African Journal of Rural Development, Vol. 7 (2): May-August, 2022: pp. 201-208. ISSN 2415-2838

Genetic variability and heritability of starch content among white fleshed and provitamin A cassava in Uganda

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ABSTRACT

High starch content and dry matter of cassava are important drivers in the application of cassava to different uses. However, information on starch content variation and heritability in Ugandan cassava cultivars is limited. Accordingly, this study was conducted to determine the variability and heritability of starch content in 112 cassava clones. The effect of genotype was investigated for one season, at two locations; Namulonge and Serere. Harvesting was done at 12 months after planting. Considerable variations were observed among clones at both sites ($P < 0.001$). Starch content ranged from 23.94 % to 75.23% dry basis at Namulonge, whereas at Serere, it ranged from 21.34% to 76.32% dry basis. Likewise, clone by location interaction was significant ($P = 0.001$). Furthermore, high broad sense heritability ($H^2 = 0.76$) was obtained. Therefore, these findings suggest that there is a significant variation for starch content in Ugandan cassava germplasm and that starch content is a heritable trait, Thus, it is important to support ongoing efforts to breed for desired high starch content cassava varieties.

Keywords: Cassava genotypes, heritability, starch content, Uganda

RESUME

La teneur élevée en amidon et en matière sèche du manioc sont des facteurs importants dans l'application du manioc à différentes utilisations. Cependant, les informations sur la variation de la teneur en amidon et l'héritabilité des cultivars de manioc ougandais sont limitées. En conséquence, cette étude était menée pour déterminer la variabilité et l'héritabilité de la teneur en amidon dans 112 clones de manioc. L'effet du génotype était étudié pendant une saison, sur deux sites ; Namulonge et Serere. La récolte était effectuée 12 mois après la plantation. Des variations considérables étaient observées entre les clones des deux sites ($P < 0,001$). La teneur en amidon variait de 23,94 % à 75,23 % de matière sèche à Namulonge, alors qu'à Serere, elle variait de 21,34 % à 76,32 % de matière sèche. De même, l'interaction entre le clone et le site était significative ($P = 0,001$). De plus, une héritabilité au sens large élevée ($H2 = 0,76$) était enregistrée. Par conséquent, ces résultats suggèrent qu'il existe une variation significative de la teneur en amidon dans le germoplasme de manioc ougandais et que la teneur en amidon est un trait héréditaire. Il est donc important de soutenir les efforts en cours pour sélectionner les variétés de manioc à haute teneur en amidon.

Mots clés : Génotypes de manioc, héritabilité, teneur en amidon, Ouganda

Cite as: Namakula, B.F., Nuwamanya, E., Kanaabi, M., Gibson, P., Chiteka, A. and Kawuki, R. S. 2022. Genetic variability and Heritability of starch content among white fleshed and provitamin a cassava in Uganda. *African Journal of Rural Development* 7 (2): 201-208.

INTRODUCTION

Cassava (*Manihot esculenta* L.), usually grown for its starchy tuberous roots, is a vital source of calories to many of the people in Africa (Bayata, 2019). Furthermore, the roots are used for industrial application such as for formation of products like starch, glucose, pastries, adhesives and feed, among others (Verma *et al*., 2022). Most of the cassava applications require starch. It suffices to note that the growing interest in cassava starch has seen the global cassava starch market increase three fold over the years (Howeler, 2015). Nevertheless, four to five tons of cassava are needed to produce one ton of starch, but the ratio may be as high as ten to one depending on quality of root (Prakash, 2011). For this reason, high starch content is the more often considered trait for adoption of new cassava varieties (Nakabonge *et al*., 2017).

Given the potential and high importance of starch content as a trait, breeding programmes in Uganda, initiated cassava improvement aimed at producing cassava varieties with high starch quantity and quality. As the first stage in breeding, genetic variation studies have been done to evaluate starch content variability among cassava genotypes in a segregating population (Nuwamanya *et al*., 2009), and also starch yield was evaluated in provitamin A clones (Atwijukire *et al*., 2017). However, these were limited to a few genotypes. The huge diversity range for the trait has not been fully explored. In addition, starch content heritability, an important aspect of breeding was not captured. This study therefore assessed variation and heritability of starch content in a diverse set of cassava germplasm comprising introduced germplasm in Uganda.

