Utilization of NIRS and chromameter in selection for increased carotenoids content in cassava roots

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Introduction

Significant progress has been made in the recent past increasing carotenoids content in cassava roots. In addition, vast information has been generated regarding carotenoids in the roots, along with improvements for efficient and reliable quantification protocols. The objective of this work was to (i) assess the efficiency of near-infrared spectroscopy (NIRS) for predicting total carotenoids content (TCC) and total β-carotene (TBC) along with other useful traits such as cyanogenic potential (HCN) and dry matter content (DMC); and (ii) further evaluate the usefulness of the Chromameter as a pre-selection tool. All data is based on fresh root samples.

Materials and Methods

Data generated by the cassava breeding project at CIAT since 2009 was consolidated and analyzed **(Table 1).** Quantification was made directly on chopped or grinded fresh root tissue (not lyophilized). The dataset was first cleaned of outlying or suspicious data points to develop reliable prediction equations (2129 data points).

NIRS: For each sample spectra were collected with a spectrometer (FOSS 6500, monochromator with autocup sampling module). Wavelength range was 400 nm to 2500 nm (acquisition step, 2nm). Root samples from year 2009 were chopped, whereas later samples were grinded with a food processor prior to NIR analysis. Each sample was duplicated (2 different samples of roots analyzed per genotype).

Color intenstity: A Minolta ChromaMeter CR-410 **(Figure 1)** was used to digitally quantify color intensity on roots samples. Three lectures per genotype were taken.

Carotenoids were extracted and quantified with a spectrophotometer and HPLC following the methodology described by Ceballos *et al.* (2012).

Figure 1. Lectures of color intensity using the Chromameter.

Table 1. Origin of information generated for different parameters used in the present study

Parameter	2009	2010	2011	2012	TOTAL
Dry matter content (%)	657	651	698	99	2105
Trichromatic lectures (L, a*, b*)			294	96	390
Phytofluene (µg/g FW)			577	99	676
Phytoene (µg/g FW)		529	626	99	1254
9-cis-β-carotene (µg/g FW)	572	666	626	99	1963
15-cis-β-carotene (µg/g FW)	572	666	198	99	1535
13-cis-β-carotene (µg/g FW)	572	666	626	99	1963
β-Criptoxantinas (µg/g FW)	572	666	49	99	1386
Violaxanthin (µg/g FW)	572	666	198	99	1535
Lutein (µg/g FW)	572	666	626	99	1963
Antheroxanthin (µg/g FW)	572	666	626	99	1963
Total β-carotene (µg/g FW)	579	666	626	99	1970
Total carotenoids by HPLC (µg/g FW)	579	666	626	99	1970
HCN Total (ppm)	647		67		714
TCC by spectrophotometer (µg/g P.F.)	657	670	707	93	2127

Results

Table 2 presents a summary of the most relevant results of this study. R^2 values between NIRS prediction and actual measurements were 0.91 for TCC; 0.93 for TBC, and 0.95 for DMC, but is less efficient for HCN (0,81). Standard error of cross validation (SECV) for TCC and TBC were acceptable (1.2 and 0.8, respectively) while the residual predictive deviations (RPD) were excellent (above 3.0). These results suggest that NIRS can be used to reliably predict different variables based on fresh root samples **(Figure 2)**.

The Chromameter can also be used for pre-selection as its R^2 values were 0.58 for TCC and 0.64 for TBC, but further research needs to be done to improve the prediction equations. If samples analyzed have lower carotenoids, levels the R^2 values improve considerably, but that is the situation where naked eye assessment also becomes very reliable.

Table 2. Efficiency of predicting equations from NIRS to explain different root quality parameters such as dry matter content (DMC), cyanogenic potential (HCN), total carotenoids content (TCC) and total β-carotene (TBC) expressed on a fresh weight (FW) or dry weight (DW) basis.

	N	Mean	SD	SEC	R^2	SECV	RPD
DMC	1964	31,4	6,4	1,4	0,95	1,6	**4,0**
HCN	675	789	636	275	0,81	309	2,1
TCC-Spect. FW	2024	9,5	3,9	1,2	0,91	1,2	**3,3**
TCC-Spect. DW	1973	30,4	12,9	4,2	0,90	4,3	**3,0**
TCC-HPLC FW	1858	10,0	4,4	1,4	0,90	1,5	**3,0**
TCC-HPLC DM	1827	32,0	14,0	4,7	0,89	5,1	2,8
TBC FW	1846	5,7	3,0	0,8	0,93	0,8	**3,6**
TBC DW	1791	18,3	9,8	2,7	0,93	2,8	**3,4**
Phytoene FW	1164	2,7	1,9	1,0	0,71	1,0	1,8
Phytoene DW	1137	8,5	6,4	3,4	0,72	3,5	1,8
Provitamin A	1894	7,3	3,5	1,0	0,91	1,1	**3,2**

