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RESEARCH

High-Throughput Phenotyping and Improvements in Breeding Cassava for Increased Carotenoids in the Roots

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ABSTRACT

Past research developed reliable equations to base selections for high β-carotene on near-infrared spectroscopy (NIR) predictions (100 genotypes d^{-1}) rather than with high-performance liquid chromatography (HPLC) (<10 samples d^{-1}). During recent harvest, CIAT made selections based on NIR predictions for the first time. This innovation produced valuable information that will help other cassava (*Manihot esculenta* Crantz) breeding programs. A total of 284 samples were analyzed with NIR and HPLC for total β-carotene (TBC) and by the oven method for dry matter content (DMC). Results indicated that NIR reliably predicted TBC and DMC. In addition, 232 genotypes grown in preliminary yield trials (PYTs) were harvested at 8.5 and 10.5 mo after planting (one plant per genotype and age) and root quality traits analyzed (by NIR only). Repeatability of results at the two ages was excellent, suggesting reliable results from NIR. In contrast to previous reports, age of the plant did not influence carotenoids content in the roots. The availability of a high-throughput NIR protocol allowed comparing results (for the first time) from seedling and cloned plants from the same genotype. Results showed very little relationship for DMC between seedling and cloned plants (R^2 = 0.09). There was a much better association for TBC (R^2 = 0.48) between seedling and cloned plants. It is postulated that variation in the environmental conditions when seedling and cloned plants (from the same genotype) may be responsible for these weak associations. Important changes in selection strategies have been implemented to overcome problems related to a lengthy harvesting season.

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Abbreviations: AYT, advanced yield trial; DMC, dry matter content; HPLC, high-performance liquid chromatography; MAP, months after planting; NIR, near-infrared spectroscopy; PLS, partial least squares; PVAC, pro-vitamin A carotenoid; PYT, preliminary yield trial; SRT, single-row trial; TBC, total β-carotene; TCC, total carotenoid content.

Cassava is an important food source for millions of people in developing countries. Cassava is the second most important food staple (in terms of calories consumed) in sub-Saharan Africa (Nweke, 2004; Tarawali et al., 2012) and is called Africa's food insurance because it gives s yields even in the face of drought, low soil fertility, low intensity management (Dixon et al., 2003: Lenis et al., 2006), and resilience to face the effects of climate change (Burns et al., 2010; Jarvis et al., 2012). However, current cassava varieties produce roots with low levels of protein, fat, minerals, and micronutrients such as pro-vitamin A carotenoids (PVAC) (Gegios et al., 2010; Thakkar et al., 2009). Since about a decade ago, however, important efforts have been made to develop biofortified cassava with improved nutritional value in the roots (Bouis et al., 2011; Montagnac et al., 2009; Dwivedi et al., 2012; Talsma et al., 2015) and important progress has been reported (Ceballos et al., 2013: Njoku et al., 2015).

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Biofortification can be achieved through conventional breeding techniques that take advantage of the genetic variability for micronutrients in different crops (Welch and Graham, 2005; Chávez et al., 2005) but also through genetic transformation (Failla et al., 2012; Welsch et al., 2010). It represents a sustainable strategy that aims at solving the root of the micronutrient problem: a deficient diet. Fortunately, the conversion of PVAC present in cassava roots into vitamin A in humans has proven to be highly efficient (Failla et al., 2008, 2012; Liu et al., 2010; Talsma et al., 2015; Tanumihardjo et al., 2010; Thakkar et al., 2007, 2009). Narrow-sense heritability of carotenoid content in cassava roots is relatively high (Akinwale et al., 2010; Ceballos et al., 2013; Morillo-C. et al., 2012; Njoku et al., 2015) with statistically significant genotype × environment interaction, which, nonetheless, does not result in drastic changes of the relative ranking of the different genotypes (Ssemakula and Dixon, 2007). Therefore, progress in increasing carotenoid content in cassava roots has been significant during the last decade. Three- or four-fold increases in total carotenoid content (TCC) and TBC, respectively, have been realized (Ceballos et al., 2013). These gains were achieved through adequate sampling and quantification procedures as well as a gradual understanding of the influence of DMC when carotenoids are quantified on a fresh weight basis (Ceballos et al., 2012b; Rodriguez-Amaya and Kimura, 2004; Ortiz et al., 2011).

Conventional breeding requires the extraction and quantification of carotenoids, which is a time-consuming and labor-intensive activity. At CIAT, up to 10 samples per day could be analyzed by HPLC. This imposes a major limitation in the size breeding populations and on the possibility to better understand the factors influencing carotenoids content in cassava roots. For example, little information has been generated regarding the relationship of carotenoids in roots from seedling (an individual originated from a germinated seed) and cloned plants. It has also been suggested that carotenoids can increase with the age of the plant but no conclusive evidence has been published (Ceballos et al., 2013).

