Screening for β-carotene, iron, zinc, starch, individual sugars and protein in sweetpotato germplasm by Near-Infrared Reflectance Spectroscopy (NIRS)

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Abstract

Vitamins and minerals are often seriously lacking in human diets, especially vitamin A, iron and zinc. It is estimated that 25% of preschool age children have vitamin A deficiency; 37% and 49% of the total world population is affected by low iron and zinc intake, respectively. Impact assessment indicated that orange-fleshed sweetpotato (OFSP) can alleviate vitamin A malnutrition. Elevation of iron and zinc levels in sweetpotato can also result in an important contribution in the human diet. To support sweetpotato breeding programs, a high throughput technique was needed to simultaneously screen, in short time, several quality traits in thousands of genotypes of the sweetpotato germplasm at CIP.

NIRS calibrations, based on several hundred samples each, were developed and showed high prediction accuracy. Calibrations were applied to the screening of 1209 accessions of the sweetpotato germplasm in 2 locations: La Molina and San Ramon. Only 246 clones were considered as OFSP clones because they have more than 20mg β-carotene/100 g, DW in at least one location. The mean concentration and distribution of β-carotene, iron, zinc and protein was higher in La Molina than in San Ramon. A group of 13 clones with high β-carotene, a significant amount of iron, high dry matter and yield and a second group of 16 clones with high β-carotene a significant amount of zinc, high dry matter and yield were identified and are recommended for dissemination and evaluation in Africa.

Keywords: Sweetpotato, β-carotene, Minerals, Near-Infrared Reflectance Spectroscopy, Germplasm Evaluation.

Introduction

Sweetpotato ranks as the world's seventh most important food crop - after wheat, rice, maize, potato, barley, and cassava. Sweetpotato is mainly produced in marginal soils in low-input subsistence farming systems of developing countries where it is a major food crop and it is consumed in large quantities (Woolfe, 1992; Grüneberg et al., 2005). CIP germplasm collection contains about 6000 sweetpotato genotypes/accessions. Sweetpotato is an important source of carbohydrates and the orange fleshed varieties are a rich source in β-carotene, a precursor of vitamin A (Kang and Priyadarshan, 2007). Socio-economists and nutritionists have estimated that breeding for enhancing β-carotene, iron and zinc concentrations in sweetpotato would have a major impact on public health (Welch and Graham, 2000; Nestel et al., 2006).

To support breeding programs for sweetpotato there is a need of high throughput techniques to screen the macro- and micronutrient concentrations of the sweetpotato germplasm and to estimate the concentrations in thousands of genotypes in relative short time. For accurate analysis of micronutrients spectrophotometer, HPLC and ICP are the methods of choice. However, these methods are time-consuming, involve low sample throughput, and are expensive if thousand of samples have to be screened.

Requiring only simple sample preparation methods (drying and milling for sweetpotato) NIRS is a rapid and relatively inexpensive technique that facilitates the analysis of several traits simultaneously, and is commonly used to estimate the main organic constituents like oil, protein and starch in various agricultural products and even in the complex matrices of processed foods (Shenk and Westerhaus, 1993; zum Felde et al., 2007). The advantages to use NIRS in screening and breeding programs are various. Application of NIRS in routine does not

need chemical reagents and avoids contamination with chemical waste! Fast analysis of several traits simultaneously in less than 2 minutes per sample are possible and several hundred samples can be analyzed per day.

The objectives of this study were:

1. To develop NIRS calibrations to estimate protein, β-carotene, iron, zinc, starch and individual sugars in sweetpotato.
2. To screen for high β-carotene, iron and zinc types in the sweetpotato germplasm of CIP.

Material and methods

Development of NIRS calibrations

Reference values for protein, β-carotene, iron, zinc, starch and individual sugars were obtained in a set of 216 (protein), 320 (β-carotene), 422 (iron and zinc), 268 (starch) and 266 (individual sugars) freeze dried and milled sweetpotato samples (Table 1). B-carotene and individual sugars were analyzed by HPLC, iron and zinc were analyzed by ICP, starch was analyzed polarimetric and protein by Kjeldahl.

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 2498 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 β-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.

