Genetic variability of root peel thickness and its influence in extractable starch from cassava (*Manihot esculenta* Crantz) roots

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Abstract

Cassava roots are the most important commercial product from this crop. Roots have two major components: the starchy parenchyma and the peel with higher amount of fiber and cyanogenic glucosides. In this study a sample of 64 clones grown in replicated trials in five locations were evaluated for peel thickness (PT) which ranged from 1.48 to 2.55 mm. Roots from a sample of 33 of these clones were further analyzed for the amount of extractable starch. Broad sense heritability for PT was high (0.93) compared with that for yield (0.63). The values obtained demonstrate that there is a very strong genetic component in the expression of peel thickness. Extractable starch depended heavily on dry matter content but also on PT. In an additional evaluation, 1448 accessions from the germplasm collection were evaluated for PT and showed a wide range of variation (from 0.79 to 5.14 mm).

Key words: ease of peel; fiber; root quality;

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Introduction

Cassava (*Manihot esculenta* Crantz), along with maize, sugarcane and rice, constitute the most important sources of energy in the diet of people from most tropical countries of the world. A new era for cassava research began for cassava with the implementation of successful breeding projects, modernization of cultural practices and development of new processing methods (Cock, 1985; Jennings and Iglesias, 2002, Ceballos et al, 2007a).

Except at crop establishment, cassava has no specific water stress sensitive growth stage as compared with grain crops, and shows a high degree of tolerance in areas with low and erratic precipitation (<600 mm annually). It can also produce well in dry air conditions during a great part of the growth cycle, high air temperatures, high potential evapotranspiration, low fertility soils (with particular capacity to withstand low-P conditions) and intense pest and disease pressures (El-Sharkawy, 2006). Cassava can be kept in the field until farmers need to harvest it. All these conditions make cassava a key crop for food security particularly in marginal conditions where grain crops would perish.

The most important commercial product of cassava is the storage root, full of starch. Cassava roots have a very short shelf life due to a process known as post-harvest physiological deterioration (PPD). PPD rapidly

renders the roots unpalatable and unmarketable (Reilly et al, 2007). Consequently, cassava roots need to be consumed or processed soon after harvesting (van Oirschot et al., 2000). The short shelf-life of the roots severely limits the marketing options by increasing the likelihood of losses and the overall marketing costs. Tolerance to PPD has also been recently reported (Morante et al, 2010). Other important characteristics of the root are starch quality traits (Carvalho et al. 2004; Ceballos et al, 2007b, 2008; Moorthy, 2004; Sánchez et al, 2009; Sriroth et al., 1999); cyanogenic glucosides (Andersen et al., 2000; Bokanga, 1994; Mkumbira et al., 2003); dry matter content (Cach et al., 2006; Jennings and Iglesias, 2002; Kawano et al. 1998) and nutritional quality (Chávez et al. 2005; Thakkar et al., 2007).

About 74 to 85% of dry root weight of cassava is starch (Rickard et al. 1991). Dry matter content strongly influences the amount of extractable starch from cassava roots. Therefore, starch factories usually pay differential price for the fresh roots depending on their dry matter content. The root can be divided into three distinctive parts (Rogers and Fleming, 1973): i) the outer layer, or phelloderm (commonly known as “peel”); ii) the parenchyma that contributes to the bulk of the root and contains most of the starch; and iii) a well-defined central vascular core. The peel is composed of an outer epidermis, a sub-epidermis and a thicker inner layer (Figure 1).

Variation in root peel thickness (PT) was recently reported by Kawiki (2009) in a large sample (> 800) of African genotypes including elite germplasm and landraces from Democratic Republic of Congo, Kenya, Tanzania, Madagascar and Uganda. PT ranged from 0.34 to 4.89 mm. It had also been reported earlier (Adetan et al. 2003; Rogers and Fleming, 1973). The inner layer of the peel contains starch, but it is suspected that it is more difficult to extract. The peel, which contains higher levels of cyanogenic glucosides (Bokanga, 1994), is considered a byproduct and is frequently used for animal feed. Riis (1997) suggested that thick root peel with high cyanogenic glucosides content could be useful to prevent or reduce damages by the burrowing bug *Cyrtomenus bergi* Froeschner. Peel thickness influences the ease of peeling which in many parts of the world is done manually and typically by women. Basic research of root characteristics required for mechanical peeling of cassava roots is available (Adetan et al. 2003).

The objectives of this study were to estimate broad sense heritability for root peel thickness and to establish the relationship of extracted starch with dry matter content of the roots and the thickness of the peel. This information would indirectly prove that the starch from the peel is more difficult to extract.

Materials and methods.

A set of 64 genotypes were evaluated in five different locations in the sub-humid environment of the northern coast of Colombia: Sampues (Sucre Department); Santo Tomas, Sabana Larga, Campeche and Pitalito (Atlantico Department). Trials had three replications. Each experimental plot had 25 plants (five rows with five plants each). Row spacing was 1 m and plants within the rows were planted 1 m apart from each other (10,000 pl ha^{-1}). Only the nine central plants were harvested for analysis. Trials were hand planted and standard fertilizations and weed-control measurements were made. No irrigation was provided. Harvest took place when plants were about 11 months of age (the typical harvesting age in this region of Colombia).