MATERIALS AND METHODS

Experimental design. Materials used in the study comprised 89 white flesh clones from CIAT Columbia, Latin America and 112 provitamin A clones from West Africa. These were introduced germplasm, sourced from Uganda's cassava breeding population.

The experiment was laid out in an augmented design, 7 blocks with 17 plots per block and three checks per block, with plots of two rows of 10 plants per row at 1m x 1m spacing. The clones were evaluated at two sites; Namulonge (0 31'47N and 32 36'9'E, 1133m above sea level) at the National Crop Resources Research Institute (NaCRRI) and Serere (1 29'57.85"N and 33 32'56.43"E, 1126 m above sea level) at the National Semi-Arid Resources Research Institute (NaSARRI). Harvesting was done at 12 months after planting and root samples for starch content analysis were sourced from plants in the middle rows.

Data collection

Sample preparation and analysis. Due to non-uniform germination, unavailability of appropriate roots for sampling and effect of cassava brown streak disease that affects roots, not all planted clones were sampled. Accordingly, out of one 201 cassava clones, 88 and 98 were sampled from Namulonge and Serere, respectively.

A sample of two fresh healthy-looking storage roots were randomly selected. Of interest were disease free, roots of 25-30 cm in diameter. Each root was washed, then the whole root was grated into paste. These were then oven dried at 105°C for 24 hours, and there after processed into flour for starch content analysis. Starch content was estimated based on acid hydrolysis and determination of sugars method according to Dubois *et al*. (1956). Duplicate samples were analyzed according to the procedure as follows. Initially, 0.5 g of cassava flour sample was weighed and transferred into a falcon tube. To the sample, 2 mls of 95% ethanol was added, then thoroughly mixed, and thereafter decanted off the filtrate. Next, 5 mls of 10% sulphuric acid was added, then incubated at 80°C for 30 minutes and samples were allowed to cool after incubation. Next, 0.5 mls of hydrolysate followed by, 1 ml of distilled water, and 0.5 ml of 5% phenol was added. Finally, 1 ml of concentrated sulphuric acid was added and mixed thoroughly. Upon cooling, absorbance at 490nm wavelength was read. A glucose standard (laboratory grade A) was then prepared. This was done by preparing varying concentrations of glucose dissolved in distilled water, namely; 40%, 35%, 30%, 25%,

20%, 15%, 10%, 5%. From these, 0.05mls of glucose, followed by 0.95 ml of distilled water, and 0.5 ml of 5% phenol was added. Finally, l ml of concentrated sulphuric acid was added and mixed thoroughly. Upon cooling, absorbance at 490nm wavelength was read. From the absorbance and concentration of the glucose solution, a standard curve of concentration against absorbance was developed. A regression equation generated from the standard curve was used to estimate starch content as follows:

$$[1] \quad \%\,Starch = \frac{(A-I)\text{x DF x } 100}{B}$$

Where A = Absorbance of sample, I = Intercept of sample, B = Slope of the standard curve, DF = Dilution factor (Dubois *et al*., 1956).

Statistical data analysis. Analyses of variance (ANOVA) for starch content were performed by least squares regression using the anova and l m function available in R stats package to test for significant differences in clone means (R Core Team, 2020). The model used to analyze data collected at single site had clone, population, root and technical replicates, as fixed effects and interaction of all other terms from the simple linear model described above. Thereafter, pooled data from Namulonge and Serere were used to determine clone by location interaction. The Tukey-Kramer honest significance (HSD) test (P-value < 0.05) was used to determine if varieties were significantly different from each other (R Core Team, 2020).

Heritability of starch content was determined on pooled data for both provitamin A and white-flesh Latin America clones. This was obtained using variances from mixed linear model fitted, considering clone, location, population, and root as random effects, while technical replicate considered as a fixed effect using lmer function in lme4 package in R (Kuznetsova *et al*., 2017).