The potential application of NIR for predicting carotenoids content and other relevant root traits in cassava was recently described (Sánchez et al., 2014). Near-infrared spectroscopy offers the major advantage that a considerably larger number of samples can be screened per day (~100 genotypes with two spectra per genotype) compared with the relatively low number of samples that can be quantified by HPLC each day (≥10 samples). The cassava breeding program at CIAT shifted, during the harvesting seasons of 2015, from quantification of carotenoids through HPLC analysis to predictions based on NIR. This modification has allowed, for the first time, for the screening of thousands of samples, which, in turn, allowed answering important questions for a more efficient breeding of biofortified cassava. The objectives of this study were to: (i) assess the reliability of NIR predictions for carotenoids and DMC, (ii) analyze the effect of age of the plant for these two traits, and (iii) relate quantifications for the same genotypes at the seedling (plants derived from botanical seed) and cloned stages.

MATERIALS AND METHODS

Rapid-cycling recurrent selection for increased levels of PVAC in the roots relied on the selection of genotypes based solely on their high-carotene content (Ceballos et al., 2013). Selection took place at the end of the growth of seedling trials 10 to 12 mo after planting (MAP). Selected genotypes were then cloned and planted in a crossing block, which was kept in the field for 18 mo. Crosses were made to produce full-sib families. Because of large variation in flowering habit of cassava, crosses can be made starting at six through 14 to 15 MAP.

Selected genotypes are then evaluated for their agronomic performance starting with single-row trials (SRTs) with eight plants per row continuing with replicated PYTs and advanced yield trials (AYTs). Data used to answer the three questions described above were pooled from three different sources of materials described below.

Seedling Nursery

The seedling nursery was grown for 10 to 11 mo and harvested from 5 March through 14 May 2015. A total of 8264 plants were vigorous enough to produce roots that were inspected at harvest time for visual selection of intensity of pigmentation in the parenchyma. A total of 1882 genotypes were selected in the field for intense yellow pulp and brought to the laboratory. Color intensity was further assessed (under more uniform and appropriate light conditions) in the lab, and only 846 samples were selected for analysis with NIR (FOSS 6500, monochromator with autocup sampling module). Wavelength range was 400 to 2500 nm (Sánchez et al., 2014).

Roots were not stored and they were handled carefully to prevent physical damage. Root samples and extracts were protected from the light as much as possible. Harvest took place during the day in two or three batches. From each genotype, two to three commercial-size roots were harvested and combined together into a homogenous sample. This prevented the root-to-root or within-root variation reported earlier (Ortiz et al., 2011). Roots were peeled and processed into a uniform paste with a food processor (Essen Skymsen Model PA-7SE) with stainless steel tools. All samples were processed by NIR as described below. Calibrations for carotenoids predictions were described by Sánchez et al. (2014). For the first batch of roots brought to the laboratory in the morning (up to 40 genotypes), NIR data was immediately analyzed to select the six samples with maximum predicted values of TBC. These samples were processed, and carotenoids were extracted in the morning hours. Quantification of TCC was done by spectrophotometer before noon, and HPLC quantifications were performed in the afternoon hours. Root samples brought later during the day could not be processed to extract and quantify carotenoids by HPLC or spectrophotometer because of time constraints.

From the total of 846 samples processed by NIR, only 197 were also processed by HPLC. This dual quantification by NIR and HPLC served two purposes: (i) validate the reliability of NIR predictions and (ii) provide further data points at the high TBC–TCC end, so that prediction by NIR could be further improved for the harvests of next year. The 197 samples analyzed by HPLC were also processed to quantify DMC by the oven method at 105°C for 24 h.

Cloned Genotypes in Single-Row Trials

The first evaluation for agronomic performance is the SRT, which typically has eight plants (Ceballos et al., 2012a). This is also the first stage where cloned plants can be evaluated for nutritional quality as well for DMC in the roots. Two SRTs were planted at different times in 2014. The first SRT was planted on 17 June and included 630 genotypes. The second trial was planted on 21 July and had 502 genotypes. The two trials were harvested between 9 and 19 June 2015. Not all genotypes from the SRTs were evaluated for carotenoids content, but only those that showed acceptable agronomic performance. A total of 379 samples were analyzed for dry matter and carotenoids contents. Root samples were handled following the same procedures described for the seedling nurseries: two to three commercial-size roots were harvested per genotype, peeled, and homogenized with a food processor.

Cloned Genotypes in Preliminary Yield Trials

A total of 288 genotypes were planted in three different PYTs in Palmira for agronomic evaluation. In addition, a multiplication plot with 15 plants per genotype had been planted. These multiplication plots were used as source of roots for assessing the age of the plant effect on TCC, TBC, and DMC. The first harvest took place by the end of February 2015 (when plants were about 8.5 mo old) when one plant was harvested and the roots processed (as described above) for screening through NIR. A second plant from the multiplication plot was harvested 19 to 21 May (~10.5 MAP) and processed in the same way. Handling of root samples was done following the procedures described above.

Cloned Genotypes in the Crossing Nursery

Every year, a new crossing block is planted with materials selected because of their high carotenoids content. Usually, each genotype is represented by 10 plants. Root samples from a total of 270 genotypes in the crossing nursery were analyzed from 22 May through 1 June 2015. Handling of root samples was done following the procedures described above.

Cloned Genotypes from Special Families for Inheritance and Molecular Markers Studies

Two full-sib families were selected because of their size and wide segregation in carotenoids content for the identification of molecular markers. These families were harvested after all the other materials had been harvested. A total of 87 genotypes were included in these families and have complete data from seedling and cloned versions of each genotype.

