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RESEARCH ARTICLE

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Variability in Starch Content, Starch Granule Morphology and Size Distribution of Three Cassava (*Manihot esculenta*) Genotypes in Relation to Yield, at Different Planting Seasons

J.I. Reuben-Kalu[1,2], E. Kokiladevi[2], M. Raveendran[2], D. Uma[3],
V. Balasubramani[2], P.S. Kavitha[4], S.G. Patil[5], T.L. Kingsley[1]

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ABSTRACT

Background: Investigation of the biochemical profile and storage root yield of three cassava genotypes (H-165, H-226 and TME-419) was carried out. Planting was done at three different seasons: summer, early monsoon and late monsoon.

Methods: The storage roots were harvested at intervals of 6, 9 and 12 months after planting to evaluate their yield. Granule morphology of the starches and biochemical characteristics of the flours were examined using electron microscopy and biochemical techniques.

Result: Significant variations were observed in the starch content, root yield and size of starch granule, which were mainly dependent on planting dates, while the variations recorded in starch granule morphology, amylose and amylopectin contents were influenced by genotype. The highest starch (33.5%) and amylose content (18.9%) were observed in TME-419 variety. The rate of storage root yield had positive correlation with maximum temperature and sunshine and negative correlation with humidity and rainfall. This study indicated that cultivation season affects starch accumulation and root yield in cassava and can be applied in developing cassava varieties with high starch and root yield, suitable for different planting seasons.

Key words: Amylopectin, Amylose, Cassava genotypes, Planting dates, Root yield, Starch granule size, Starch.

INTRODUCTION

In the tropics, cassava (*Manihot esculenta*) is one of the most significant food crops. It is drought-tolerant, adaptable to a wide range of climatic conditions and effective in producing carbohydrates. In the world, cassava is a major source of nutrition and income for over 800 million people. Its storage root is the edible part that can also be employed as raw material for making of different traditional fermented foods, energy foods, animal feed, or in industrial products like bioethanol (FAO, 2024; Fathoni *et al.,* 2020).

Cassava can be cultivated throughout the year in the tropics using irrigation, but it is recorded that December-January planting season is better (Enesi *et al*., 2022). It is mostly harvested at 12 months after planting (MAP) or depending on the market needs, irrespective of their actual maturity date. The early-maturing cassava varieties can be harvested at six or seven months after planting, while the late-maturing varieties are harvested at eleven or twelve months after planting (Nzola *et al.,* 2022; Biduski *et al.,* 2018).

The climatic conditions affect the level of starch accumulation in cassava plants cultivated in various environments (Sawatraksa *et al.,* 2018). Recorded evidence indicates that diverse nutrient management practices exert substantial influence on the physiological response observed across the distinct stages of cassava growth (Aravind Ashokh et al., 2024). There have been some efforts to identify suitable genotypes of cassava for specific environments and to improve cassava varieties for high starch content, dry matter content and wide adaptation to a variety of growing seasons, with the goal of maximizing cassava storage root yields at harvest (Sawatraksa *et al.,* 2019). The rate of starch accumulation and size of starch

[1]Biotechnology Research and Development Center, National Root Crops Research Institute Umudike, Abia State, Nigeria.
[2]Department of Plant Biotechnology, Center for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[3]Department of Biochemistry, Center for Plant Molecular Biology and Biotechnology. Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[4]Nammazhvar Organic Farming Research Centre, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[5]Department of Physical Sciences and Information Technology, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: J.I. Reuben-Kalu, Biotechnology Research and Development Center, National Root Crops Research Institute Umudike, Abia State, Nigeria.
Email: janereubenkalu@gmail.com

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granules of different cassava genotypes may respond differently across growing seasons and growth stages. Another method for increasing yield per unit area of cassava at harvest is to grow cassava under supplementary irrigation.

Ascertaining starch accumulation patterns in cassava at different growth stages and different growing seasons is important to provide more insight into the starch accumulation abilities of different cassava genotypes for particular growing season. The results presented will give better insights to cassava growers and researchers in identifying the specific growing season suitable for different cassava genotypes for optimum starch content and storage root yield.