Variance components obtained were used to estimate broad sense heritability (repeatability) on an entry-mean basis (Holland *et al*., 2003). Heritability was estimated as the broad-sense heritability, calculated using the formulae below:

$$H^2 = \frac{genotypic\ variance}{phenotypic\ variance}$$

$$H^2 = \sigma^2 G/(\sigma^2 G + \sigma^2 error)$$

Where σ2 *G* is the genotype variance and σ^2 *error* is the error variance

RESULTS

Genetic variation of starch content. There was significant variation in starch content among both white fleshed-LA and provitamin A-WA clones ($p < 0.001$) at both locations (Namulonge and Serere), (Table 1). Furthermore, there was a strong clone by location interaction ($p < 0.001$), implying that starch content was dependent upon location. Populations were significantly different ($p < 0.001$) at Serere, however, not significantly different at Namulonge (Table 1).

At Namulonge, provitamin A clones had slightly wider variability of starch content ranging from 24.71 to 75.23 % dry basis compared to white flesh-LA clones whose starch content ranged from 23.94% to 73.93 % (Table 2). Furthermore, provitamin A clones had a higher mean (56. 14%) compared to white flesh-LA clones with a mean of 50.86 (Table 2, Figure 1).

At Serere, white-flesh-LA clones had slightly wider starch content variability (22.43 % to 76.32%) than provitamin A clones (21.34 % to 73.66%), (Table 2). Furthermore, white flesh-LA clones had a higher mean (51.19%) than provitamin A clones with a mean of 49.22% (Table 2, Figure 1).

Generally, for the cassava accessions (both white flesh and provitamin A clones), there was higher performance at Namulonge with average starch content of 54.07% than at Serere where mean starch content of 50.52 % was registered (Figure 1). However, there was a higher starch content diversity range at Serere (21.34% to 76.32%) than at Namulonge from (23.94% to 75.23%), (Figure 1). The mean value of starch content between Latin America clones and PVAC clones was significantly different at Namulonge ($p = 0.00$, 95% C.I. = [0.93, - 6.82]), and Serere ($p = 0.03$, 95% C.I. = [-6.39, - -0.13]), implying differences in locations and populations (Table 3).

Table 1. Mean squares associated with starch content (dry matter basis) of cassava accessions planted in two locations

	Namulonge		Serere	
SOV	d.f	Mean Square	d.f	Mean square
Clones	88	1205.47***	97	1612.8***
Population	1	99.34NS	1	3958.0***
Rep	1	77.79NS	1	2.8NS
Tech-rep	2	2.39NS	2	0.1NS
Accession X Rep	88	60.64**	97	42.4***
Error	521	38.1	449	25.8
Mean		54.07		50.52
CV		11.47%		10.06%
H^2		0.82		0.62

SOV = source of variation; d.f = degrees of freedom; *** represents significance at p = 0.001; CV = coefficient of variation, H^2 = broad sense heritability. Data based on analysis in two locations; Namulonge (central region) and Serere (eastern region); data on starch content, dry basis on 89 and 98 genotypes of both white flesh-Latin-American germplasm and provitamin A Clones-West Africa planted in Namulonge and Serere, respectively

Table 2. Distribution of starch content among cassava genotypes

		Starch content			
Location	Clones	No of clones	Mean (%)	SD	Range (%)
Namulonge	White flesh-LA	37	50.86	6.26	23.94 - 73.93
	Provitamin A-WA	52	56.14	6.05	24.71 - 75.23
Serere	White flesh-LA	60	51.19	6.34	22.43 - 76.30
	Provitamin A-WA	38	49.22	0.688	21.34 - 73.66

Data based on analysis in two locations; Namulonge (central region) and Serere (eastern region); data on starch content, dry basis. LA-Latin America; WA-West Africa

Table 3. Tukey post -hoc multiple comparison table of cassava starch content by location and population

					95% CI	
Location	Population(I)	Population (J)	Mean difference (I-J)	sig	lower bound	Upper bound
Namulonge	Latin America	PVAC-WA	3.80	0.00	0.93	6.82
Serere	Latin America	PVAC-WA	-3.26	0.03	-6.39	-0.13

PVAC- provitamin A, WA- West Africa, sig – significance, CI – confidence Interval