Carotenoids and Dry Matter Quantification by Near-Infrared Spectroscopy

All samples described in this study were processed by NIR (FOSS 6500, monochromator with autocup sampling module). Wavelength range was 400 to 2500 nm (Sánchez et al., 2014). Carotenoid contents improved progressively across years. To account for these increasing maximum levels, in the analysis we employed the LOCAL regression algorithm rather than using predicting equations based on classical partial least squares (PLS) regression. The specificity of the data, with increasing content of the constituent of interest year after year, clearly showed the limitation of PLS regression approach. The increasing range of the constituent forced the model to work in extrapolation inducing a greater error of prediction. The LOCAL procedure is designed to search and select *n* samples similar to the sample to predict. The *n* samples are then used to develop a model (based on PLS regression) specific to the sample being analyzed providing more accurate predictions (Davrieux et al., 2016; Shenk et al., 1997).

Carotenoids were extracted and TCC quantified using spectrophotometry and HPLC following the method described by Ceballos et al., (2012b) and Sánchez et al. (2014). Different carotenoid pigments were also quantified with HPLC, which allowed estimation of TBC. Carotenoids were extracted and quantified only in few samples each day. A sample from roots (either chopped or a paste) was taken for the quantification of DMC. To estimate it, two samples of ground root tissue (total combined weight of 55–65 g) was dried in an oven at 105°C for 24 h. Dry matter was expressed as the percentage of dry weight relative to fresh weight.

RESULTS

Reliability of Near-Infrared Spectroscopy Predictions for Carotenoids and Dry Matter Content

Average TBC values of the 846 samples from the seedling nursery screened by NIR was 10.7 $\mu g\ g^{-1}$ (fresh wt. basis) ranging from 4.4 to 17.9 $\mu g\ g^{-1}$. As explained in the Materials and Methods section, data from 197 samples was available from NIR prediction and HPLC (TBC), spectrophotometer (TCC), and oven (DMC) quantifications from the seedling nursery. In addition, during the harvesting season, 87 cloned genotypes (24 clones from the PYTs, 42 clones from the crossing block, and 21 from the special families) were simultaneously analyzed with NIR and HPLC. Therefore, the data available to assess the predictive precision of NIR were based on 284 samples for carotenoids content. For DMC, there was missing information for one sample and therefore, $n = 283$ for this trait. Figure 1A presents DMC predictions by NIR and the actual data obtained by drying two samples (40–60 g) in the oven. The predicting accuracy of NIR is demonstrated by a large coefficient of determination ($R^2 = 0.82$). There are just a couple of data points in which NIR overestimated DMC in this large sample of genotypes. Figure 1B illustrates the relationship between NIR predictions and HPLC quantifications for TBC, the most relevant nutritional trait. The predictions by NIR were satisfactory with a coefficient of determination value of $R^2 = 0.74$.

