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Near infrared spectroscopy, a suitable tool for fast phenotyping – The case of cashew genetic improvement

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ABSTRACT

Cashew apple (*Anacardium occidentale* L.) quality traits such as firmness, pH, total soluble sugar (SSC), soluble solid (SS), titratable acidity (TA), SS/AT ratio (flavor), vitamin C (VC), total carotenoids (TC), total flavonoids (TF), total polyphenols (TP) and total antioxidant activity (TAA) are critical factors for fruit quality assessment. We are reporting here a set of results obtained with a near infrared spectrometer using the 830–2,500 nm range, showing good prediction of the quality traits cited above in the ripe early dwarf cashew clones by partial least squares (PLS) algorithm. The intact cashew apples spectra resulted in good predictions of firmness (R^2 = 0.92; RMSEP = 0.71), pH (R^2 = 0.84; RMSEP = 0.17), SSC (R^2 = 0.86; RMSEP = 0.99), SS (R^2 = 0.90; RMSEP = 0.70), TA (R^2 = 0.96; RMSEP = 0.055), flavor (R^2 = 0.87; RMSEP = 7.5), VC (R^2 = 0.92; RMSEP = 38), TC (R^2 = 0.97; RMSEP = 0.089), TF (R^2 = 0.95; RMSEP = 0.86), TP (R^2 = 0.94; RMSEP = 27) and TAA (R^2 = 0.93; RMSEP = 34). Near Infrared Spectroscopy (NIR) is a valid approach to study the physiology of early dwarf cashew clones, and the presented NIR methodology is expected to be an alternative for cashew germoplasm banks as a tool to support the database of images spectra for a rapid and robust phenotyping.

1. Introduction

Cashew (*Anacardium Occidentale* L.) fruit belongs to the Anacardiaceae family, widely distributed and adapted species in the Northeast region of Brazil as an economically important agricultural crop (Daramola, 2013). The characterization and evaluation of the genetic relationships of accesses maintained in cashew germoplasm banks are extremely important for the conservation of their genetic diversity and their use in breeding programs.

The Brazilian Cashew Germoplasm Bank (BCGB) maintained by Embrapa Agroindustria Tropical, holds plus than 700 accessions, most of which belong *Anacardium occidentale*, and the genetic variability contained in this collection has allowed development of early dwarf cashew clones. These clones are recommended for commercial cultivation in the northeastern Brazil since the 1980s until today (Castro et al., 2011). Nevertheless, there are spreading in all regions of the country. The accessions characterization comprises the morphological, biochemical, agronomic and molecular descriptors, and this phenotyping task long evaluation time and high coasts.

The use of new phenotyping approaches as visible/near-infrared (vis/NIR) and near infrared spectroscopy (NIR) have been extensively investigated in recent years for evaluate de quality assessment of microtom tomato (Ecarnot et al., 2013), grapevine variety discrimination and evaluation of plant water status (Gutiérrez et al., 2016), nutraceutical properties of fruits as apples, pears and persimmons (Beghi et al., 2013; Eisenstecken et al., 2015; Jannok et al., 2014) and measurements of water contents, chlorophyll, minerals or products of metabolic pathways (Teixeira Dos Santos et al., 2013).

The vis/NIR and NIR are rapid and non-destructive techniques that require minimal sample processing before analysis appear to be one of the most convenient and straightforward analytical tools for study the phenotyping through relies on absorption of energy from molecules in regions of the electromagnetic spectrum (Wang et al., 2014). Chemometric methods assists plant metabolism analyses through developed prediction models to estimate the target parameters, and NIRS associated with chemometrics have become a useful tool to quickly quantify

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non-destructively phytochemicals in agricultural products (Cozzolino, 2015).

Cashew apple phenotyping for genetic breeding programmes based on genome wide selection, in general, uses the same traits investigated in this work, as soluble solid, total acidity, SS/TA ratio, ascorbic acid and polyphenols which are variables relevant for the selection of new clones of cashew apple with higher nutritional quality, attractive sensorial characteristics and extended shelf life. However, from the point of view of genomic selection programmes of cashew clones, actually is necessary only a method capable to rank cashew tree, that is, a good method that gives predictive values and not necessarily an absolute value. In this way, the NIR emerges as a suitable tool, reason which led, recently, Ribeiro et al. (2016) to investigate the NIR as technique for evaluate the quality traits as soluble solids, titratable acidity, SSC/TA ratio and ascorbic acid in cashew apples aiming provide an initial information for genome wide selection in cashew apple breeding programmes. Caramês et al. 2017 showed the NIR as technique to evaluate the main parameters of identity and quality of cashew apples nectar, aiming with a short time and a minimal sample preparation, give to the food industry a new technology capable to reduce the time and amount reagent necessary in the food analysis, thus, in this case, NIR can be exploited by agro-industrial sector as rapid tool to verify the quality control of cashew apple nectar required by Brazilian legislation.

In this work, we purpose the use of NIR spectrophotometer as a useful, robust, of low coast, and non-destructive alternative to predict the primary (firmness, pH, total soluble sugar, soluble solid, titratable acidity, SS/AT ratio), and secondary (vitamin C, total carotenoids, total flavonoids, total polyphenols and total antioxidant activity) metabolism traits of early dwarf cashew clones (commercial and belonging to the BCGB), aiming deepen the results already presented by Ribeiro et al. (2016), searching enrich the BCGB with image spectra data and help the laboratories worldwide are looking for tools for a rapid and more complete phenotyping of cashew apples clones.