In situ *data collection.*

Trials were harvested following the standard procedure (Ceballos et al., 2007a). Several variables were measured: fresh root yield (**FRY**); dry matter content (**DMC-G**) by the gravimetric method (Kawano et al., 1978); plant height (**PHT**); a score for plant type (**PTY**) combining plant architecture, stay green at harvest and overall plant health was taken using a 1-5 score (1=excellent; 5=very poor); and harvest index (**HIX**) were measured in the locations where the trials were conducted (ratio of FRY over total biomass of the plant). In addition a sample of three

commercial-sized roots from three random plants from each experimental plot was taken to quantify PT. Roots were cut in the mid-section and a disc was taken. A digital caliper was used to measure PT at three different points in these root slices from the middle section of the root (**Figure 1**).

Data collected at CIAT's Experimental Station in Valle del Cauca Department.

Because of limitations on the number of roots that could be transported and processed at a given time, a set of 33 genotypes (from the total of 64 genotypes included in this study) was used for more detailed analysis on extractable starch. The sub-sample of 33 genotypes was randomly selected from the original set of 64 genotypes.

From each experimental plot a sample of about one kg of roots (typically 2-3 roots) was packed and shipped by plane to prevent PPD. Processing of the roots, therefore, could take place the day after harvest. Roots from each genotype were identified with plastic pearls of different color inserted at their proximal extreme of (neck of the root). All roots from a given entry were weighted before washing and then washed in a rotating cylinder common in many small-sized (fermented) starch factories of Colombia. This process typically rasps some of the outer epidermis and sub-epidermis and, to a lesser extent, portions of the

inner layer. Therefore the weight of the roots after washing is lower than before washing, not only because of the soil washed away but also because of losses mostly in peel tissue (average weight of roots from a given plot was reduced from 1093 down to 1007 g or about 7.9%). All entries from a given location were processed simultaneously in the same day.

After washing the roots they were regrouped per genotype (using the colored pearls for identification). Three PT measurements in the mid section of the roots were taken on each root as it was done in the *in situ* evaluations. As stated above, the washing process basically removes part (not all) of the outer epidermis and sub-epidermis therefore the PT at this stage is slightly smaller than the unwashed roots. Roots were then manually peeled with an ordinary knife and the weight of the peel (**PW**) and parenchyma recorded. This allowed estimating the relationship between peel thickness and weight. Once the weighting was made, peel and parenchyma from the roots of a given genotype were pooled together for starch extraction. The tissue (peel and parenchyma) was crushed in an Osterizer blender. The slurry was filtered through a 100µm sieve. The starch was allowed to settle and the supernatant decanted off and dried in an oven with fan-forced ventilation at 40°C during 48h (Sánchez et al, 2009). The extracted starch (**EXS**) was expressed as Kg of dry starch per Kg of fresh root.

Screening of roots from a sample of the germplasm collection at CIAT

CIAT holds in trust the worldwide germplasm collection of cassava and other Manihot species (more than 6000 accessions). In July 2010 a sample of 1448 accessions from the collection were harvested as part of ongoing efforts to characterize the entire collection (unreplicated evaluation based on four plants per genotype). Three measurements of PT were made per genotype to assess the range of variation for this variable.

Data Analysis

Statistical analysis was conducted with Statistixs (2003) software. Locations and genotypes were considered random and fixed effects, repsectively (Steel and Torrie, 1960). Standard analysis of variance and stepwise regression analyses were conducted. Broad sense heritability was estimated using the expectations of the mean squares in the analysis of variance to obtain estimates of genetic and phenotypic variances (Nyquist, 1991). For PT, plant to plant variation within a rep, root to root variation within a plant, and variations of measurements within a root do not have any bearing on the heritability values *per se* but are relevant for understanding and developing adequate sampling strategies for future work. Broad sense heritability was estimated using plot averages, which in the case of PT included as many as 27

observations (three quantifications per root, three roots per plant and three plants per plot). Heritability was estimated as follows (Nyquist, 1991):

$$h^2_{\text{(Broad Sense)}} = \sigma^2_{\text{Genetic}} / \sigma^2_{\text{Phenotypic}}$$

Variances were obtained from the analysis of variance for each variable and using the expected mean squares (Steel and Torrie, 1960). Heritability estimates were based on genotype averages across locations.

Results

General conditions of the experiments were satisfactory with adequate plant densities and normal plant growth. The average PT across the entire experiment was 1.923 mm (**Table 1**). Average PT (across the five locations) for individual genotypes ranged from 1.48 to 2.55 mm.

The average standard deviation for the three PT measurements taken in the middle of each root was 0.084 mm and ranged from 0.000 to 0.781 mm (standard deviation of three measurements averaged across 2880 roots). The average standard deviation for the PT of the three roots of a given plant was 0.138 mm and ranged from 0.000 to 0.760 (standard deviation of three roots measured per plant averaged across 960 plants).

Finally the average standard deviation for the 3 plants of a given rep was 0.127 mm and ranged from 0.003 to 0.686 mm (average of three plants sampled in a total of 320 plots) (**Table 1**).