Screening for high β-carotene, iron and zinc in sweetpotato germplasm

The germplasm evaluation, 1209 clones in total, was carried out in two environments of Peru (La Molina and San Ramon) with two replications and ten plants per plot in 2006. The β-carotene, iron, zinc, starch, glucose, fructose and sucrose concentrations in storage roots were estimated by the developed calibrations in freeze dried and milled samples of 1209 germplasm accessions. Additional traits recorded were: Storage root yield, upper biomass yield and dry matter. Descriptive statistics of the mean concentration of all traits evaluated and multivariate analysis on both locations, San Ramon and La Molina, was done.

Results and discussion

Development of NIRS calibrations

Mean values, standard deviations and ranges of the reference values and the statistics of the NIRS calibration and of the cross-validation are shown in Table 1.

NIRS calibration equations developed on the basis of 216-422 selected samples showed high coefficients of determination for the calibrations (R^2_c) (0.81 to 0.98) with slightly lower coefficients of determination for cross-validations (R^2_{cv}) (0.80 to 0.97). The highest R^2_c and R^2_{cv} were found for β-carotene (0.98 and 0.97, respectively), starch (0.97 and 0.96, respectively), and for protein (0.97 and 0.95). The standard errors of calibration (SEC) and the standard errors in cross validation (SECV) were low for all traits (Table 1). Independent and external validations (Bonierbale et al., 2008) confirmed the values of cross validation (results not shown).

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.

Based on several hundred samples each, NIRS calibrations to estimate protein, β-carotene, iron, zinc, starch and individual sugars in freeze dried and milled sweetpotato root samples were developed. Applied calibrations are ongoing extended by including samples from different African and Peruvian environments.

The iron and zinc calibrations for freeze dried and milled sweetpotato material have high precision close to those for protein, β-carotene, starch and individual sugars. Extension of existing calibrations for freeze dried sweetpotato samples for protein, β-carotene, iron, zinc, starch and individual sugars is done every year with at least 50 samples, each. The available calibrations for freeze dried storage roots are ready to be used in a sweetpotato NIRS-network simultaneously and have been already installed on the NIRS equipment at NARO (National Agricultural Research Organization) in Namulonge, Uganda and are applied for routine analysis (Figure 1).

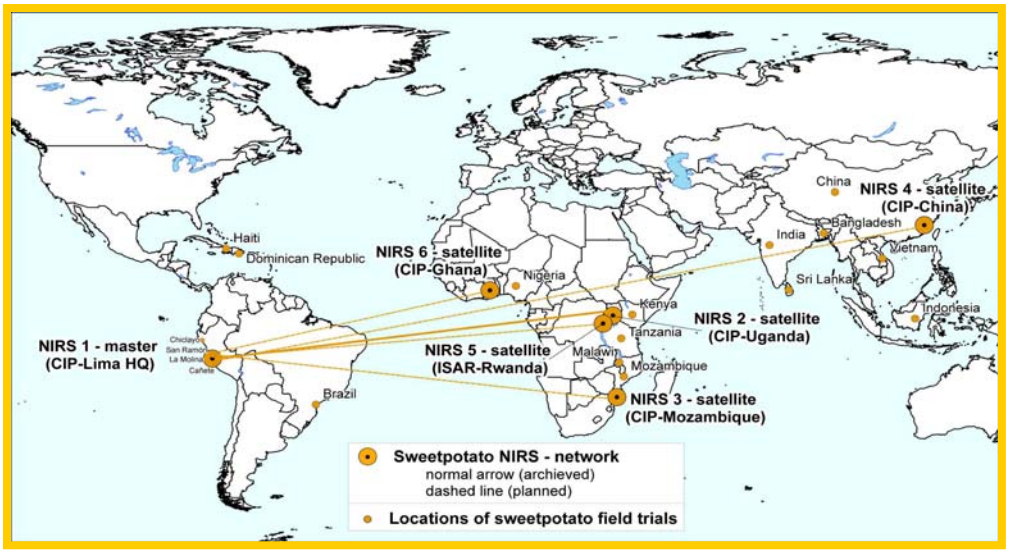

Figure 1. Established and planned sweetpotato NIRS network

Calibrations were applied for the documentation of descriptive and potentially beneficial characteristics of sweetpotato genebank accessions, the assessment of the food value of present farmers' varieties, and the selection of parents in breeding programs oriented to nutritional enhancement of sweetpotato.

