Dataset Report

Work Package 3
High-Throughput Phenotyping Protocols (HTPP)
Deliverable 3: Report On Existing Calibrations For Quantitative & Qualitative Traits (Raw Sweetpotato – Dried And Milled)

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Work Package 3

High-throughput phenotyping protocols (HTPP)

DELIVERABLE 3: Report on existing calibrations for quantitative & qualitative traits **(raw sweetpotato – DRIED and milled)**

General identification

Constituents	Protein, Starch, Iron, Zinc, Glucose, Fructose, Sucrose, Maltose, beta – carotene
Institute/Partner	CIP
Country	Peru, Uganda, Ghana, Mozambique
Project (s)[1]	HarvestPlus, SASHA (BMGF)
Product	Sweet potato
Presentation of product	Sweet potato flour (freeze dried, milled)

Calibration description

Product	Sweet potato	
Product presentation	Sweet potato flour (freeze dried, milled)	
Constituents	Protein, Starch, Iron, Zinc, Glucose, Fructose, Sucrose, Maltose, beta – carotene	
Unit	% (Protein, Starch, Glucose, Fructose, Sucrose, Maltose), mg/100g DW (beta – carotene, Iron, Zinc)	
Number of developing year	12 years	
Number of values	n=216 for protein, n=258 for starch, n= 264 for fructose, glucose, sucrose, maltose, n=422 for iron, zinc, n=320 for beta – carotene in Peru (n=103 in Ghana, n=79 in Mozambique, n=53 in Uganda)	
Range of values	Protein Starch Fructose Glucose Sucrose Maltose Iron Zinc beta – carotene	1.7 – 9.1 % 22.3 – 73.7 % 0.1 – 19.1 % 0.0 – 28.3 % 3.0 – 44.1 % 0.0 – 2.1 % 0.8 – 4.5 mg/100g 0.5 – 3.1 mg/100g 0.0 – 157.2 mg/100g
Spectrometer Brand/model	FOSS XDS	
Spectral range	Protein Starch Fructose Glucose Sucrose Maltose Iron Zinc beta – carotene	1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 1100 to 2500nm 400 to 2500nm
Software used for calibration	WinISI II Project Manager 1.50, WinISI 4 (FOSS)	
Regression model	MPLS	

[1] Names of projects in which the calibrations were established

Numeric formats available for equation and b coefficients	WinISI 4, Excel, ASCII
Calibration published in scientific papers (DOI + Reference)	Proceedings ISTRC
Observation	

Calibration summary:

Each freeze dried and milled sample was scanned by NIRS within the range of 400 to 2500 nm using a NIRS monochromator (model FOSS 6500; NIRSystems Inc., Silver Spring, MD, USA) and using small ring cups with a sample autochanger. Calibration equation for β-carotene were developed under WinISI II Project Manager 1.50, with spectral information from 400 to 2500 nm and using modified partial least squares (MPLS) regression and cross validation techniques. Calibration equation for protein, iron, zinc, starch and individual sugars were developed with reduced spectral information from 1100 to 2500nm. The derivative and mathematical treatments were 2, 5, 5 and 1 for beta-carotene and 1, 4, 4 and 1 for protein, iron, zinc, starch and individual sugars. The first number is the derivative, the second the gap, and the third and fourth numbers are the smooth. The results of the calibration calculation were checked observing the t-outliers with t > 2.0, GH- and X-outliers >8. The number of outlier elimination passes was two. Samples with t > 2.0 were deleted from the sample file. A lower than usual t-outlier value of 2 was chosen because no extra care was taken during the reference analysis, e.g. duplicate analysis of the same samples.

Table 1. Variation of concentrations as measured by reference methods, NIRS-calibration and cross validation statistics for the content of protein, -carotene, iron, zinc, starch and individual sugars concentrations in sweetpotato in the calibration sets

Trait	Reference Values			Calibration		Cross Validation	
	Range [a,b]	Mean [a,b]	SD [a,b]	R^2_c	SEC [a,b]	R^2_{cv}	SECV [a,b]
Protein (N=216) [b]	1.7 – 9.1	4.1	1.7	0.97	0.30	0.95	0.36
-carotene (N=320) [a]	0.0 – 157.2	33.7	37.9	0.98	4.25	0.97	5.69
Iron (N=422) [a]	0.8 – 4.5	2.0	0.7	0.81	0.26	0.80	0.27
Zinc (N=422) [a]	0.5 – 3.1	1.3	0.5	0.91	0.14	0.89	0.15
Starch (N=268) [b]	22.3 – 73.7	58.0	9.3	0.97	1.41	0.96	1.58
Fructose (N=266) [b]	0.1 – 19.1	2.88	3.0	0.95	0.55	0.94	0.61
Glucose (N=266) [b]	0.0 – 28.3	3.9	4.4	0.95	0.67	0.94	0.72
Sucrose (N=266) [b]	3.0 – 44.1	13.8	6.7	0.82	2.60	0.80	2.76

SD = standard deviation, R^2_c = coefficient of determination in calibration, SEC = standard error of calibration, R^2_{cv} = coefficient of determination in cross validation, SECV = standard error of cross validation, [a] = mg 100 g^{-1} in dry weight, [b] = % in dry weight.

