experimental errors, because roots are left at room temperature (Wheatley et al., 1985; Zapata, 2001) for 7 days. Current measurements on PPD had to be carried out at different harvesting times, because of restriction in the availability of planting material from the germplasm bank and limitations in the number of clones that

could be processed at any given time. Therefore, PPD estimates were probably affected by variations in the environmental conditions under which they were taken.

Other associations involving dry matter content

Van Oirschot et al. (2000) reported a negative correlation between dry matter and sugar contents in cassava roots. This relationship was established on six cultivars and upon preharvest pruning of stems. In this paper, the association between these two variables is further confirmed but on a much larger sample (1374 clones) and with no pruning being involved.

Trace mineral concentrations

All measurements were taken in mg kg⁻¹ or % and on a dry tissue basis. Because of their nutritional relevance, results from few elements were highlighted. Roots averaged 17.1 mg kg⁻¹ for iron, 7.5 mg kg⁻¹ for zinc, and 0.076% for calcium (Table 4). Significant but weak relationships between total carotene content and Mn, and Ca were found (correlation coefficients of 0.15 and 0.13, respectively). In general, the correlations between PPD and mineral concentrations in roots were low. The higher correlation coefficients found were negative: K ($\rho = -0.29$); S ($\rho = -0.24$); and N ($\rho = -0.21$). There seemed to be an inverse relationship between these minerals and PPD.

Protein contents

Table 4 also presents the estimates of crude protein content in root tissue. Averages for roots are slightly higher than those reported in the literature (Buitrago, 1990). A weak positive correlation ($\rho = 0.14$) was

Table 4. Simple descriptive statistics (minimum, maximum, mean and standard deviation) for mineral and protein concentrations (dry weight basis) in roots from 600 cassava genotypes^a, and their correlations with carotene content and PPD

Mineral	Minimum	Maximum	Mean	Standard deviation	Correlations with	
					carotene	PPD
Element content measured in mg kg ⁻¹						
Fe	6.0	230.0	17.1	15.2	0.09*	-0.12**
Mn	0.45	5.0	1.4	0.6	0.15**	-0.19**
B	1.14	9.91	2.0	0.6	0.07ns	-0.14**
Cu	0.79	40.31	5.8	5.4	-0.02ns	-0.06ns
Zn	2.63	37.52	7.5	3.6	-0.07ns	-0.03ns
Na	18.6	1230.0	129.2	147.3	-0.02ns	-0.04ns
Al	4.4	330	11.5	20.4	na	na
Element content measured in%						
Ca	0.031	0.250	0.076	0.032	0.13**	0.06ns
Mg	0.052	0.240	0.105	0.028	0.02ns	0.00ns
K	0.410	2p.500	1.172	0.321	-0.02ns	-0.29**
P	0.071	0.320	0.165	0.036	-0.08*	-0.10*
S	0.012	0.055	0.027	0.008	0.04ns	-0.24**
Crude protein content (%) ^b						
	0.769	8.313	3.063	1.418	0.02	-0.21**

***Significant at $P < 0.05$ and $P < 0.01$ probability level respectively. ns: nonsignificant. na: nonavailable.

^aSample sizes for root evaluation of B = 580; for Cu = 599, and for Al = 460.

^bCorrelations with carotene content and PPD estimated using N content.

Table 5. List of the best 33 clones regarding crude protein content in the roots from a sample of 600 cassava clones

Clone and protein (%)	Clone and protein (%)	Clone and protein (%)	Clone and protein (%)	Clone and protein (%)	Clone and protein (%)
CM 5620-3	8.31	MCOL 2436	6.25	MBRA 101	5.94
SM 1406-1	8.13	MBRA 26	6.25	MCOL 219	5.94
MCOL 689B	7.75	MCR 136	6.13	MGUA 33	5.94
MCOL 1563	7.38	MGUA 9	6.13	CM 7310-1	5.88
MGUA 76	6.94	MGUA 91	6.06	MCOL 678	5.88
MCR 142	6.63	MMEX108	6.06	MMEX 95	5.81
CM 696-1	6.44	SM 629-6	6.00	MGUA 79	5.81
CM 3199-1	6.44	SM 673-1	6.00	MBRA 300	5.75
SM 734-5	6.44	MCOL 2532	6.00	MCOL 2459	5.75
MCR 38	6.31	MGUA 19	6.00	MBRA 1384	5.75
MGUA 86	6.31	CM 3236-3	5.94	MCOL 2694	5.75

CM and SM codes identify clones derived from CIAT's cassava breeding project. The remaining clones are from the germplasm bank collection.

also observed between nitrogen and HCN contents in the roots. Table 5 lists the best 30 clones regarding protein content in the roots. A high frequency of these clones come from Meso-America.