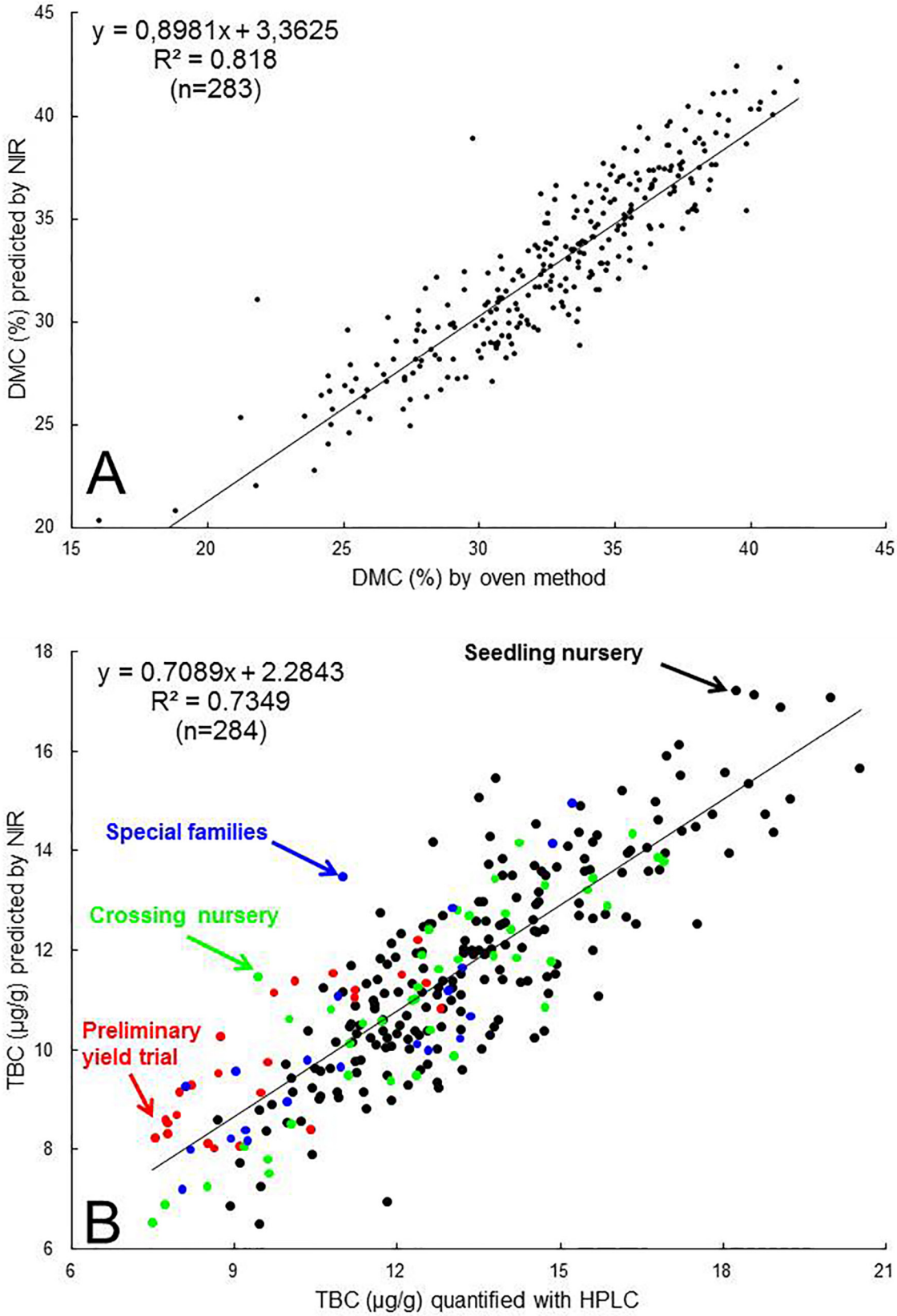

Fig. 1. Precision of predictions made by near-infrared spectroscopy (NIR). (A) Dry matter content (DMC) by NIR compared with estimations based on the oven method; (B) Total β-carotene (TBC) predicted by NIR on a fresh weight basis compared with high-performance liquid chromatography data. Black dots indicate data from the seedling nursery harvested in 2015. In blue is the information coming from the special families, whereas green points are those from the materials currently in the crossing block. Finally, in red, are clones included in the preliminary yield trials.

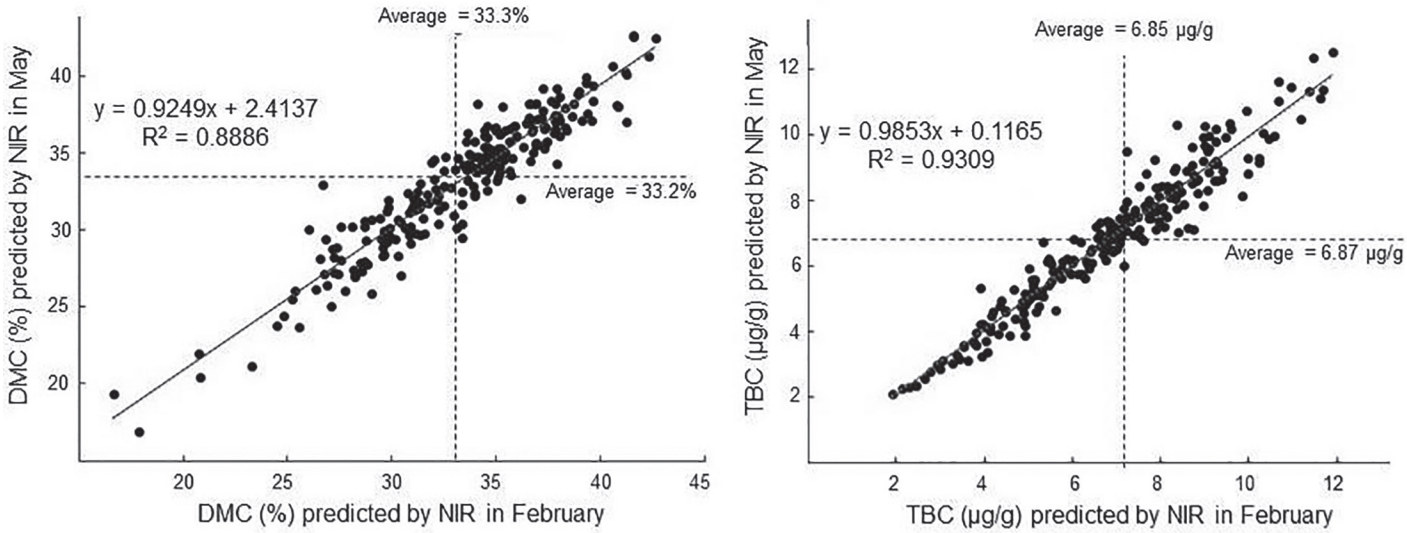

Fig. 2. Predictions for dry matter content (DMC, on the left) and total β-carotene (TBC, on the right) by near-infrared spectroscopy (NIR) in 232 genotypes harvested at 8.5 (February) and 10.5 (May) months after planting.

The actual average TBC quantified through HPLC was 12.9 μg g^{-1}, whereas for NIR predictions the average was 11.4 μg g^{-1} indicating an underestimation of actual TBC values by NIR. There were only a few cases where NIR overestimated TBC. In general, the higher the TBC values the larger the underestimation by NIR (data not presented). The NIR predicting equation for carotenoids content in cassava root needs to be continuously improved as the maximum levels keep increasing year after year, a unique situation for NIR use. This is precisely the reason why the samples with maximum predicted values were processed to provide actual HPLC data that would help improving the precision of the predictions. Also, this is the reason why the LOCAL regression algorithm is used instead of the standard prediction equations based on PLS regression (Davrieux et al., 2016). If data were split between samples from seedling (197 observations) and cloned plants (87 genotypes), the relationship between NIR and HPLC data was the same, indicating that the predicting capacity of NIR was equally satisfactory in seedling and cloned plants (data not presented).