Screening for high β-carotene, iron and zinc in sweetpotato germplasm

The β-carotene concentration of the 1209 clones evaluated in this study ranges from 0 to 101.05 mg/100g DW in La Molina and from 0 to 63.48 mg/100g DW in San Ramon (Table 2). Since 12 µg of β-carotene to be equivalent to 1 µg of retinal (IOM, 2001) we consider that a variety with 20 mg/100g, DW (5 mg/100g, FW, considering 25% of dry matter) provide nearly 100% of the RDI of vitamin A for children under five years old (450 µg RE/ day; FAO/WHO, 2002) and hence defined it as a high β-carotene variety. Under this assumption only 246 clones out of 1209 were considered as OFSP clones because they have more than 20mg β-carotene/100 g, DW in at least one location.

The iron and zinc concentration ranges from 1.05 to 3.94 and from 0.63 to 2.78 mg/100g DW, respectively in La Molina and from 0.72 to 2.55 and from 0.36 to 1.54 mg/100g DW, respectively in San Ramon (Table 2). The mean concentration and distribution of β-carotene, iron, zinc and protein was higher in La Molina than in San Ramon (Figure 2, Table 2). We expect that this is associated with higher nitrogen supply in La Molina. B-carotene is slightly positive correlated with iron and zinc; however protein was closely correlated with iron and zinc (results not shown).

The dry matter percentage (DM) range from 15.58 to 44.02% and the starch concentration from 29.97 to 76.15 g/100g DW in La Molina and from 15.01 to 51.08% and 27.76 to 76.12 g/100g DW, respectively in San Ramon. The individual sugars range from 0 to 12.12 mg/100g DW in La Molina and from 0 to 18.85 g/100g, DW in San Ramon for fructose, from 0 to 17.53 g/100g DW in La Molina and from 0 to 25.57 g/100g DW in San Ramon for glucose and from 0 to 35.34g/100g DW in La Molina and from 0.02 to 30.64g/100g in San Ramon for sucrose (Table 2).

Table 2. Variation of concentrations as measured by NIRS in 1209 germplasm accessions

Trait	La Molina			San Ramon		
	Min.[a,b,c]	Max.[a,b,c]	Mean[a,b,c]	Min.[a,b,c]	Max.[a,b,c]	Mean[a,b,c]
Protein[b]	2.97	15.46	**8.72**	1.05	6.14	**2.63**
-carotene[a]	0.00	101.05	**16.58**	0.00	63.48	**12.17**
Iron[a]	1.05	3.94	**2.19**	0.72	2.55	**1.31**
Zinc[a]	0.63	2.78	**1.48**	0.36	1.54	**0.79**
Starch[b]	29.97	76.15	**61.47**	27.76	76.12	**65.51**
Fructose[b]	0.00	12.12	**1.71**	0.00	18.85	**1.87**
Glucose[b]	0.00	17.53	**2.05**	0.00	25.57	**2.49**
Sucrose[b]	0.00	35.34	**10.86**	0.02	30.64	**11.06**
DM[b]	15.58	44.02	**30.79**	15.01	51.08	**36.43**
FYLD[c]	0.44	204.4	**35.56**	0.44	111.1	**10.40**
RYLD[c]	0.44	97.78	**18.70**	0.22	82.67	**18.64**