Discussion

The results presented in this study are exploratory in nature. The correlations among different variables, because of the size of samples involved, are very useful in suggesting associations that can be exploited to facilitate cassava genetic improvement. Furthermore, one of the main purposes of this study was to evaluate the nutritional properties of cassava both for human and animals. It was of particular interest to determine the potential of cassava to provide carotenes through the diet as a contributing factor for alleviating vitamin A deficiency in human populations.

The lack of replication for the large number of genotypes screened is a strong limitation in this study. However, the variation associated with the experimental error of carotene concentration in the roots, has been measured (CIAT, 1999). Standard deviations for measurements of roots from different plants of the same clone, of different roots from the same plant and of different samples from the same root represented 7.7, 7.0 and 2.8% of the mean carotene concentrations, respectively. Carotene content is a stable trait and genetic differences remain relatively constant even when clones are grown in different locations, as indicated by a preliminary study to measure the importance of genotype by environment interaction (CIAT, 2002). Likewise, estimates of the experimental errors associated with crude protein estimations are available. Crude protein content has been measured in roots from 132 clones in repeated occasions (from 2 to 4 measurements in the same genotype) always in different years and often using a different biochemistry laboratory. Mean protein content was 3.561 and the average standard deviation in these measurements was 0.282. Coefficient of variability for crude protein content in cassava roots was low (8.72%). Observed differences in crude protein content from the sample of 600 genotypes reported here, therefore, are expected to be largely genetic in nature. CIAT is currently growing a set of contrasting clones to measure the stability of Fe and Zn measurements and the relative importance of genotype by environment interaction for these two traits.

Jalal et al. (1998) demonstrated that carotene rich, orange fleshed sweet potato (*Ipomoea batatas* (L) Poir) supplied for 3 weeks to a group of human individuals in Indonesia, resulted in changes in blood serum amount of retinol. The analysis of different mineral elements was also relevant, particularly in light of the emerging evidence of a synergistic effect between carotene, Fe and Zn. García-Casal et al. (1998) demonstrated that

vitamin A and beta-carotene increased iron absorption from different sources. Also vitamin A has been shown to contribute increasing hemoglobin content (a typical symptom of Fe-deficiency) as reported by Kolsteren et al. (1999) and Mwanri et al. (2000).

Results observed in the large samples analyzed demonstrate that cassava roots are a valuable source of carotene, which can help alleviating chronic vitamin A deficiency in human populations suffering from it. Although the negative association between carotene content and PPD is still preliminary it is a relevant issue: if higher carotenes in the roots reduce or delay PPD, this would encourage farmers to grow cassava clones with yellow roots, therefore helping to overcome the frequent reluctance by subsistence farmers to adopt new varieties. Further studies, under better-controlled conditions for measuring PPD, however, are needed for corroborating the preliminary evidence found and are already underway.

The range of variation for carotene content observed in the 1789 measurements was narrower than that reported by Iglesias et al. (1997). The highest value observed in the current analysis was 1.040 mg/100 g FT, whereas in the previous report as much as 2.55 mg/100 g FT have been reported for MBRA 516. In the current analysis, carotene content in the clone MBRA 516 was measured in two different opportunities providing values of 0.78 and 0.83 mg/100 g FT. After Iglesias et al. (1997) publication, which was a preliminary report, carotene quantification was changed to be based on the spectrophotometry because of problems with the HPLC protocol employed that had become evident through time. The current results, therefore, are more consistent and reliable than those of Iglesias et al. (1997).

Regarding protein content in the roots (estimated through *N* measurements), the mean crude content of 3.06% agrees with those reported in the literature. However, the few clones with high protein content (ranging from 5.75 to 8.31%) are remarkable. New root samples from the same clones will be evaluated again to confirm current expectations, and to have a better estimation of the effect of genotype by environment interaction in the expression of this trait. The weak correlation between nitrogen content and cyanogenic potential would suggest that a fraction of the nitrogen detected originated in the cyanogenic glucosides. This association, if confirmed, seems to be low enough to allow for the possibility of developing clones with high protein and low HCN in their roots.

A remarkable feature regarding protein content in the roots is that 12 out of the best 30 clones originated in Meso-America: Costa Rica, Guatemala and Mexico (Table 5). This proportion (40%) is much higher than that of clones representing this region (6.3%) in the total sample of 600 clones. This would suggest that a genetic introgression from Meso-American, noncultivated *Manihot* species might have occurred, resulting in a high frequency of cassava clones with increased protein content in their roots. About a dozen *Manihot* species grow wild in Meso-America (mainly *M. aesculifolia*, *M. gualanensis*, *M. isoloba*, *M. pringlei*, and *M. oaxacana*), and can readily cross with *M. esculenta* (Brücher, 1989). Distinctive characteristics of cassava clones from this region (particularly Guatemala) have been reported using simple sequence repeat markers (CIAT, 2001). Cassava clones from this region are currently recovered from the *in vitro* collection and will be carefully analyzed for their protein content in the roots in November 2004. If only a few of these clones did reproduce the high concentrations (above 5%) reported in this study, it would already be a major finding in cassava research with enormous potential in Asia, Africa and Latin America.