Report about improvement of existing Near-Infrared Spectroscopy (NIRS) calibrations to support worldwide sweet potato quality breeding

Thomas zum Felde and Eduardo Porras

The sweet potato breeding program at CIP is working on developing clones with good yield, resistances to biotic and abiotic stresses and keeping and improving high nutritional value. Ten thousands of breeding lines per year have to be analyzed for their nutritional quality. Near-Infrared Spectroscopy (NIRS) was chosen in 2006 to serve as fast and cost-effective method in estimating the nutritional components of sweet potato samples. A NIRS analytical network has been established to facilitate and homogenize the analysis of sweet potato samples in African target regions. NIRS calibration models have been developed and transferred to CIP's sweet potato breeding platforms in Uganda, Mozambique and Ghana. However, it is mandatory to maintain and extend the calibrations through the years with new samples grown under target region conditions. Furthermore, it is important to keep sampling and sampling preparation procedures before the NIRS analysis updated and on high standards.

The Quality and Nutrition Laboratory at CIP-HQ in Lima manages the NIRS network established to support "satellite" labs involved in sweet potato quality breeding in Maputo (Mozambique), Namulonge (Uganda) and Kumasi in Ghana (Figure 1). NIRS calibrations for fast and cost-effective analysis of beta-carotene, protein, starch, fructose, glucose and sucrose have been developed and maintained at CIP HQ starting in 2007. Annual extensions with samples from target regions were done to improve the robustness of the estimations of nutrients in sweet potato samples. Recent calibrations base on reference samples of n=216 for protein, n=258 for starch, and n= 264 for fructose, glucose and sucrose and serve to date at CIP-HQ and the 3 African platforms for sweet potato quality evaluation (Table 1). Around 530,000 samples were evaluated the past 12 years.

Table 1: Variation of concentrations as measured by reference methods, NIRS-calibration and cross validation statistics for protein, starch and individual sugars concentrations

Trait	Reference Values			Calibration		Cross Validation	
	Range [a]	Mean [a]	SD [a]	R^2_c	SEC [a]	R^2_{cv}	SECV [a]
Protein	1.7 – 9.1	4.1	1.7	0.97	0.30	0.95	0.36
Starch	22.3 – 73.7	58.0	9.3	0.97	1.41	0.96	1.58
Fructose	0.1 – 19.1	2.88	3.0	0.95	0.55	0.94	0.61
Glucose	0.0 – 28.3	3.9	4.4	0.95	0.67	0.94	0.72
Sucrose	3.0 – 44.1	13.8	6.7	0.82	2.60	0.80	2.76

SD = standard deviation, R^2_c = coefficient of determination in calibration, SEC = standard error of calibration, R^2_{cv} = coefficient of determination in cross validation, SECV = standard error of cross validation, [a] = % in dry weight.

For technical reasons, beta-carotene calibrations cannot be transferred from one NIRS instrument to another. Beta-carotene calibrations therefore have been developed for each African sweet potato breeding platform individually using reference HPLC values obtained at QNLAB in Lima in freeze dried and milled sweet potato samples prepared and scanned by NIRS in Mozambique, Uganda and Ghana. Responsible technicians and research assistants from CIP's African sweet potato breeding platforms were trained in their labs and at QNLAB in Lima individually on high-throughput NIRS analysis including field sampling and sample preparation procedures
(http://cipotato.org/wp-content/uploads/2014/07/006160.pdf).