[a] = mg 100 g^{-1} in dry weight, [b] = % in dry weight, [c] = t /ha

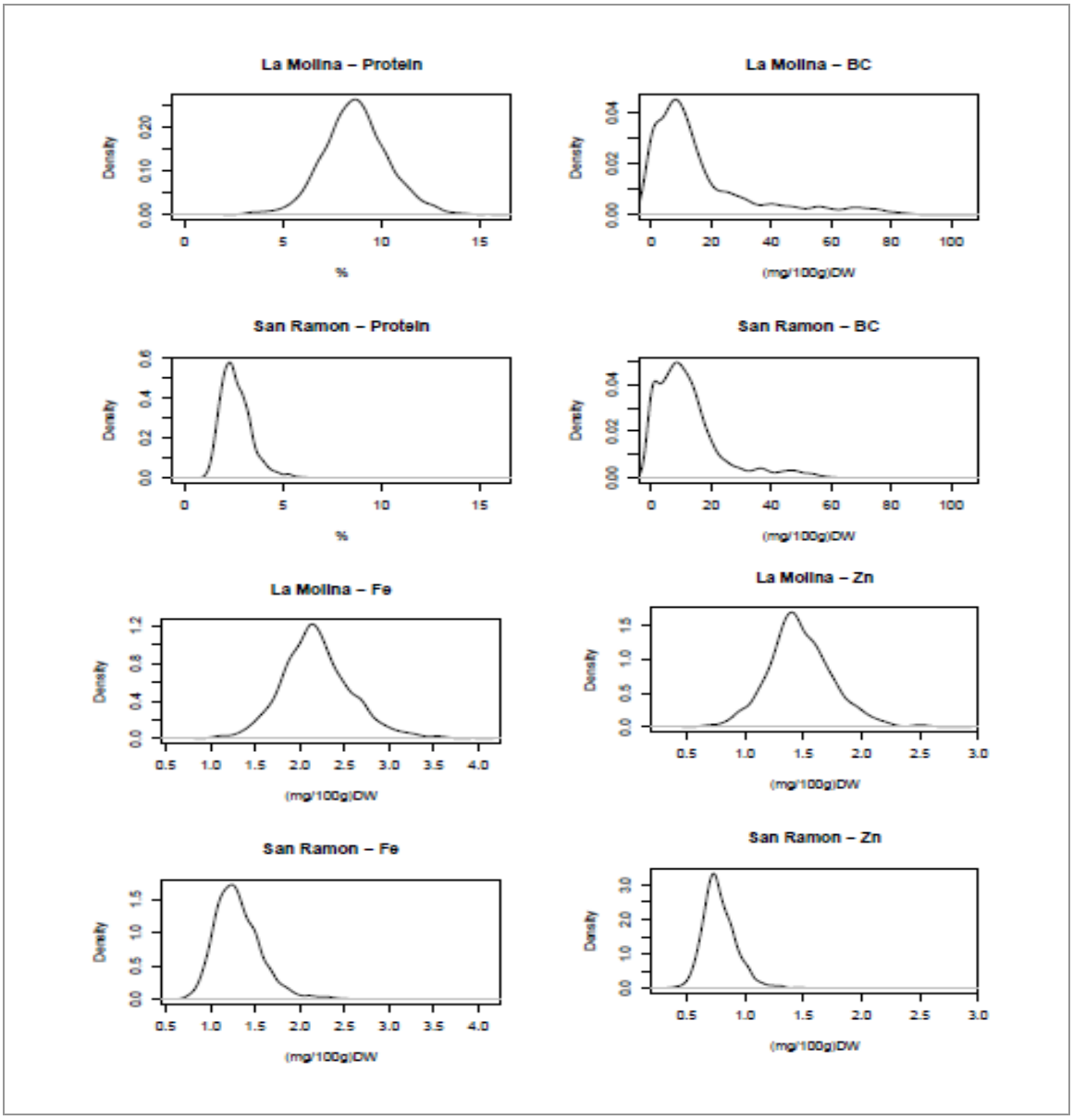

Figure 2. Density plots for protein, -carotene (BC), iron (Fe) and zinc (Zn) in San Ramon and La Molina

Multivariate analysis on each location was performed on the 1209 accessions and the 11 variables evaluated in this study. In La Molina, the 2 first principal components (PC) explain the 66% of the total variance, PC1 (41%) and PC2 (25%) (Figure 3a) while in San Ramon the 2 first principal components explain also 66%, PC1 (37%) and PC2 (29%) (Figure 3b).

In both locations, β-carotene showed similar vector directions to sucrose, iron, zinc and protein indicating positive relations between these compounds. However the β-carotene vector was opposite to the dry matter vector indicating a negative relation between β-carotene and dry matter (Figures 3a and 3b). This finding support what has been found in other studies for a reduced number of clones.