Figure 1B identifies the different samples analyzed with distinctive colors. Low-TBC samples involve older genotypes (preliminary yield trials, crossing nursery, and the special families), whereas the black dots identify data from 2015 seedling nursery (newer genotypes). The highest levels of TBC come from the latter, illustrating the progress achieved increasing TBC in the latest cycle of selection.

Effect of Age of the Plant in Total Carotenoid Content, Total β-Carotene and Dry Matter Content

The potential effect of age of the plant on TBC and DMC was assessed using data from the PYTs. Figure 2 presents NIR predictions for these two variables for samples from 232 genotypes harvested at 8.5 and 10.5 MAP. The analysis of data is based on values predicted by NIR. Two features in this figure are worth emphasizing. For both variables, NIR predictions seem to be very consistent, which suggests a good repeatability of measurements even when taken 2 mo apart from each other. A second conclusion that can be drawn from these figures is that there does not seem to be much change for TBC or DMC as the plant ages. Average DMC was 33.2 and 33.3% in the February and March harvests, respectively. Similarly, TBC averages were 6.85 and 6.87 μg g^{-1} respectively for these two harvesting dates.

Nutritional Value of Roots from Seedling and Cloned Plants From the Same Genotypes

It has always been assumed that nutritional quality of cassava roots would not change drastically between seedling and cloned plants from the same genotype. This assumption was based on the observation that genotypes whose roots had yellow parenchyma at the seedling stage would also show the same pigmented coloration when plants were cloned. However, little quantitative data has been published, as efforts had to be invested in quantifying new segregating populations. Near-infrared spectroscopy predictions allowed us, for the first time, to quantify a large number of samples in a single season. To answer the question of the relationship between carotenoids content and DMC quantified in seedling and cloned plants from the same genotype, a large dataset was created using NIR predictions from the different sources of cloned plants described in the Materials and Methods section, and grown in 2015, and the original data from the seedling stage of the same genotypes, which comes from different years.

Figure 3A illustrates the relationship of DMC at the seedling stage as well as in cloned plants from the same 717 genotypes. There is a positive association, as expected,

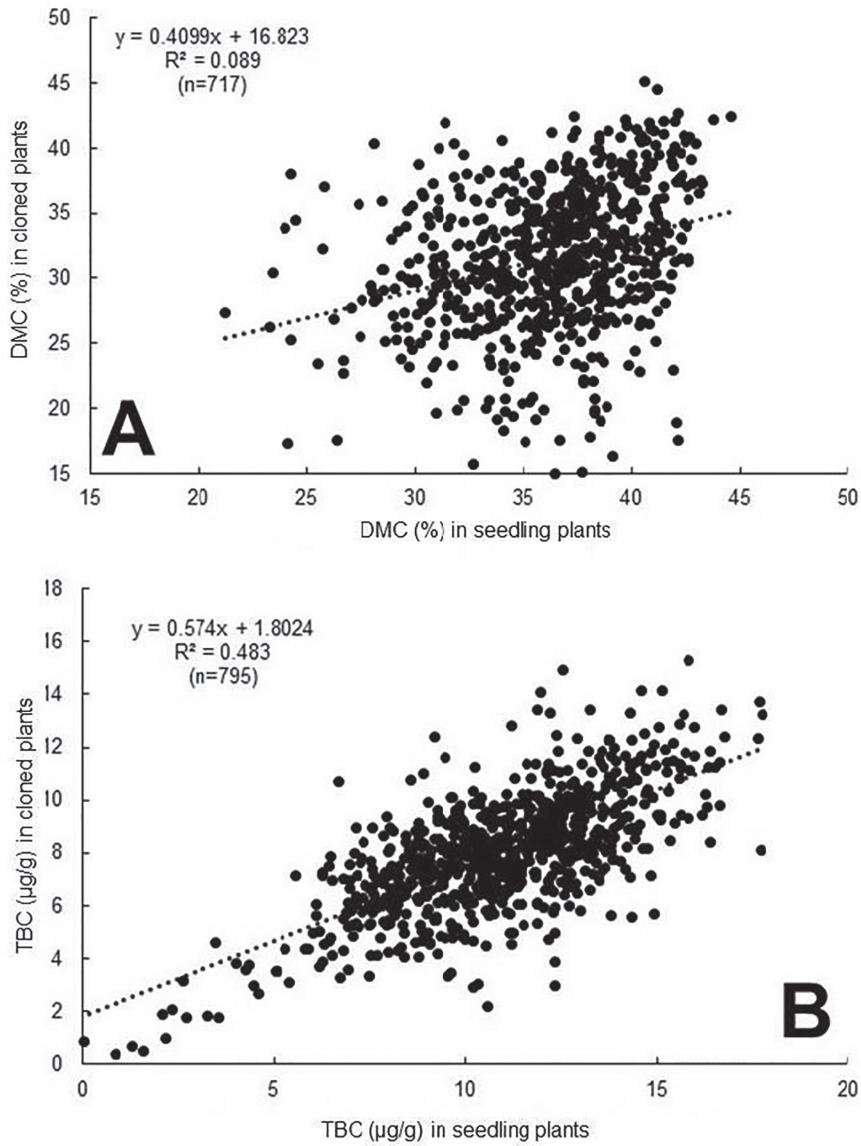

Fig. 3. Relationship between seedling and cloned plants from the same genotypes. (A) Predictions by near-infrared spectroscopy (NIR) for dry matter content (DMC); (B) Predictions by NIR for total β-carotene (TBC) on a fresh weight basis. Cloned plants data taken in 2015. Seedling plants data taken from nurseries harvested 2009 through 2014.