Acknowledgments

The authors wish to express their gratitude to Danish International Development Assistance (DANIDA) and USAID for the financial support through a grant coordinated by the International Food Policy Research Institute (IFPRI). This research is part of the Harvest Plus initiative.

References

- ACC/SCN, 1992. Second report on the world nutrition situation. United Nations, Administrative Committee on Coordination, Subcommittee on nutrition. Geneva. ACC/SCN in collaboration with IFPRI.
- ACC/SCN, 2000. Forth report on the world nutrition situation. United Nations, Administrative Committee on Coordination, Subcommittee on nutrition. Geneva. ACC/SCN in collaboration with IFPRI.
- Allen, C.A., 1994. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). *Gen Res Crop Evol* 41: 133–150.
- Babu, L., & S.R. Chatterjee, 1999. Protein content and amino acid composition of cassava tubers and leaves. *J Root Crops* 25(20): 163–168.
- Beaton, G.H., R. Martorell, K.J. Aronso, B. Edmonston, G. McCabe, A.C. Ross & B. Harvey, 1993. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in

- developing countries. ACC/SCN State of the arts series, Nutrition Policy Paper No. 13. Geneva.
- Beeching, J.R., H. Yuanhuai, R. Gómez-Vázquez, R.C. Day & R.M. Cooper, 1998. Wound and defense responses in cassava as related to post-harvest physiological deterioration. In: J.T. Romeo, K.R. Downum & R. Verpoorte (Eds.), *Recent Advances in Phytochemistry* (vol. 32). *Phytochemical Signals in Plant-Microbe Interactions*, pp. 231–248. Plenum Press, New York, London.
- Brücher, H. 1989. *Useful Plants of Neotropical Origin and Their Wild Relatives*. Springer-Verlag, Berlin and New York, p. 296.
- Buitrago, A.J.A., 1990. La yuca en la alimentación animal. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia p. 446.
- Buschmann, H., M.X. Rodriguez, J. Tohme & J.R. Beeching, 2000. Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of cassava (*Manihot esculenta* Crantz). *Ann Bot* 86: 1153–1160.
- CIAT, 1999. Improved Cassava for the Developing World. Annual Report, 1999.
- CIAT, 2001. Improved Cassava for the Developing World. Annual Report, 2001.
- CIAT, 2002. Improved Cassava for the Developing World. Annual Report, 2002.
- Cock, J., 1985. Cassava. New Potential for a Neglected Crop. Westview Press, Boulder, CO., USA.
- Combs, G.F., 1998. The Vitamins. *Fundamental Aspects in Nutrition and Health*. Academic Press. p. 618.
- Cronin, D.A. & S. Smith, 1979. A simple and rapid procedure for the analysis of reducing, total and individual sugars in potatoes. *Potato Res.* 22: 99–105.
- El-Sharkawy, M.A., 1993. Drought-tolerant cassava for Africa, Asia and Latin America. *BioScience* 43: 441–451.
- Essers, A.J.A.M., R.M. Bosveld, R.M. van der Gift & A.G.J. Voragen, 1994. A new chromogen for the assay of cyanogens in cassava products. In: M.O. Akoroda (Ed.), *Root crops for food security in Africa*. Proceedings of the Fifth Triennial Symposium of the International Society for Tropical Root Crops, African Branch (ISTRAC-AB), The Technical Centre for Agricultural and Rural Cooperation (CTA) and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, pp. 314–317.
- FAO/FIDA, 2000. La economía mundial de la yuca. Hechos, tendencias y perspectivas. Fondo Internacional de Desarrollo Agrícola. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma, Italia.
- García-Casal, M.N., M. Layrisse, L. Solano, M.A. Baron, F. Arguello, D. Llovera, J. Ramirez, I. Leets & E. Tropper, 1998. Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat, and corn by humans. *J Nutr Bethesda* 128(3): 646–650.
- Gomez, G., J. Santos & M. Valdivieso, 1983. Utilización de raíces y productos de yuca en alimentación animal. In: C.E. Domínguez (Ed.), *Yuca: Investigación, producción y utilización*. Working Document No. 50. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- Graham, R.D. & J.M. Rosser, 2000. Carotenoids in staple foods: Their potential to improve human nutrition. *Food Nut Bull* 21(4): 404–409.
