DEVELOPMENT

Measuring Provitamin A Content in Crops

Hernan Ceballos (CIAT) and Elizabeth Parkes (IITA)

Cassava is grown in areas where mineral and vitamin deficiencies are widespread, especially in Africa. A marginal nutrient status increases the risk of morbidity and mortality. Beta-carotene, the most potent and widespread form of provitamin A (1), is the predominant carotenoid in cassava, occurring as a mixture of *trans*- and *cis*-forms (2).

HarvestPlus supports breeding work to improve the nutritional quality of different crops, including increasing carotenoid content in cassava roots (3). Significant progress has been made over the past 10 years, including achieving almost a triplication of the original concentration of carotenoids in cassava roots and gaining a better understanding of the impact of processing cassava roots on bioavailability (4,5).

In the past decade, the International Center for Tropical Agriculture (CIAT) has produced thousands of segregating progenies, which were evaluated in the field. Initially, data were analyzed simultaneously by high-performance liquid chromatography (HPLC) and spectrophotometry. Both measurements were taken for purposes of comparison, although HPLC data is more informative because it quantifies various types of carotenoids. HarvestPlus has recommended HPLC as a reference method and the spectrophotometer reading for Total Carotenoid Content (TCC). Regression analysis on more than 3,000 data points showed, as expected, a very close relationship between TCC as measured by HPLC and by spectrophotometry (regression coefficient 1.07 and R^2 value above 0.93). The protocol for carotenoids quantification is well established, and data has been found to be reliable and replicable (6).

A bottleneck in breeding emerged as the visual selection for root color method implemented initially became obsolete with the gradual development of large populations with deep yellow roots. Fresh roots are needed for quantification of carotenoids, as dried samples lose carotenoids in the process of drying and/or storage (7,8). Carotenoid content can be reliably quantified (through spectrophotometer or HPLC) but only for a limited number of samples per day. In most cases, breeding projects have a defined harvesting season because dry matter content (DMC) fluctuates depending on rainfall patterns and variation in DMC affects carotenoid quantification. Together, these limitations create a bottleneck in the number of samples that can be analyzed in each cycle of selection. An efficient system for pre-selection of the few samples to analyze is, therefore, highly desirable.

There are several approaches for pre-selection. At CIAT, two prediction strategies were tested: near-infrared spectroscopy (NIRS) and Hunter color quantification with a chromameter. Predictions and carotenoid quantifications were based on fresh root samples. This is a key feature because freeze-drying equipment is not always available and there is the potential for carotenoid losses through the processing of samples, as stated above.

Predictions based on NIRS were found to be highly satisfactory (9). The R^2 values for TCC were above 0.92 and for total beta-carotene (TBC) even better (0.93). In other words, more than 90% of the variation in the quantified levels of TCC or TBC can be predicted by the NIRS. Another advantage of NIRS is that analysis of a given sample can predict several other traits, as well. DMC, for example, was very reliably predicted by NIRS (R^2 = 0.96) and improving predictions of cyanogenic glucosides content is underway (currently R^2 values are around 0.86). Drawbacks to NIRS include the expense of the equipment and the need to develop predictive equations.

The second pre-selection strategy that CIAT evaluated was the measurement of Hunter color with a chromameter. This is a simple and portable device that is considerably less expensive than the NIRS. Current predicting equations for TBC are very promising (R^2 > 0.70), independent of the levels of TBC actually quantified. For TCC, the chromameter has thus far produced less reliable results (9).

The International Institute of Tropical Agriculture (IITA) uses HPLC, spectrophotometer, and NIRS. IITA has also developed an alternative method - iCheck™ Carotene - introduced by BioAnalyt, which is used to quickly screen large populations especially at seedling and clonal stages of breeding. The test-kit consists of a portable photometer and ready-to-use reagent vials. This combination allows for cost-effective, simple, user-friendly, and rapid screening of large sample numbers, including at field locations with no electricity or refrigeration.

To use iCheck™, roots are harvested early in the morning and labeled, then washed and peeled. Samples are prepared, and 0.4 mL is taken from the slurry sample and injected into the reagent vial (iEx Carotene) included in the test kit. The vial is shaken and allowed to stand for a minimum of five minutes for carotenoids extraction before measurement is taken. Training conducted from 6–9 March 2014 showed that the reading could be taken from 5 to 60 min during which time the carotenoids are stable in the vial. The vial is inserted into the device and measured. The device displays the result in mg carotenoids per liter (mg/L). To get the concentration of TCC in cassava root, the result is multiplied by the dilution factor (total sample volume in water/sample weight).

The iCheck™ device needs to be handled carefully, but it is durable enough for field conditions. The device's calibration should be periodically checked with a solid photometric standard.

More detailed methodological information for iCheck™ will be available during the roundtable discussion.

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