2. Material and methods

2.1. Plant material

Cashew apples (*Anacardium occidentale* L.) at ripe stage were collected at Experimental Station of Embrapa Agroindustria Tropical in Pacajus (lat. 4°11′26,62″S, lon. 38°29′50,78″W), Brazil. Seventeen cashew apples genotypes with different peel color were evaluated in this work, as follow: commercial early dwarf cashew clones (CCP 76, CCP 1001, BRS 189, BRS 226, BRS 265) and early dwarf cashew clones of Embrapa Germoplasm Bank (COMUM_05101, EPACE 49, H7.1022, H7.1023, H7 1024, H7.1033, PRO 51211, PRO 51221, PRO 51232, PRO 5124, PRO 51,443 and PRO 52,022). It is relevant to know if there is a significant variability among the sampling regions. This may allow the possibility to identify one specific region for measurement, which is able to represent the whole fruit, and, at the same time, reveal some information on the distribution of the different chemical species associated with the quality traits along the fruit. To achieve better representativeness of the cashew apple inner content, the spectra were collected from top, middle and basal regions of the peduncles, one for each sampled region (Fig. 1). Then, the cashew apples were individually identified for reference analysis, which included primary and secondary metabolism.

2.2. Reference analysis

Metabolites from primary and secondary metabolism were evaluated. Firmness pulp was evaluated with a texturometer (Stable Micro Systems TA-XT2) equipped with 6 mm diameter cylindrical flat-tipped steel plunger. Results were expressed in Newton (N). The pH was measured using an automatic pHmeter (Labmeter PHS-3B®, São Paulo, Brazil) as recommended by AOAC (2005). Total soluble sugar (TSS) was

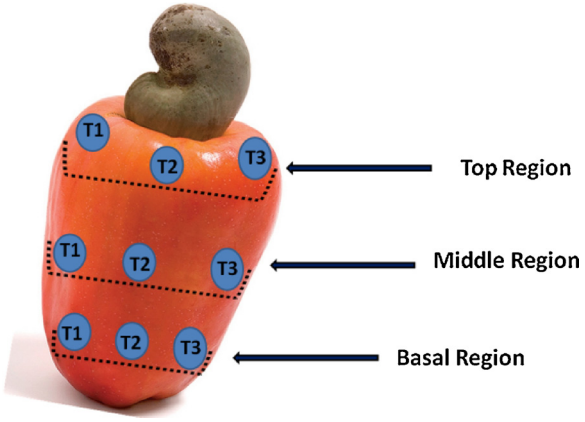

Fig. 1. Peduncle on the top, middle and basal regions of cashew apple.

quantified by Anthrone method through absorbance at 620 nm, and results were expressed as % glucose (Yemm and Willis, 1954). Soluble solids (SS) content was determined using a digital refractometer (Atago N1, USA) with automatic temperature compensation and results were expressed in Brix (concentration of sucrose w/w). Titratable acidity (TA) was determined by titration with NaOH solution 0.1N and results were expressed as a percentage (%) of malic acid AOAC (2005). Flavor is a parameter responsible for palatability and was obtained by the quotient between soluble solids (SS) and titratable acidity (TA). Total vitamin C was determined by titrating a 1.0 mL aliquot of 1:50 diluted juice with Tillman solution (2,6-dichlorophenolindophenol reagent, 0.02%) as described by Strohecker and Henning (1967). Results were expressed as mg 100 g^{-1} of fresh weigh (FW). Total carotenoids (TC) were extracted and determined as described by Higby (1962). Pulp was homogenized in isopropyl alcohol and hexane. The content was transferred to a separation funnel of 125 mL completed with distilled water, and it allowed to rest for three 30 min periods followed by three subsequent filtrations performed in cotton previously sprayed with anhydrous sodium sulphate; acetone was added and then, the volume of 50 mL was completed with hexane. Absorbance was measured at 450 nm, and the results were expressed as mg of total carotenoids per 100 g^{-1} FW. Total flavonoids (TF) were evaluated using aluminium nitrate nonahydrate according to the procedure reported by Woisky and Salatino (1998). The sample for determination was prepared by extract methanol and a mixture of (C_2H_5OH, 80%, $Al(NO_3)_3{\cdot}9H_2O$, 10% and $C_2H_3KO_2$ 1 M). After 40 min of incubation at room temperature, the absorbance was measured at 415 nm, and the total flavonoids were calculated from quercetin hydrate (Qu) calibration curve and expressed as μg g^{-1} of quercetin FW. The total polyphenols (TP) were measured by a colorimetric assay using Folin-ciocalteu reagent as described by Obanda and Owuor (1997). Before the colorimetric assay, the samples were subjected to a procedure of extraction in 50% methanol and 70% acetone (Larrauri et al., 1997). For the colorimetric assay, Folin-ciocalteu and Na_2CO_3 20% were added to extract. After incubation in the dark for 30 min, absorbance was measured at 700 nm. Gallic acid was used as the standard, and results were expressed as gallic acid equivalents (GAE) mg 100 g^{-1} FW. The total antioxidant activity (TAA) was determined using the ABTS method as described by Re et al. (1999). This method measures the ability of lipophilic and hydrophilic antioxidants to quench a 2,2′-azinobis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{*+}$) radical cation. The radical solution was formed using 7 mM $ABTS^{*+}$ and 140 mM potassium persulfate and incubated for 16 h protected from light. Absorbance at 734 nm was measured (results close to 0.700) to check $ABTS^{*+}$ formation. Once the radical was formed, the reaction was started by adding 30 μL of extract to 3 mL of radical solution. Absorbance was measured at 734 nm after 6 min, and the decrease in absorption was used to calculate the TAA. A calibration curve was prepared and different trolox concentrations

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