- Graham, R.D., D. Senadhira, S. Beebe, C. Iglesias & I. Monasterio, 1999. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crop Res* 60: 57–80.
- Hock-Hin, Y. & T. Van-Den, 1996. Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. *J Sci Food Agric* 70: 51–54.
- Iglesias, C., J. Mayer, A.L. Chávez & F. Calle, 1997. Genetic potential and stability of carotene content in cassava roots. *Euphytica* 94: 367–373.
- Jalal, F., M.C. Nesheim, Z. Agus, D. Sanjur & J.P. Habitch, 1998. Serum retinol concentrations in children are affected by food sources of beta-carotene, fat intake, and anthelmintic drug treatment. *J Clinical Nutr* 68(3): 623–629.
- Jennings, D.L. & C.H. Hershey, 1985. Cassava breeding: A decade of progress from international programs. In: G.E. Russell (Ed.), *Progress in Plant Breeding*, pp 89–116. Butterworths. London, Boston.
- Kakes, P., 1990. Properties and functions of the cyanogenic system in higher plants. *Euphytica* 48: 25–43.
- Kawano, K., W.M. Gonçalves Fukuda & U. Cempukdee, 1987. Genetic and environmental effects on dry matter content of cassava root. *Crop Sci* 27: 69–74.
- Kawano, K., K. Narintaraporn, P. Narintaraporn, S. Sarakarn, A. Limsila, J. Limsila, D. Suparhan & W. Watananonta, 1998. Yield improvement in a multistage breeding program for cassava. *Crop Sci* 38: 325–332.
- Kolsteren, P., S.R. Rahman, K. Hilderbrand & A. Diniz, 1999. Treatment for iron deficiency anemia with combined supplementation of iron, vitamin A and zinc in women of Dinajpur, Bangladesh. *Euro J Clin Nutr* 53(2): 102–106.
- MI/UNICEF/Tulane University, 1998. Progress in controlling vitamin A deficiency. Ottawa: Micronutrients Initiative.
- Mwanri, L., A. Worsley, P. Ryan & J. Masika, 2000. Supplemental vitamin A improves anemic school children in Tanzania. *J Nut* 130: 2691–2696.
- Olsen, K.M. & B.A. Schaal, 2001. Microsatellite variation in cassava (*Manihot esculenta*, Euphorbiaceae) and its wild relatives: further evidence for a southern Amazonian origin of domestication. *Am J Bot* 88: 131–142.
- Renvoize, B.S., 1972. The area of origin of *Manihot esculenta* as a crop plant – a review of the evidence. *Econ Bot* 26: 352–360.
- Rodríguez Amaya, D., 1989. Critical review of provitamin A determination in plant Foods. *J Micronut Anal* 5: 191–225.
- Rodríguez Amaya, D., 1990. Provitamin A determination problems and possible solutions. *Food Nut Bull* 12(3): 246–250.
- Rodríguez-Amaya, D.B., 2001. A guide to carotenoid analysis in foods. ILSI Press, Washington, pp. 64.
- Safo-Katanga, O., P. Aboagye, S.A. Amartey & J.H. Olaham, 1984. Studies on the content of yellow-pigmented cassava. In: E.R. Terry, E.V. Doku, O.B. Arene & N.M. Mahungu (Eds.), *Tropical Root Crops Production and Uses in Africa*, pp. 103–104. IDRC, Ottawa, Canada.
- Scott, K.J. & D.J. Hart, 1993. Further observations on problems associated with the analysis of carotenoids by HPLC. *Food Chem* 47: 403–40.
- Scott, G.J., M.K. Rosegrant & C. Ringler, 2000. Global projections for root and tuber crops to the year 2020. *Food Policy* 25: 561–597.
- Van Oirschot, Q.E.A., G.M. O'Brien, D.D. Dufour, M.A. El-Sharkawy & E. Mesa, 2000. The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. *J Sci Food Agric* 80: 1866–1873.

- West, K.R. Jr., 2001. The magnitude of vitamin A deficiency disorders: Overview. XX IVACG Meeting. Hanoi, Vietnam. February 12–15.
- Wheatley, C., C. Lozano & G. Gomez, 1985. Post-harvest deterioration of cassava roots, In: J.H. Cock & J.A. Reyes (Eds.), Cassava: Research, Production and Utilization. pp. 655-671. UNDP-CIAT, Cali.
- WHO/UNICEF, 1995. Global prevalence of vitamin A deficiency. Micronutrient Deficiency Information System Working Paper 2. Geneva WHO.
- Zapata, G., 2001. Disminución de deterioro fisiológico postcosecha en raíces de yuca (*Manihot esculenta* Crantz) mediante almacenamiento controlado. B.S. Thesis, Universidad de San Buenaventura, Facultad de Ingeniería Agroindustrial. Cali, Colombia.