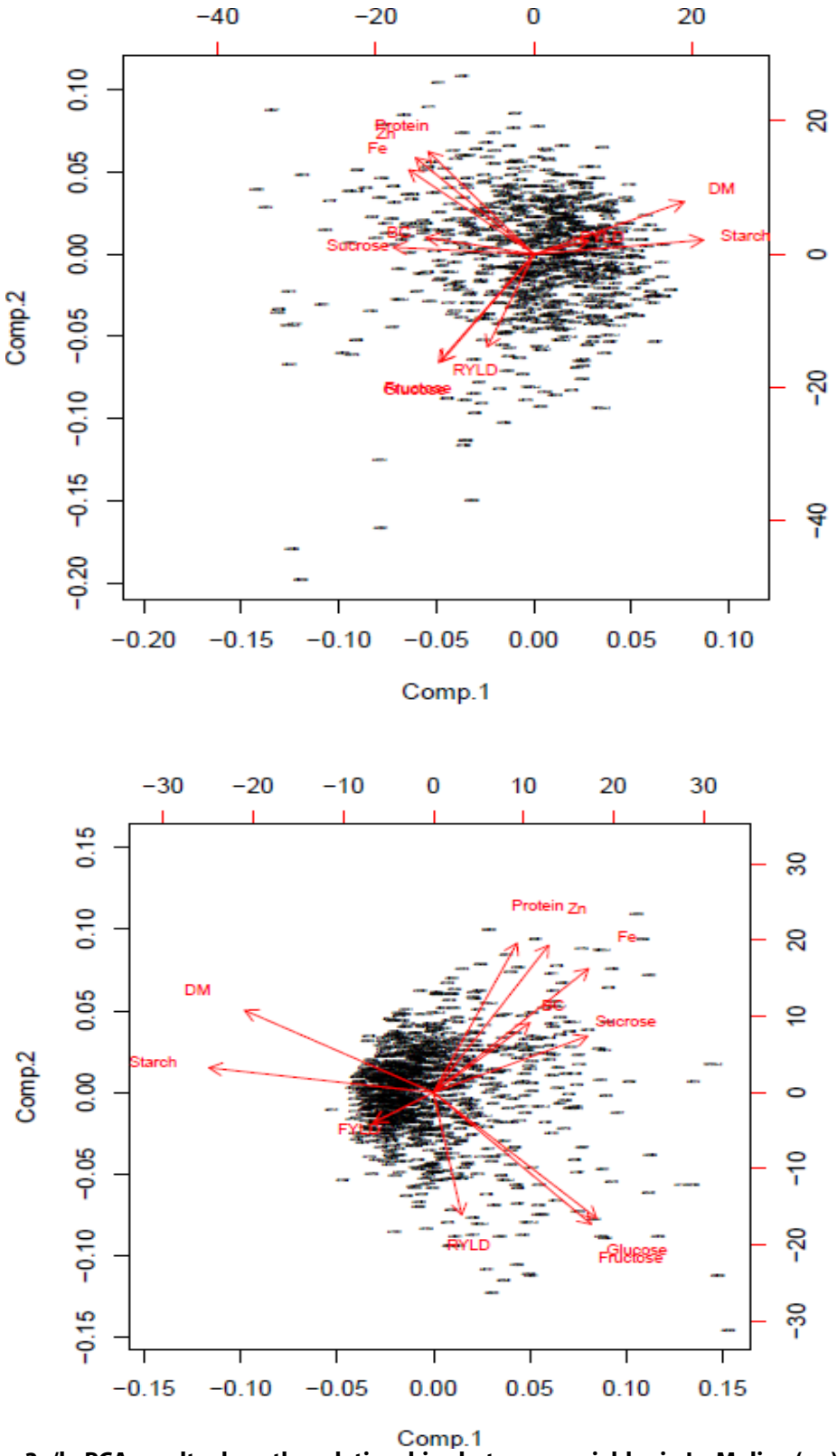

Figure 3a/b. PCA results show the relationships between variables in La Molina (up) and San Ramon (down)

A group of 13 clones with high β-carotene, a significant amount of iron, high dry matter and yield was identified. These clones were selected on the basis of single means estimations for storage root yield (>= 9 t /ha), β-carotene (>=20 mg / 100g), iron (>=1.8 mg/100g DM) concentrations and DM% (>25%) in storage roots. These clones have the following CIP-numbers: 401430, 440001, 440008, 440012, 440018, 440020, 440092, 440135, 440139, 440315, 440394, 441724, 441725.

A second group of 16 clones with high β-carotene a significant amount of zinc, high dry matter and yield was identified. These clones were selected on the basis of single means estimations for storage root yield (>= 9 t /ha), β-carotene (>=20 mg / 100g), zinc (>=1.8 mg/100g DM) concentrations and DM% (>25%) in storage roots. These clones have the following CIP-numbers: 420081, 440001, 440002, 440008, 440010, 440012, 440018, 440020, 440090, 440092, 440135, 440139, 440315, 440394, 441724, 441725.

These in total 17 different OFSP clones are recommended for dissemination and evaluation in Africa and for use in sweetpotato breeding programs.

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