Analyses of variance for the data collected *in situ* (PT, FRY, DMC, HIX, PTY, and PHT) on the 64 entries of this study are presented in **Table 2**. Broad sense heritability ranged from 0.63 (FRY) to 0.93 (PT). Broad sense heritability has limited value in predicting actual genetic progress as a considerable fraction of the genetic variance it is based on cannot be fully exploited by the phenotypic recurrent selection used in cassava. These h^2 values, however, are useful for understanding the relative influence of the non-genetic sources of variation in the phenotypic expression of traits. In the case of PT, heritability was estimated using the plot averages (arising from a total of 27 measurements in each plot). Genotype-by-environment interactions were highly significant ($P < 0.01$) for all traits as it was the clone source of variation. Location effects were highly significant for all traits, except for PT (non significant) and PTY (significant at 5% probability level).

Average FRY (30 t ha^{-1}) was outstanding and combined with an average DMC of 32.2% resulted in an average dry matter production of about 10 t ha^{-1}. The range of variation for average FRY across locations was 25.60 (Location 3) to 37.46 t ha^{-1} in Location 2 (**Table 2**). DMC was

generally uniform across locations (around 31.5%) except for Location 4 which had a much higher value (34.52%). Average plant height ranged from 1.75 m in Location 3 up to 2.72m in Location 1.

Harvest index has been reported as a useful tool, particularly in early stages of selection (Kawano 1990; 2003; Kawano et al., 1998). The relationship between HIX and FRY is illustrated in **Figure 2**. There is a clear positive association between the two traits. However, the association becomes negligible when HIX ranges between 0.50 and 0.70, which are the typical values for improved germplasm.

Table 3 presents the results of the 33 entries analyzed at CIAT's Experimental Station in Palmira. Heritability values for DMC and PT were similar to those measured *in situ* in the five different locations were trials grew. **Table 3** presents three additional parameters that could not be estimated *in situ*: amount of extractable starch (**EXS**), peel weight (**PW**) after washing the roots, and **DMC-O** estimated by drying a 100 g sample per genotype/replication. Since the weight of roots from each plot ranged around 1 kg (1093 g) but was not exactly uniform (ranging from 536 to 2544 g) **EXS** and **PW** were standardized on a per kg of fresh root basis.

Location effects were highly significant ($P < 0.01$) for all variables presented in **Table 3**, except for EXS (significant at 5% probability level). Genetic effects (variation among 33 clones), was highly significant ($P < 0.01$) for all traits. Genotype-by-environment interaction was also highly significant for PT and DMC (estimated by the gravimetric method), significant ($P < 0.05$) for EXS and non-significant for DMC (oven method) and PW. Heritability values were (as it is frequently the case for broad sense heritability) high, ranging from 0.70 for EXS to 0.95 for PT. Interestingly, heritability was higher for DMC estimated by the indirect gravimetric method than by drying samples in the oven (0.87 and 0.83, respectively). Although the oven method is a direct measure of DMC, results from this study suggest that it is not as precise as the indirect gravimetric method. This is likely to be the result of sample size (100 grams for DMC-O versus around 1000 grams in DMC-G). **Figure 3** illustrates the relationship between the results obtained through the two methods to quantify DMC. Differences tended to be slightly higher at DMC values below 33%. This is convenient because precision at higher levels of DMC is what breeders need the most, considering the generalized interest to increase DMC in improved cultivars (Ceballos et al., 2007a; Kawano et al., 1987). **Figure 4** presents the relationship between PT and PW. There is an obvious relationship but still there was a lot of variation for PW which is not accounted for by PT.

The average of EXS per kg of fresh root varied widely among clones (146 to 206 g of dry starch per kg of fresh root) as presented in **Table 3**. The best genotype yielded about 21% of starch (data not presented). Stepwise regression analysis was conducted to explain as much as possible the factors influencing the variation in EXS. The best model was EXS = 30.12 + 5.17 (DMC) – 11.53 (PT). The adjusted R^2 value was only 0.36 indicating that many other factors influence the amount of EXS in addition to those included in the model. The most important factor, as expected was DMC whose sequential sum of squares was 239558, followed by PT with a sum of squares clearly smaller (10476). Both factors were significantly ($P < 0.01$) different from zero and as expected the coefficient for DMC was positive, whereas that for PT was negative. Therefore higher DMC and thinner peels tended to increase the amount of EXS.

Genetic correlations among relevant variables measured in the *in situ* evaluation (64 genotypes) are presented in **Table 4**. There was a highly positive correlation (0.87) between fresh root yield and harvest index and a very negative one with dry matter content (-0.83).

The range of variation for PT within the germplasm collection is presented in **Table 5**. As expected the variation was considerably wider than in the replicated trials which only had 64 genotypes. PT ranged

from 0.79 to 5.14 mm, with an average higher (2.55 mm) than that observed in the replicated trials (1.92 mm).