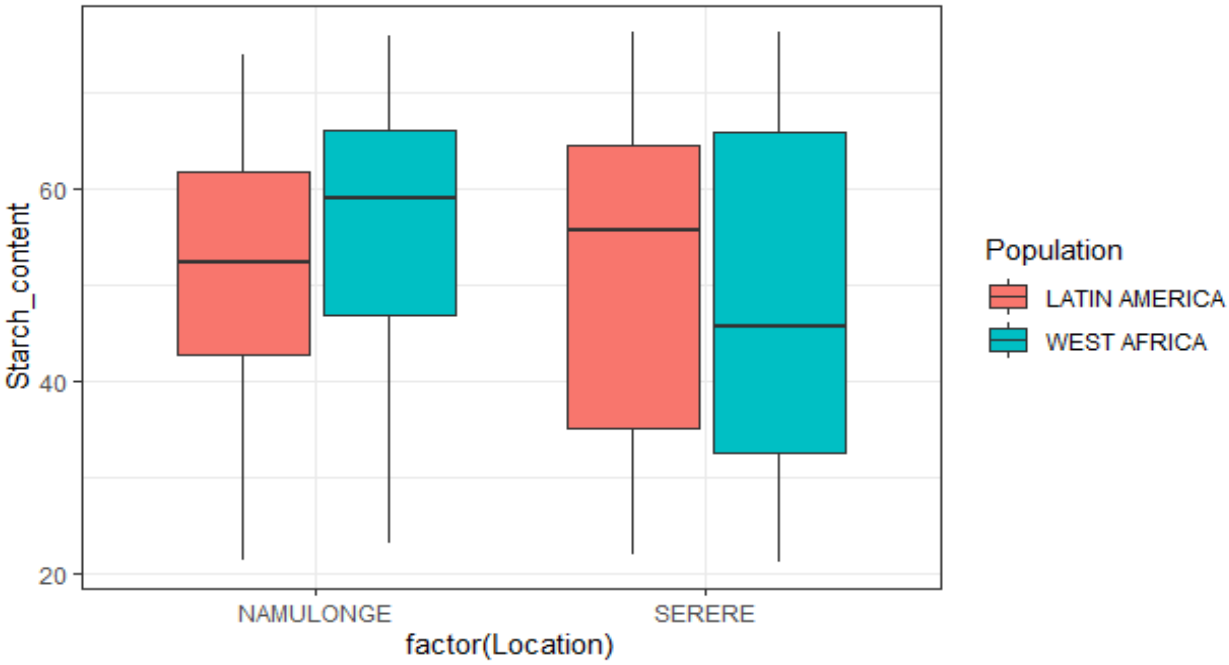

Figure 1. Cassava starch content distribution at Namulonge and Serere

Table 4. Mean squares associated with starch content (dry matter basis) of cassava accessions planted in two locations

	Starch content	
SOV	D.f	Mean Square
Clones	146	1470.7***
Location	1	6201.0***
Population	1	1113.2***
Rep	1	27.1NS
Tech_rep	2	1.6NS
Clone*location	39	1163.1***
Location*population	1	2969.9***
Error	1158	35.3
Mean		52.00%
CV		11.39%
H^2		0.76

SOV = source of variation; D.f = degrees of freedom; *** represents significance at P = 0.001; CV = coefficient of variation, H2 = broad sense heritability. Data based on analysis in two locations; Namulonge (central region) and Serere (eastern region); data on starch content, dry basis on 89 and 98 genotypes of both white flesh-Latin-American germplasm and provitamin A Clones-West Africa planted in Namulonge and Serere, respectively

Starch content was significantly different across locations (P = 0.001) and there was a highly significant clone by location interaction (P = 0.001), (Table 4). Entry mean-based single site broad sense heritability estimates were moderate to high (H^2= 0.62 – 0.82), (Table 1). Highest heritability (H^2=0.82) was obtained at Namulonge and moderate heritability was observed at Serere (H^2=0.62), (Table 1). Overall, heritability was high (H^2 = 0.76), implying that starch content is a highly heritable trait (Table 4).

DISCUSSION

Appreciable variability in starch content was observed among clones at both locations (Table 1). Related studies have indicated that the genetic composition of the cultivars, cultural practices on the field as well as a combination of environmental factors influence starch content (Benesi *et al.* (2004). Starch content ranged from 21.34% to 76.30 % with most of the clones ranging from 41.35% to 75.23% (Figure 1). This is consistent with findings by Nuwamanya *et al.* (2009), who observed similar trends, i.e., starch yield percent ranged from 47% to 90% starch content on dry matter basis.