but the coefficient of determination was negligible (R^2 = 0.089). Average at the seedling stage (36.1%) was considerably higher than in cloned plants (31.5%). This is a surprising result, as it has always been assumed that DMC at the seedling stage and after the genotypes are cloned was relatively high. However, there is limited information published in this regard (CIAT, 1988).

Figure 3B provides the information for TBC (predicted by NIR) in 795 genotypes quantified at the seedling stage and then as cloned plants. There is a positive association with a coefficient of determination (R^2 = 0.48), clearly better than for DMC but still lower than expected. More importantly, average TBC values at the seedling stage were considerably larger (10.86 µg g^{-1}) than in cloned plants (8.04 µg g^{-1}). Quantification of carotenoids on a fresh weight basis has been requested by nutritionists to facilitate and standardize retention and bioavailability studies. Quantifying carotenoids on a fresh weight basis, however, is drastically influenced by fluctuations in DMC (Ceballos et al., 2012b, 2013). Converting TBC on a fresh weight basis into a dry weight basis improved the relationship between seedling and cloned plants but not by much (R^2 = 0.50, further data not presented).

The reduction of TBC values from seedling to cloned plants from the same genotype tends to increase with higher levels of TBC (data not presented). This makes sense, as samples with higher initial levels of TBC (e.g., at the seedling stage) have more space to diverge at the cloned stage. When TBC at the seedling stage was >14.0 µg g^{-1}, TBC values for the respective samples in cloned plants were nerve higher than at the seedling stage.

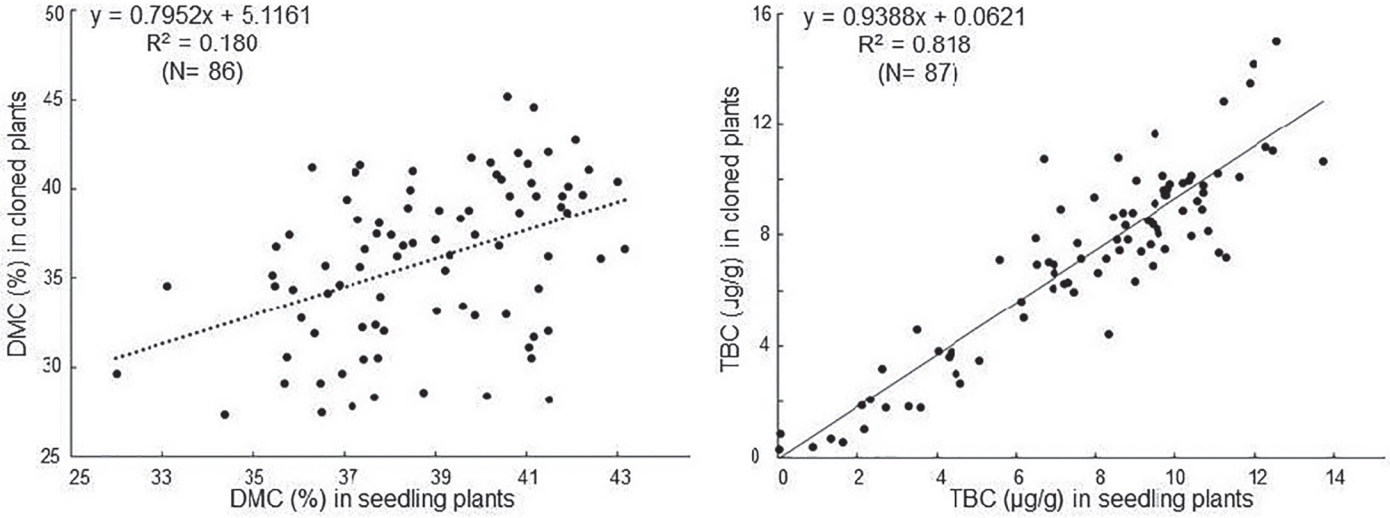

Fig. 4. Relationship between seedlings and cloned plants from the same genotypes in two full-sib families. (A) Predictions by near-infrared spectroscopy (NIR) for dry matter content (DMC); (B) Predictions by NIR for total β-carotene (TBC) on a fresh weight basis.