Discussion

As reported by Kawiki (2009) there is a clear genetic variability for PT in cassava. This trait seems to be highly heritable in spite of the variations reported within clones, among roots from the same plant and even within the same root (**Table 1**). Since the number of data to collect quickly becomes large a sensible recommendation would be to take data from three roots (from different plants) and take 2-3 measurements in the middle section of each root. Heritability for this trait was the highest. The range of variation for PT observed in the replicated trial (1.48-2.55 mm), however, is considerably smaller than that reported by Kawiki (0.35mm to more than 4.5 mm) and the range of variation in the 1448 accessions from the germplasm collection (0.79 to 5.14 mm). This should be taken into consideration because the impact of PT on EXS may be larger in other populations with wider range of variation for PT.

Data for this study came from a Regional Trial (except the evaluation of the accessions from the germplasm collection). Materials included survived clonal evaluation, preliminary and advanced yield trials. It can be speculated that the selection process indirectly selected for

intermediate PT values. It is interesting to note that the 147 improved from the germplasm collection evaluation had and average of 2.56 mm but showed a considerably wide range for PT from 1.12 to 4.55 mm (**Table 5**). The improved clones whose PT was > 4.00 mm are relatively old (from crosses made more than 30 years ago). The most recent of the improved clones in Table 6, originated in crosses made less than 20 years ago and showed a PT ranging from 2.0 to 3.5 mm.

Heritability for HIX was considerably higher than for FRY (0.85 vs. 0.63, **Table 2**). This has been and remains an important distinction that justifies the inclusion of HIX as a selection criterion, particularly in early phases of selection (Kawano 1990; 2003). The two variables showed very high genetic correlations (**Table 4**). However, **Figure 2** indicates that the association between HIX and FRY vanishes for HIX values typical for improved and adapted germplasm (>0.50). The widest range of variation for FRY (17 to 58 t ha^{-1}) was observed among genotypes with HIX around 0.70. As expected HIX above a threshold (around 0.75) tends to be undesirable as they are correlated with a reduction in productivity. They are also rather infrequent. Results presented in **Figure 2** supports the prevailing criteria that most productive clones usually have a HIX ranging from 0.55 to 0.75.

The most relevant aspect of this research focuses on the relationship between PT (or PW) and EXS. As expected, DMC played a very important role in defining EXS. This article also provides evidence of the statistically significant role played by PT on EXS, although it was considerably less important than DMC. It has to be emphasized that the impact of PT on EXS should be much larger whenever a wider variation for PT is considered (such as the variation presented in **Table 5**). It should also be mentioned that thicker peels are not necessarily undesirable. Thicker peels are easier to separate from the parenchyma facilitating the labor for those processing pathways that require peeling the roots. This is an activity that is typically carried out by women in many areas of the world and is labor intensive, time consuming and unsuitable for large scale processing (Adetan et al., 2003). A study to correlate peel thickness and ease of peel (requiring relatively large number of roots for statistically robust parameters) is underway. When roots are peeled, the peel is frequently used for animal feeding with the caution that the levels of cyanogenic glucosides in higher in the peel than in the parenchyma (Bokanga, 1994)**.** Finally, thick peel has been linked to tolerance/resistance to certain types of insects feeding on the roots (Riis, 1997).

Finally, the relative relationship between PT, PW and EXS is affected by the shape of the root, which changes the relative proportion between

surface and volume. This is one of the reasons for the relatively poor correlation between PT and PW shown in Figure 3. Length and diameter of the roots were measured and this information was introduced into the regression model (data not presented). Results however were not conclusive probably because the few roots per plot were used and the inherent variation of root shape and size for cassava. Further analysis should therefore, be made, with fewer genotypes (and larger number of roots) to measure how the relative proportion of PW changes with the shape of the root, in addition to the PT.

References

Adetan, D. A., L. O. Adekoya, and O. B. Aluko, 2003: Characterisation of some properties of cassava root tubers. Journal of Food Engineering **59**, 349-353.

Andersen, M.D., P.K. Busk, I. Svendsen, and B.L. Møller, 2000: Cytochromes P-450 from cassava (*Manihot esculenta* Crantz) catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin. The Journal of Biological Chemistry **275**, 1966-1975.

Bokanga, M. 1994: Distribution of cyanogenic potential in cassava germplasm. International Workshop on Cassava Safety. IITA, Ibadan, Nigeria. Acta Horticulturae **375**, 117-123.

Cach T. N., J. I. Lenis, J. C. Pérez, N. Morante, F. Calle and H. Ceballos, 2006: Inheritance of relevant traits in cassava (*Manihot esculenta* Crantz) for sub-humid conditions. Plant Breeding **125**, 177-182.

Carvalho, L.J.C.B., C.R.B. de Souza, J.C.M. Cascardo, C.B. Junior, and L. Campos, 2004: Identification and characterization of a novel cassava (*Manihot esculenta* Crantz) clone with high free sugar content and novel starch. Plant Molecular biology **56**, 643-659.

Ceballos, H., M. Fregene, J.C. Pérez, N. Morante, and F. Calle, 2007a: Cassava Genetic Improvement. In: M.S. Kang and P.M. Priyadarshan (eds), Breeding Major Food Staples, 365-391. Blackwell Publishing, Ames, IA.