MATERIALS AND METHODS

Field study area and establishment

Three cassava genotypes (H-165, H-226 and TME-419) were used in the study and planting was carried out in three batches at the organic farm of Tamil Nadu Agricultural University, Coimbatore, India. The first batch was planted in May 01, the second batch in August 01 and the third batch in November 01 2021, with drip irrigation. Harvesting was done after six, nine and twelve months of planting.

Estimation of starch content

Starch content of the cassava genotypes was determined using the anthrone method following the procedure reported by (Landhäusser *et al*., 2018).

Determination of amylose and amylopectin

The amylose content of the cassava flour samples was determined by the iodine method recorded by Rosado-Souza *et al.* (2019) with appropriate modifications.

Starch granule size analysis

Analysis of the starch granule size of the cassava starch samples was carried out using a particle size analyzer (Horiba Scientific India).

Analysis of starch granule morphology by scanning electron microscope

Starch samples extracted from the cassava roots were analyzed by using scanning electron microscope was employed. The samples were observed and photographed using (SEM FEI, Czech Republic. Model: Quanta 250. Vacuum: $3.99e^{-4}$ Pa).

Determination of cassava storage root yield at harvest

Each of the cassava varieties was harvested at six, nine and twelve months after planting (MAP) to determine the storage root yield. Tuber yield per hectare was calculated as follows:

$$\text{Yield per hectare}\frac{t}{ha} = \frac{\text{Yield per plot (kg)} \times 10{,}000\ m^2}{6\ m^2\ \text{(net plot size)} \times 1{,}000\ \text{(kilogram/tone)}}$$

1 hectare = 10,000 m^2 and 1 tone = 1,000 kilogram.

Statistical analysis

The results obtained were statistically analyzed through analysis of variance (ANOVA) using R 4.3.0 software 2021.

RESULTS AND DISCUSSION

We describe a comparative study of starch, amylose, amylopectin, starch granule morphology, starch granule size distribution and root yield of three cassava varieties cultivated at different dates.

Result of the analysis of variance for the mean squares of starch content, amylose content, amylopectin content and starch granule size are recorded in Table 1. Significant difference at p=0.001 was observed in the starch, amylose and amylopectin contents for the three cassava genotypes, whereas significant difference at p=0.01 was observed in the starch granule size. Also, significant difference at p=0.001 was observed in the planting dates (PD) and the interaction between the planting dates (PD × G) and the genotypes (G) across the three cassava genotypes. Results show that starch content and amylose content showed a strong association with genotype. This shows the existence of variability among the casava varieties and variations in response of the cassava genotypes to each growing season. In previous study by Janket *et al.* (2020), variation was reported in starch yield, granule size and amylose/amylopectin ratio among cassava cultivars (Kasetsart 50, Rayong 11 and CMR38-125-77) planted in two distinct growing seasons (early rainy season and post-rainy season).

The changes observed in starch, amylose, amylopectin contents and starch granule size distribution of the cassava genotypes harvested at 6, 9 and 12 months after planting is presented in Table 2. The starch content was between 22.2

Table 1: Analysis of variance for starch, amylose and amylopectin contents and starch granule size of the three cassava varieties at harvest across the three seasons of planting.

Source of Variance	Df	Starch (mg/g)	Amylose (%)	Amylopectin (%)	Starch granule size (µm)
G	2	27050.50 (94.71)***	2343.00 (99.38)***	2325.00 (99.40)***	65.60 (12.89)**
PD × G	2	227.00 (0.79)***	5.50 (0.23)***	9.00 (0.38)***	402.20 (79.01)***
PD	4	641.00 (4.49)***	4.25 (0.36)***	2.50 (0.21)***	20.58 (8.08)***
Residuals	18	0.15 (0.01)	0.06 (0.02)	0.02 (0.00)	0.01(0.02)

Significant differences are indicated by asterisk, ***Significant difference at p=0.001 level, **Significant difference at p=0.01 level. Df= Degree of freedom. G: Genotype. PD × G: Planting date × genotype. PD: planting dates. Numbers within the parenthesis are the percentage variation of the mean squares.

to 33.5% (Table 2a), amylose content ranged between 15.65 to 18.93% with TME-419 recording the highest value (18.93%) whereas H-165 variety recording the lowest value (15.65%) (Table 2b). The amylopectin content ranged from 81.3 to 84.3% with H-165 having the highest value (84.3%) (Table 2c), while the starch granule size distribution ranged between 13.47-31.88 µm and TME-419 had the largest starch granule size (31.88 µm) (Table 2d).