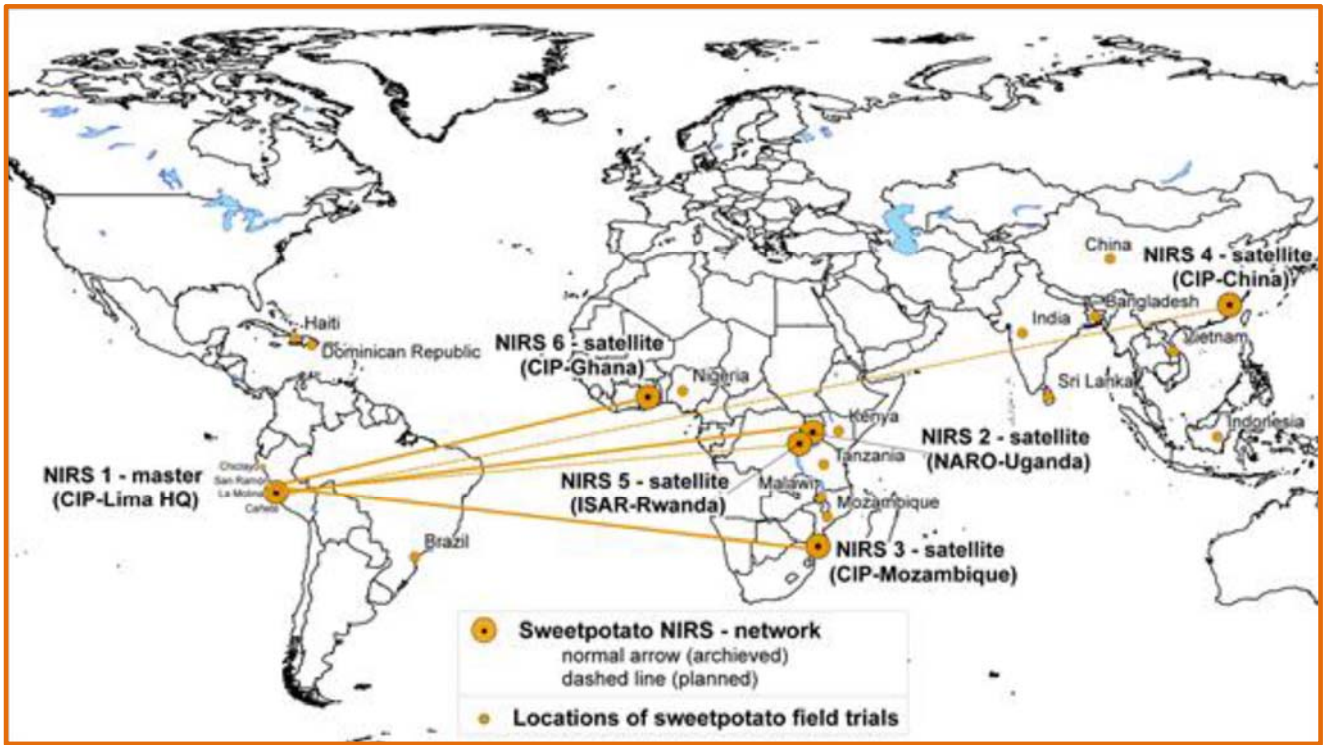

Figure 1. Established NIRS network

NIRS calibration to estimate beta-carotene in sweet potato samples **in Ghana**

A set of 103 samples coming from orange fleshed sweet potato clones grown in Ghana were analyzed in duplicate for its beta-carotene concentration by HPLC at QNLAB in Lima. Using these reference values and the NIRS scans obtained in Ghana of each sample, NIRS calibration was developed (Table 2). As indicated by the high coefficients of determination of the calibration and of the cross validation, the developed calibration is robust and was installed in the NIRS lab in Ghana for application in routine use.

Table 2: Variation of concentrations, NIRS-calibration and cross validation statistics for beta-carotene concentrations in sweet potato samples from Ghana (103 samples)

Trait	Reference Values			Calibration		Cross Validation	
	Range [a]	Mean [a]	SD [a]	R^2_c	SEC [a]	R^2_{cv}	SECV [a]
Beta-carotene	0.37 – 65.01	15.18	15.73	0.96	2.75	0.94	3.19

SD = standard deviation, R^2_c = coefficient of determination in calibration, SEC = standard error of calibration, R^2_{cv} = coefficient of determination in cross validation, SECV = standard error of cross validation, [a] = mg/100g in dry weight

NIRS calibration to estimate beta-carotene in sweet potato samples **in Mozambique**

A set of 79 samples coming from orange fleshed sweet potato clones grown in Mozambique were analyzed in duplicates for its beta-carotene concentration by HPLC at CIP QNLAB. Using the reference values and the NIRS scan of each sample, NIRS calibrations to estimate beta-carotene concentrations were developed (Table 3). As indicated by the high coefficient of determination of the calibration and of the cross validation, the developed calibration is robust. This calibration was installed in the NIRS lab in Maputo, Mozambique and is in routine use.

Table 3. Variation of concentrations, NIRS-calibration and cross validation statistics for b-carotene concentrations in sweet potato samples from Mozambique (79 samples)

Traits	References values			Calibration		Cross-validation	
	Range [a]	Mean [a]	SD [a]	R^2_c	SEC [a]	R^2_{cv}	SECV [a]
Beta-carotene	0.00 – 54.28	13.57	13.57	0.94	2.31	0.91	2.54

SD = standard deviation, R^2_c = coefficient of determination in calibration, SEC = standard error of calibration, R^2_{cv} = coefficient of determination in cross validation, SECV = standard error of cross validation, [a] = mg/100g in dry weight

NIRS calibration to estimate beta-carotene in sweet potato samples **in Uganda**

A NIRS calibration to estimate beta-carotene was developed in 2014 using a set of 53 samples coming from orange fleshed sweet potato clones grown in Uganda. These samples were analyzed in duplicates for its beta-carotene concentration by HPLC at CIP QNLAB and scanned at the new NIRS-XDS equipment in Namulonge, Uganda.

Table 4. Variation of concentrations, NIRS-calibration and cross validation statistics for beta-carotene concentrations (53 samples)

Traits	References values			Calibration		Cross-validation	
	Range [a]	Mean [a]	SD [a]	R^2_c	SEC [a]	R^2_{cv}	SECV [a]
Beta-carotene	0.00 – 85.77	42.67	14.37	0.92	4.16	0.89	4.83

SD = Standard deviation, R^2_c = determination coefficient calibration, SEC = standard error calibration, R^2_{cv} = determination coefficient cross-validation, SECV = standard error cross-validation, [a] = mg/100g on dry weight basis

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