Ceballos H., T. Sánchez, N. Morante, M. Fregene, D. Dufour, A.M. Smith, K. Denyer, J.C. Pérez, F. Calle, and C. Mestres, 2007b: Discovery of an Amylose-free Starch mutant in cassava (*Manihot esculenta* Crantz). Journal of Agricultural and Food Chemistry **55**, 7469-7476.

Ceballos, H., T. Sánchez, K. Denyer, A.P. Tofiño, E.A. Rosero, D. Dufour, M.A. Smith, N. Morante, J.C. Pérez, and B. Fahy, 2008: Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* **Crantz).** Journal of Agricultural and Food Chemistry **56**, 7215-7222.

Chávez, A.L., T. Sánchez, G. Jaramillo, J.M. Bedoya, J. Echeverry, E.A. Bolaños, H. Ceballos, C.A. Iglesias, 2005: Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euphytica **143**:125-133

Cock, J, 1985: Cassava. New potential for a neglected crop. Westview Press. Boulder, CO., USA, 240 pp

El-Sharkawy, M.A., 2006: International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stress in the tropics. Photosynthetica **44**, 481-512.

Jennings, D.L., and C.A. Iglesias 2002: Breeding for crop improvement. In: R.J. Hillocks, J.M. Tresh and A.C. Bellotti (eds), Cassava: biology, production and utilization, 149-166. CABI Publishing, Wallingford.

Kawano, K., 1990: Harvest index and evolution of major food crops cultivars in the tropics. Euphytica **46**, 195-202.

Kawano, K. 2003: Thirty years of cassava breeding for productivity-biological and social factors for success. Crop Sci. **43**, 1325-1335.

Kawano, K., P. Daza, A. Amaya, M. Ríos, and M.F. Gonçalvez, 1978: Evaluation of cassava germplasm for productivity. Crop Sci. **18**, 377-380.

Kawano, K., K. Narintaraporn, P. Narintaraporn, S. Sarakarn, A. Limsila, J. Limsila, D. Suparhan, V. Sarawat, W. Watananonta, 1998: Yield improvement in a multistage breeding program for cassava. Crop Sci **38**, 325-332.

Kawiki, R.S., 2009: Variation in cassava (*Manihot esculenta* Crantz) based on single nucleotide polymorphisms, simple sequence repeats and phenotypic traits. Ph.D. Disertation, Department of Plant Sciences, Faculty of Natural and Agricultural Sciences, University of Free State. Bloemfontein, South Africa. pp. 56-89

Mkumbira, J., L. Chiwona-Karltun, U. Lagercrantz, N. Meso Mahungu, J. Saka, A. Mhone, M. Bokanga, L. Brimer, U. Gullberg, and H. Rosling, 2003: Classification of cassava into ‘bitter’ and ‘cool’ in Malawi: From farmers' perception to characterisation by molecular markers. Euphytica **132**, 7-22

Moorthy, S.N., 2004: Tropical sources of starch. In: A.C. Eliasson (ed), Starch in food, 321-359. CRC Press, Boca Ratón.

Morante, N., T. Sánchez, H. Ceballos, F. Calle, J.C. Pérez, C. Egesi, C.E. Cuambe, A.F. Escobar, D. Ortiz, A.L. Chávez, 2010: Tolerance to post-harvest physiological deterioration in cassava roots. Crop Sci. **50**, 1333-1338.

Nyquist, W.E., 1991: Estimation of heritability and prediction of selection response in plant populations. Critical Reviews in Plant Sciences **10**, 235-322.

Reilly, K., D. Bernal, D.F. Cortes, R. Gomez-Vasquez, J. Tohme, J.R. Beeching, 2007: Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. Plant Molecular Biology **64**, 187-203.

Rickard, J.E., M. Asaoke, and J.M.V. Blanshard, 1991: The physico-chemical properties of cassava starch. Trop. Sci. **31**, 189-207.

Riis, L., 1997: Behaviour and population growth of the burrower bug *Cyrtomenus bergi* Froeschner: effects of host plants and abiotic factors. Ph.D. thesis, Royal Veterinary Agricultural University, Copenhagen.

Rogers D.J., H.S. Fleming, 1973: A monograph of *Manihot esculenta* with an explanation of the taximetrics methods used. Economic Botany **27**, 1-113.

Sánchez, T., G. Mafla, N. Morante, H. Ceballos, D. Dufour, F. Calle, X. Moreno, J.C. Pérez, D. Debouck, 2009: Screening of starch quality traits in cassava (*Manihot esculenta* Crantz). Starch/Stärke **61**, 12-19.

Statistix 8, 2003: User's Manual. Analytical Software. P.O.Box 12185 Tallahassee, Fl., USA, pp 167-188.

Sriroth, K., V. Santisopasri, C. Petchalanuwat, K. Kurotjanawong, K. Piyachomkwan, and C.G. Oates, 1999: Cassava starch granule structure-function properties: influence of time and conditions at harvest on four cultivars of cassava starch. Carbohydrate Polymers **38**, 161-170.

Steel, R.G.D., and J.H. Torrie, 1960: Principles and procedures of statistics. McGraw-Hill Book Company. New York, Toronto, London.