The values we obtained have close range with previous studies on different cassava cultivars, where notable variations were also reported in previous studies by Pérez and Bertoft (2010); Breuninger *et al*. (2009) and Rolland-Sabaté *et al.* (2012).

Starch physicochemical properties and the fine structure of amylose and amylopectin in cassava are significantly influenced by various factors, with genotype and environment being among the primary contributors (Piengtawan *et al.,* 2020). Starch content is one of the key criteria for identifying desirable genotypes in cassava (Hasmadi *et al.,* 2021).

In the correlation analysis between starch, amylose, amylopectin and starch granule size distribution (Fig 1), it was observed that starch and amylose contents were highly positively correlated. Amylopectin content was highly negatively correlated with starch and amylose, while the starch granule size distribution was positively corrected with amylose, but non-significantly correlated with starch and amylopectin contents. Similar finding was given by Shadrack *et al.* (2019) where positive association was reported between starch, amylose and starch granule size distribution and negative association between amylopectin and starch granule size in the characterization of starches from different cassava varieties. Also, Hasmadi *et al.* (2021) highlighted a positive correlation between starch and amylose content, positive association between starch granule size and amylose content and negative relationship between starch granule size and amylopectin.

Planting cassava in the early rainy season resulted in rapid growth rates of starch yield and granule size during the initial growth stages, while planting in the post-rainy season led to accelerated growth rates of these traits during the middle to late growth stages Janket *et al.* (2018). We observed that when the cassava varieties were planted in August (early monsoon), they produced starches whose amylose contents increased gradually as the age of the plant increased, while the amylose contents were recorded to be relatively stable when planted during the summer season (May).

As a minor component of starch's polyglucan structure, amylose makes up roughly 16 to 38% of the carbohydrate content in most starches (Seung *et al.,* 2020). The differences in starch content, amylose content, starch granule morphology and physicochemical properties of cassava starch from different cultivars affects the functions of these cassava genotypes when used as raw materials for both food and industrial purpose (Akonor *et al.,* 2023).

Table 2 (a-d): Changes in starch, amylose, amylopectin contents and starch granule size distribution of the cassava genotypes harvested at 6, 9 and 12 months after planting.

Genotype	06 MAP	09 MAP	12 MAP	
a. Starch				
H-165	221.6	235.47	223.5	226.86 c
H-226	286.23	246.93	253.37	262.18 b
TME-419	332.8	334.8	335.63	334.41 a
	280.21 A	272.4 B	270.83 C	
b. Amylose				
H-165	157.57	157.63	156.47	157.22 c
H-226	163.33	164.60	166.27	164.73 b
TME-419	186.63	188.33	189.50	188.16 a
	169.18 C	170.19 B	170.74 A	
c. Amylopectin				
H-165	842.53	843.57	842.57	842.89 a
H-226	833.50	835.27	836.57	835.11 b
TME-419	810.67	811.77	813.53	811.99 c
	828.90 C	830.20 B	830.89 A	
d. Starch granule size				
H-165	13.47	20.72	20.78	18.32 c
H-226	12.64	22.41	26.79	20.61 b
TME-419	14.26	25.14	31.71	23.70 a
	13.46 C	22.76 B	26.43 A	

Different lowercase letters in the last columns represent significant difference between the genotypes. Different uppercase letters in the last row represent significant difference between the planting dates (LSD). MAP: Months after planting.

The morphological study of the shape, size and pattern of starch granule distribution of the three cassava varieties is shown in Fig 2. As revealed by the scanning electron microscope, the morphological surface appearance of the starch granules was smooth, spherical, rounded, oval-shaped, truncated and exhibited different starch granule sizes at different radial locations ranging from 12.64 to 31.87 µm. The starch granules had no evidence of holes