There were no significant differences in starch content among the provitamin A clones and the white flesh clones at Namulonge, however, significant differences were observed at Serere (Table 1). These differences could be attributed to differences in the distribution of rainfall at the two locations (Benesi *et al.,* 2008). Extended dry weather at Serere might have forced the plants to use their food reserves by breaking down some of the starch into sugars for survival during the dry season (Benesi *et al.,* 2008). Furthermore, an impact of disease might have influenced the differences.

Higher mean performance was observed at Namulonge (54.07%) than at Serere (50.52%), (Table 1 and 3, Figure 1). These differences could be attributed to root bulking in the different locations. Studies by Tumuhimbise *et al*. (2014), showed that locations effects were significantly different for fresh storage root yield (bulking).

Overall, White flesh-LA clones performed better than Provitamin A-WA at both locations (Figure 1). These differences could be due to the fact that there was increased utilization of glucose for carotenoid synthesis that could impact on the amount of glucose available for starch synthesis among provitamin A-WA clones. Synthesis of phytoene, a carotenoid precursor in plants, requires isopentenyl pyrophosphate (IPP). The IPP is synthesized either from acetyl-CoA or from pyruvate and glyceraldehyde-3-phosphate(Cunningham and Gantt, 1998) , all of which are obtained from metabolism of glucose. Thus, it is very likely that the lower performance of provitamin A clones could have resulted from the higher utilization of glucose for carotenoid synthesis.

Very low starch contents (< 30% dry basis), were observed in some clones (Table 2). This could be due to the high dry matter displayed by these progenies and, hence, low digestibility and creation of side products during hydrolysis such as isomaltose and maltose (Van Der Veen *et al*., 2006) which are not detected by glucose specific tests used in this analysis.

There was a highly significant clone by location interaction (Table 4), suggesting that when and where cassava is grown and harvested for starch extraction and starch content determination will be important in maximizing the starch yield and content from tubers.

Generally, there was high broad sense heritability for Namulonge (H^2=0.812), and moderate broad sense heritability at Serere (H^2=0.62), (Table 4.1). This difference could be due to variations in sample handling effects at Serere, since it is an off-station site, that contributed to the lower repeatability than at Namulonge. Overall heritability was high (across both locations, H^2 = 0.76), (Table 4). This is in line with results obtained by Oliveira *et al*. (2014), where broad sense heritability estimates for starch yield were

reported to be of medium magnitude, that is (0.50 ± 0.05). However, heritability obtained in this study was much higher, and this could be due to accuracy of the phenotyping method (Dubois *et al*., 1956; Moorth and Padmaja, 2002).

CONCLUSION

There is indeed significant variation in starch content among cassava germplasm in Uganda. Both white flesh-LA clones and Provitamin A clones are diverse enough for starch content assessment. Nonetheless, white flesh clones had higher starch content than Provitamin A clones, thus should be considered as parents in breeding for the traits. Most of the clones had starch content ranging from 41.35% to 75.23% on dry basis which is a typical range for most cassava varieties. Furthermore, starch content is a highly heritable trait ($H^2 = 0.76$), thus, there is good genetic control of the expression of the trait, and this shows great potential for selection in breeding. There is significant variation in starch content among introduced cassava germplasm in Uganda and thus, justifying systematic genetic improvement for starch content, a key end-user quality trait.

ACKNOWLEDGEMENT

This research was undertaken as part of Roots, Tubers and Bananas (RTB) research, and funded by the CGIAR Research Programme on RTB and supported by CGIAR Trust Fund contributors. Additional funds were provided by the Next Generation Cassava Breeding Project through funds from the Bill and Melinda Gates Foundation and the Makerere University Regional Center for Crop Improvement under the World Bank project-ACEII. We thank the technical staff of National Crops Resources Research Institute (NaCRRI) Root Crops Programme, who assisted with field trial establishment, maintenance, and data collection both in the field and laboratory.

STATEMENT OF NO CONFLICT OF INTEREST

The authors declare that there are no competing interests in this publication.

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