Thakkar, S.K., B. Maziya-Dixon, A.G.O. Dixon, M.L. Failla, 2007: β-carotene micellarization during *in vitro* digestion and uptake by Caco-2 cells is directly proportional to β-carotene content in different genotypes of cassava. Journal of Nutrition **137**, 2229-2233.

van Oirschot, Q.E.A., G.M. O'Brien, D. Dufour, M.A. El-Sharkawy, E. Mesa, 2000: The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. J Sci Food Agric **80**, 1866-1873.

Table 1. Average peel thickness (mm) in each of the five locations (three replications per location) from 64 cassava genotypes. From each entry, three plants per replication were sampled. Three roots per plant were taken and from each root three individual measurements of peel thickness were taken. This large number of measurements allowed an assessment of the variation (standard deviations) within a root, between roots of a given plant, and between plants sampled in each replication.

Parameter / Location	Loc1	Loc2	Loc3	Loc4	Loc5	All
Average peel thickness	1.919	2.049	1.825	1.933	1.889	1.923
Averages for standard deviations						
Within root (three measurements averaged across 8640 roots)	0.126	0.070	0.074	0.081	0.071	0.084
Within plant (three roots from a total of 2880 plants)	0.168	0.143	0.130	0.124	0.123	0.138
Between plants within replication (averaged across 960 plots)	0.157	0.126	0.121	0.126	0.105	0.127
Between replications within locations 320 obs: 64 entries and 5 locs)	0.206	0.189	0.151	0.302	0.163	0.202

Table 2. Mean squares from the combined analysis of for relevant agronomic traits estimated from 64 genotypes (cassava clones) evaluated across five locations. In the last row of the table the estimated h^2 values for each variable (based on plot averages or totals) are presented.

Source of Variation	df	Mean squares					
		PT mm	FRY t ha^{-1}	DMC-G %	HIX 0-1	PTY 1-5	PHT m
Location	4	1.28^{NS}	3799.7**	335.5**	1.20**	11.87*	23.76**
Rep(Loc)	10	0.69	124.4	23.2	0.01	2.60	0.56
Clone	63	1.03 **	206.5**	35.8**	0.04**	5.85**	1.20**
Clone*Loc	252	0.08 **	75.8**	4.5**	0.01**	0.98**	0.13**
Error	630	0.06	41.1	3.0	0.00	0.51	0.09
Average		1.92	30.0	32.2	0.60	2.66	2.17
h^2		0.93	0.63	0.87	0.85	0.83	0.91
Loc.1		1.92	28.36	31.32	0.49	2.79	2.72
Loc.2		2.05	37.46	31.97	0.65	2.65	2.06
Loc.3		1.83	25.60	31.54	0.64	2.94	1.75
Loc.4		1.93	29.30	34.52	0.68	2.66	2.11
Loc.5		1.89	29.11	31.62	0.56	2.27	2.21

NS= statistically non significant; * Significance at 0.05 level of probability; ** Significance at 0.01 level of probability.

Table 3. ANOVA for variables measured at CIAT based on roots from 33 clones.

Source of Variation	df	Mean squares				
		Peel		DMC (%)		Extracted
		Thickness mm	Weight g	DMC-O %	DMC-G %	Starch kg (kg root)$^{-1}$
Location	4	1.12**	7245.5**	386.15**	187.20**	0.026*
Rep(Loc)	10	0.09	1030.8	41.94	20.89	0.006
Clone	32	1.48**	4395.6**	61.88**	36.30**	0.004**
Clone * Loc	128	0.08**	445.29	10.516	4.60**	0.001*
Error	320	0.05	420.0	10.648	2.87	0.001
Average		1.79	168.2	32.36	31.78	0.177
Max. clone average		2.47	220.4	36.07	34.24	0.206
Min. clone average		1.35	140.0	28.16	28.09	0.146
h^2		**0.95**	**0.90**	**0.83**	**0.87**	**0.70**

* Significance at 0.05 level of probability; ** Significance at 0.01 level of probability.

Table 4. Genetic correlations among relevant variables estimated across the 64 genotypes evaluated in five locations.

Variable		Harvest. index	DMC (gravimetry)	Dry matter yield	Peel thickness
Fresh root yield	t ha^{-1}	0.872	-0.831	0.899	-0.157
Harvest index	0-1	1.000	-0.475	0.923	-0.023
DMC (gravimetry)	%		1.000	-0.511	0.079
Dry matter yield	t ha^{-1}			1.000	-0.171
Peel thickness	mm				1.000

Table 5. Variation in peel thickness in a sample of 1448 accessions of the germplasm collection), discriminated by country or region. The 99 accessions from the Caribbean region include Costa Rica, Cuba, Guatemala, Panama and Puerto Rico. Asian accessions come from Fiji Islands, Indonesia, Malaysia, Philippines, and Thailand.