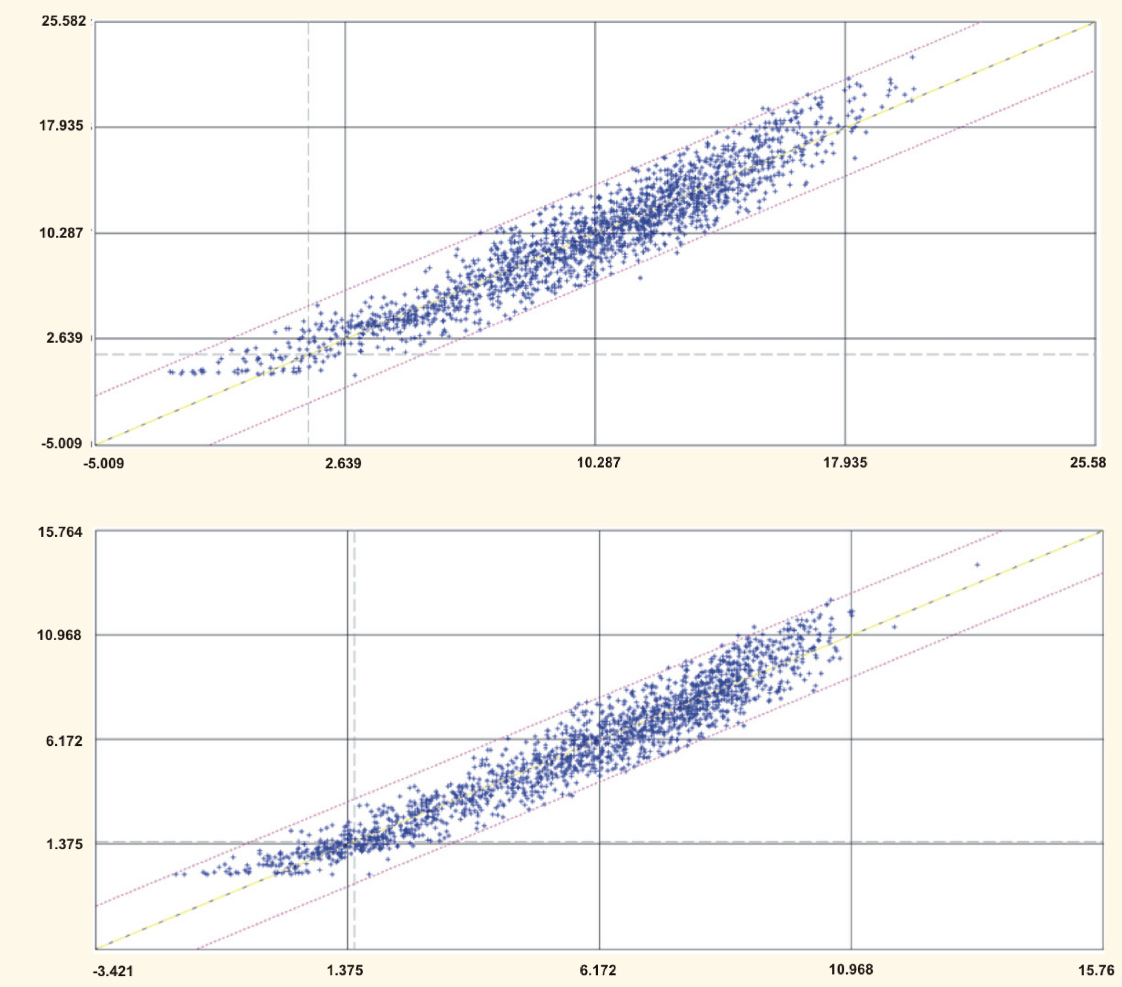

Figure 2. Relationship between predicted (NIRS) and measured values (from HPLC) for total carotenoids content (top) and total β-carotene (bottom). Data on fresh weight basis.

Conclusions

1. NIRS can be efficiently used to predict DMC, TCC and TBC in fresh cassava root samples. Previous work (Bonierbale *et al.*, 2009; Davey *et al.*, 2009; Tumwegamire *et al.*, 2011) was done on **dried** biofortified roots, tubers and bananas.
2. The ChromaMeter can be used only as a pre-selection tool until better predictions can be made with the trichromatic lectures it provides.
3. Similar results were obtained in chopped or grinded fresh root samples (not appreciable difference in spectra).

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