Figure 4 presents information of a different study that was performed after the main harvests reported earlier in this article. The information in Fig. 4 comes from 87 genotypes of two special full-sib families that have been selected for genetic studies in the segregation and inheritance of carotenoids content in cassava roots. Two features make this study distinctive. The analysis of the two families at the seedling stage took place between 11 and 18 June 2014. The same genotypes, but as cloned plants, were evaluated between 30 June and 1 July 2015. The environmental conditions at the seedling and cloned stages were therefore more uniform than previous data. The second distinctive feature of this analysis is that for TBC, all genotypes were evaluated regardless their carotenoids content (e.g., roots with white parenchyma were also analyzed). The coefficients of determination for DMC and TBC (R^2 = 0.18 and 0.82, respectively) were considerably better that the equivalent information presented in Fig. 3A and 3B (R^2 = 0.09 and 0.48 for DMC and TBC, respectively). These improvements could be partially explained because the timing of evaluation was more uniform.

To better understand the timing of harvesting and rain patterns in CIAT Experimental Station, Fig. 5 presents the historic monthly precipitation averages (1980–2014) as well as the data from 2015, which showed an unusual pattern as a result of the El Niño phenomenon.

DISCUSSION

Results presented in this study are relevant for breeding biofortified cassava and other root and tuber crops. The use of NIR allows a significant increase in the number of samples that can be screened with acceptable levels of precision.

Regarding the reliability of NIR predictions for carotenoids and DMC, results presented confirm that they are indeed precise enough for selection purposes (Fig. 1). Data comes from different kinds of trials, including seedling and cloned plants, and harvests taking place throughout a lengthy period of time. The relative efficiency of NIR predictions for DMC and TBC was expected. The population of data points for DMC is not expected to change drastically over time with averages around 35% and a range of variation from 20 to 44%. Every cycle of selection provides an additional number of samples but for a relatively fixed biological condition. A reasonable target for DMC is 35 to 40%, but it is unlikely that further progress beyond these values is feasible. On the other hand, TBC has been increasing constantly during the past decade (Ceballos et al., 2013) and, as maximum levels attained keep increasing, only a few representative samples are available for the NIR equations to properly predict these higher values. This is a reasonable explanation for understanding why the underestimation of TBC by NIR tends to be higher at higher levels of TBC (quantified by HPLC). To adapt NIR to this constantly moving target (maximum levels for TBC) the LOCAL regression algorithm is being implemented for more efficient predictions (Davrieux et al., 2016). This is a rather unique situation for the applications of NIR.

The repeatability of measurements at different ages of the plant presented in Fig. 2 suggests stable and reliable NIR spectra for TBC and DMC. Results from this study failed to provide evidence that there is a significant change in DMC and TBC as the plants aged from 8.5 to 10.5 MAP contrary to previous suggestions made by Ceballos et al. (2013). Tackling this problem is difficult, as TBC is influenced by DMC and, in turn, DMC is strongly influenced by the environmental conditions. Dry matter content typically reaches a maximum at the end of the dry period, just before the arrival of the rains (Ceballos et al., 2012a). With the arrival of the rains, cassava restarts growing, and to do so, it recycles the energy stored in the roots. As a result, DMC usually drops by >5% for several weeks after the arrival of the rains. Then, as the plants start to

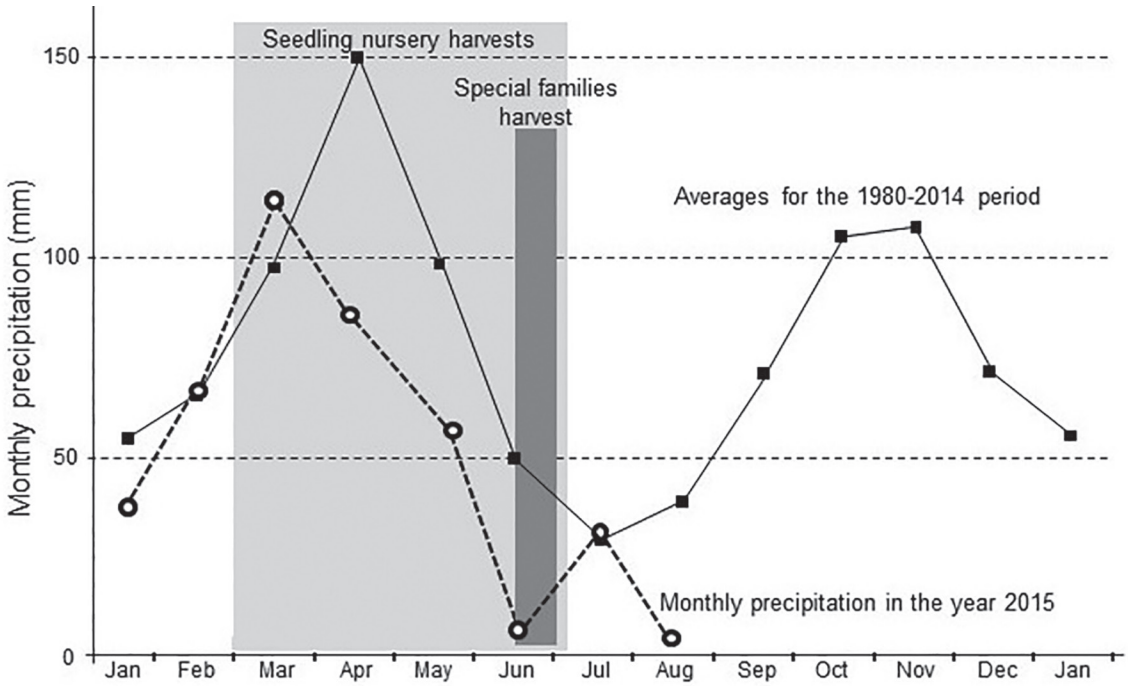

Fig. 5. Average monthly precipitation (mm) at CIAT Experimental Station and the timing of seedling nursery harvests every year compared with the timing of the harvest of the special families that took place at the end of the harvesting season. Solid lines show the historic averages (1980–2014), while dashed lines present the rainfall data for year 2015.