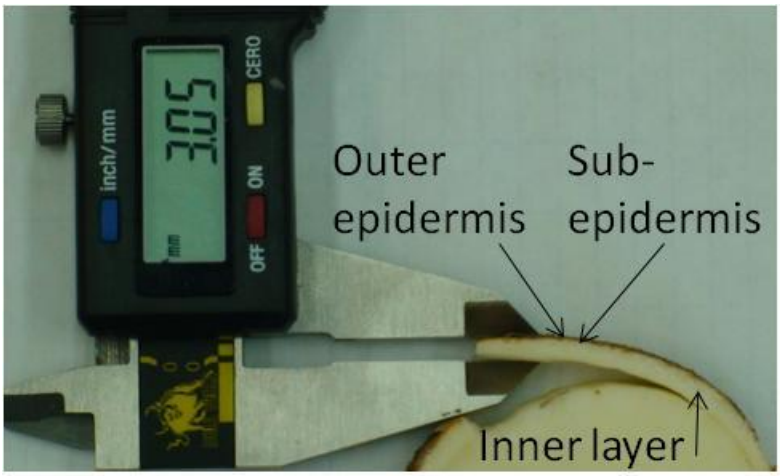
Origin	Maximum	Minimum	Average	St.Deviation	# Clones
Improved	4.55	1.12	2.56	0.63	147
Brazil	5.03	0.79	2.41	0.58	438
Colombia	4.18	1.05	2.63	0.59	474
Peru	4.25	1.68	2.77	0.55	85
Venezuela	4.60	1.72	2.85	0.75	40
Ecuador	4.26	1.53	2.62	0.69	21
Argentina	4.00	1.10	2.05	0.59	31
Paraguay	4.10	1.44	2.33	0.56	41
Mexico-USA	5.14	1.65	2.72	0.73	32
Caribbean	4.70	1.65	2.72	0.61	97
Asia	3.86	1.51	2.60	0.61	39
Africa	3.43	2.04	2.80	0.70	3
TOTAL	**5.14**	**0.79**	**2.55**	**0.62**	**1448**

Figure 1. Illustration of a section of the cassava roots showing the three components of the phelloderm or “peel” and the digital caliper used to measure it.

Figure 2. Relationship between averages (across three reps) for harvest index and fresh root yield evaluated in 64 cassava clones from trials grown in five locations.

Figure 3. Relationship between DMC estimated by the oven and by the gravimetric methods. Divergence of results tended to be larger at low levels of DMC (< 33%). Each data point represents the averages (across three replications) for each clone at each location (total of 33 clones x 5 locations = 165 data points)

Figure 4. Relationship between peel thickness (PT) and peel weight (PW). Each data point represents the averages (across three replications) for each clone at each location (total of 33 clones x 5 locations = 165 data points)



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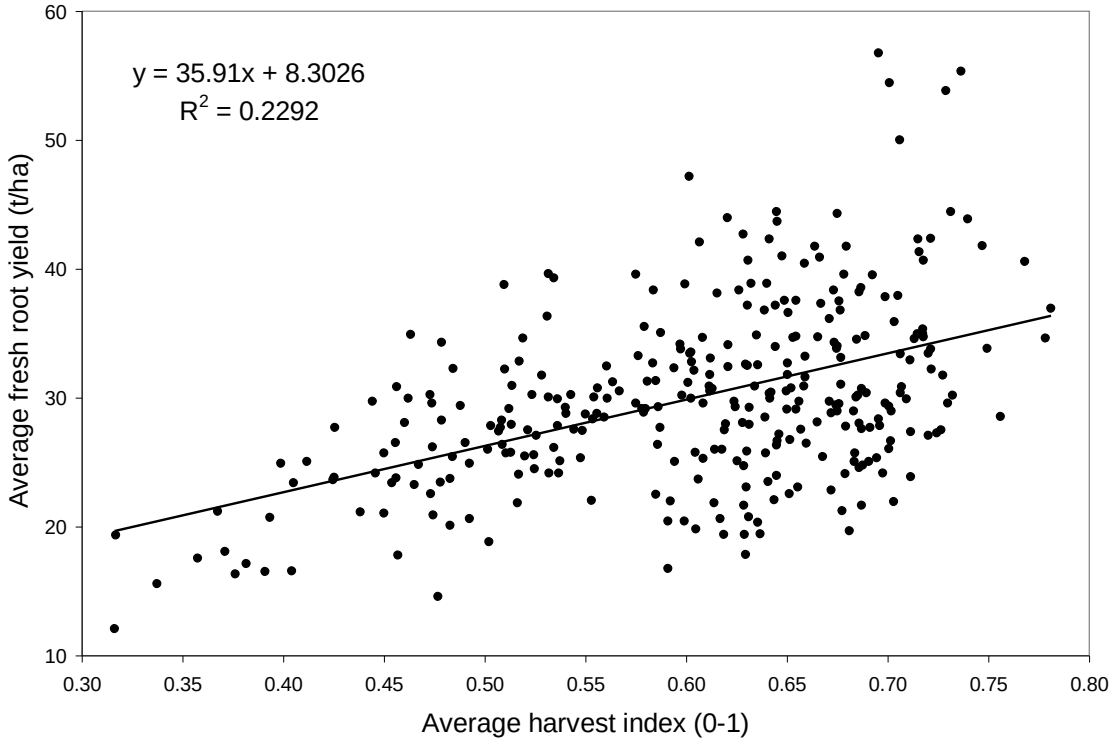