store starch in the roots, DMC gradually increases again. Unfortunately, the harvest of trials is conducted around the critical time of the transition from the dry to the wet seasons (Fig. 5). To make matters worse, not all genotypes respond similarly to the change of seasons, therefore, creating large genotype × environment interactions that likely contribute to variation of results. The harvests to study the effect of age of the plant took place in February (dry season) and then in May, well after the arrival of the rains. Year 2015, however, showed an unusual rainfall pattern as a result of the strong El Niño phenomenon. The peak of the first rainy season moved ahead 1 mo and was considerably lower than average values (Fig. 5).

Harvests to produce biofortified cassava at CIAT during the past decade lasted for 3 to 4 mo (Fig. 5). The harvest had to be extended considerably so that an appropriate number of samples could be analyzed. Unavoidably, some samples were harvested at the end of the dry season (e.g., March) and some later after the arrival of the rains (May–June). The problems described above in relation to the interactions between TBC, DMC, age of the plant, and timing of harvesting in relation to the seasons is likely to have affected the data presented in Fig. 3. It is clear that the relationship between data taken at the seedling stage and in cloned plants is better for TBC than for DMC. In a way, this was expected, as there are many reports in the literature linking fluctuation in DMC to the environmental conditions, particularly the arrival of the rains or increase of temperature during the spring in subtropical conditions (Ceballos et al., 2011; Kvitschal et al., 2009; Sagrilo et al., 2008). The negligible coefficient of determination for the relationship between DMC in seedling and cloned plants presented in Fig. 3A contradicts reported data for the same experimental station: DMC in seedling and cloned plants had a correlation of $R^2 = 0.59$ when yield trials were harvested at the recommended time (CIAT, 1988). In the current study, the correlation coefficient was considerably lower ($R^2 = 0.09$). It is suggested that, in part, the weak association for DMC between seedling and cloned data from the same genotypes is due to the unusually long harvesting period used for the screening of the seedling nurseries over the years as well as the different trials to obtain data on cloned plants during 2015. Total β-carotene quantifications will be affected, as reported in the literature, by fluctuations in DMC (Ceballos et al., 2012b), which suggest that the actual relationship for TBC between seedling and cloned plants of the same genotype may in fact be better than the regression presented in Fig. 3B ($R^2 = 0.483$).

The performance of the two special families would support the hypothesis that the lengthy harvesting season weakens the relationship between seedling and cloned plants from the same genotype. As illustrated in Fig. 4, the coefficient of determination for TBC increased considerably (from 0.483 to 0.818), while those for DMC also increases (from 0.089 to 0.180).

Results from this study led to the implementation of several modifications for breeding biofortified cassava. Changes begin with the planting time of the seedling nursery. Previously, botanical seeds for the seedling nursery were germinated in February to March each

year. Germinated seedlings were transplanted to the field around April to May (2 mo after germination) and grown for about 11 mo when they were harvested. These dates and ages are not exact and activities were conducted in batches so plants would be harvested across a 4-mo period but still be ~11 mo of age at harvest time. Starting in 2015, however, seeds will be germinated in September (just one batch, as harvesting will be concentrated in 1–2 mo) and transplanted in November. Seedling plants will be harvested in April to May. Since these plants are relatively young, only three stem cuttings can be taken and planted. No data will be taken at this time, although selection for acceptable vigor and high-heritability traits, such as resistance to thrips (Thysanoptera), will take place. In Africa, a selection against cassava mosaic disease could be made at this stage. Selection against plants producing roots with white, cream, or pale yellow parenchyma may also take place.

In February through March the following year, the three (cloned) plants from each genotype would be ready for harvest (11 MAP on average). However, only one of them will be harvested for quantification by NIR during the dry season (February–March), which is ideal, particularly for DMC. Selection will be made, as usual, for high TBC and adequate DMC levels based on NIR predictions. The remaining two plants of each selected genotype will be harvested late in April to May for the usual planting time around May to June. There are several advantages in implementing these changes: (i) whatever the difference between seedling and cloned plants is, it will no longer affect the selection process because selection will be made on cloned plants not seedlings; (ii) NIR predictions allow for a shorter harvesting season (1–2 mo depending on the number of genotypes that need to be screened), therefore offering a more uniform condition (regarding age of the plants and environmental conditions at harvesting time; (iii) because there are three plants per genotype available, quantification of carotenoids can be made in the ideal conditions (one plant harvested by the middle of the dry season), but harvesting of plants that serve as source of planting material is delayed until the arrival of the rains, thus generating a short storage period of the planting material and, therefore, optimal conditions for the agronomic performance trials that follow; and (iv) the availability of two standing plants in the field as source of planting material for the following agronomic evaluations offers the possibility of planting two separate SRTs in two different locations. Growing seedling plants for only 6 mo was an established practice for the cassava breeding program at CIAT and was called F1C1 (Jennings and Iglesias, 2002).

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