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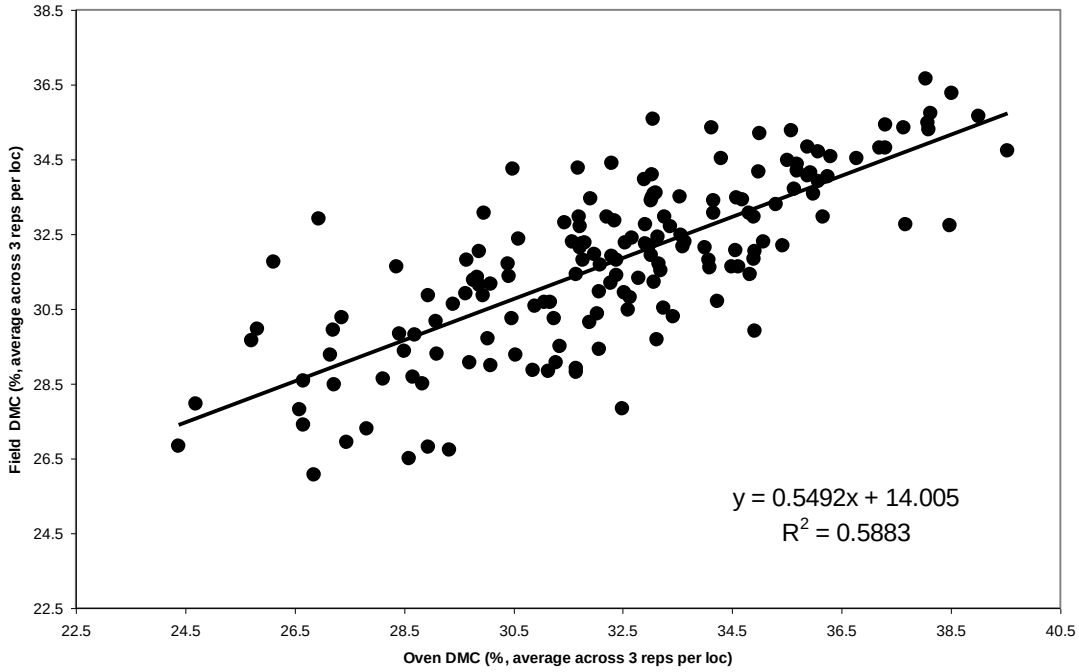

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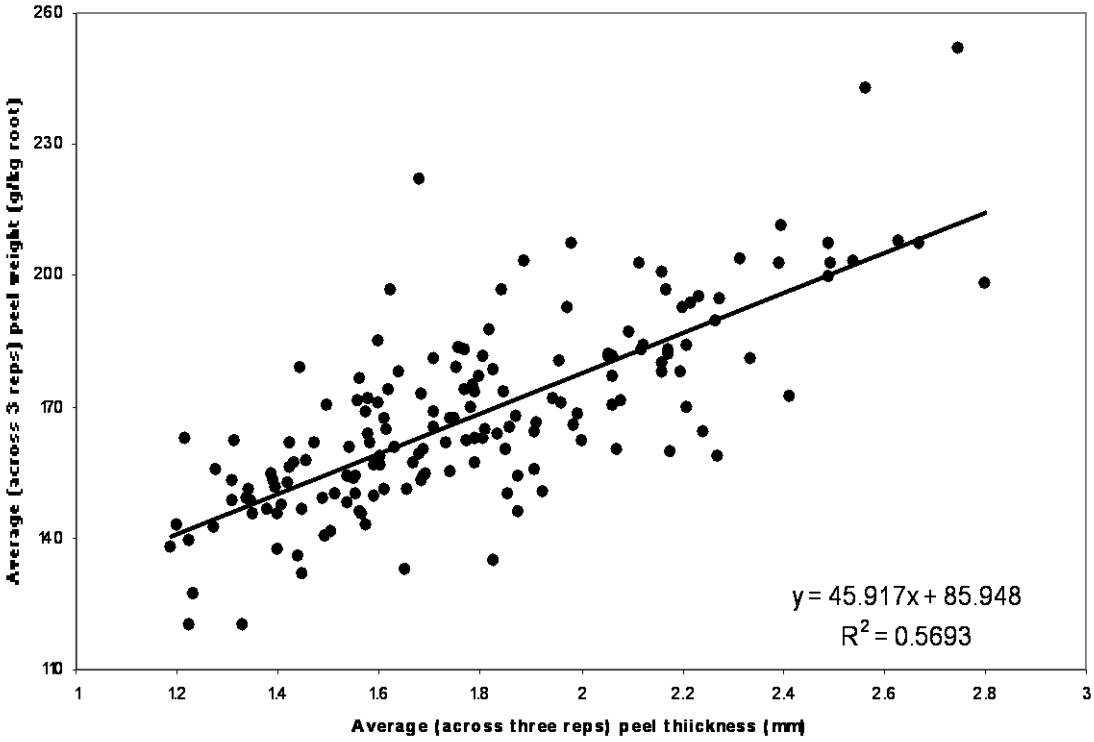

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