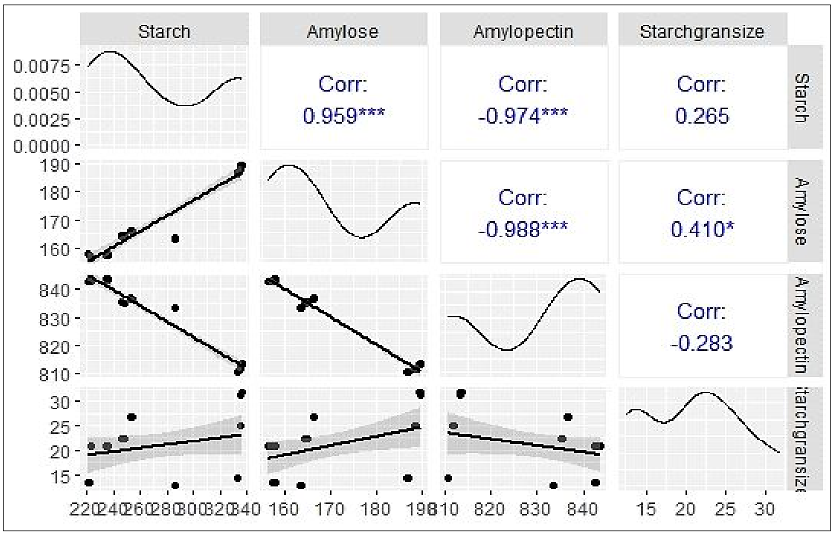
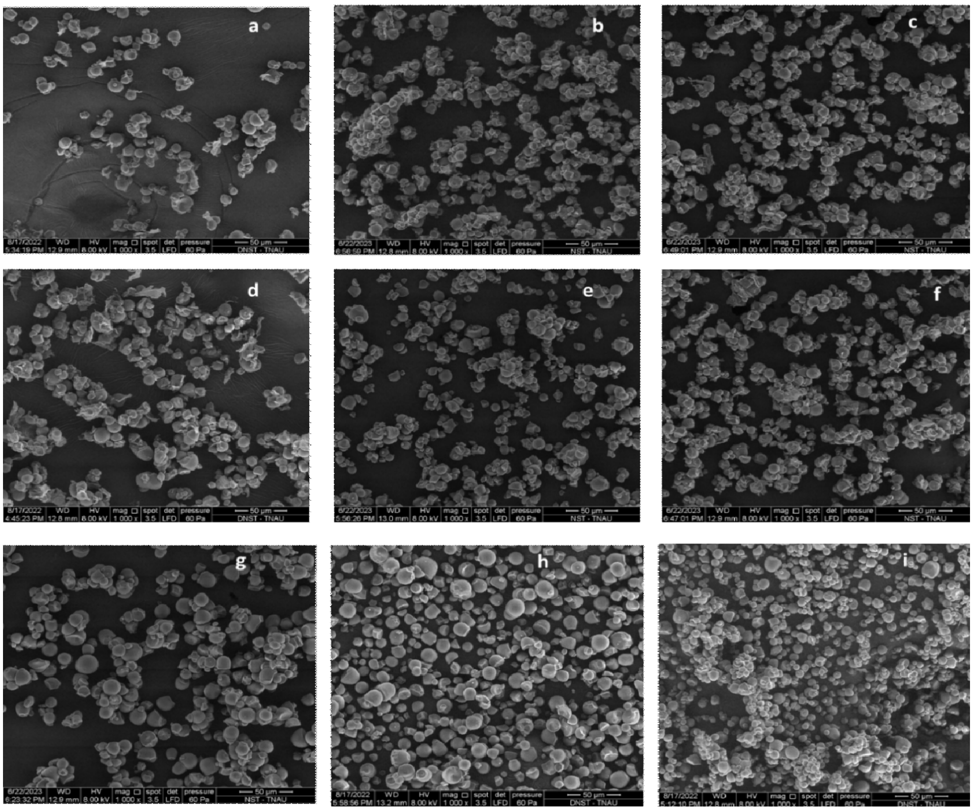


Fig 1: Correlation between starch, amylose, amylopectin and starch granule size distribution.



Magnification: 1000 X. a-c: Starch granule morphology of H-165 cassava variety at 6, 9 and 12 MAP, d-f: Starch granule morphology of H-226 cassava variety at 6, 9 and 12 MAP, g-i: Starch granule morphology of TME-419 cassava variety at 6, 9 and 12 MAP respectively.

Fig 2 (a-i): Starch granule morphology of the cassava genotypes at three sampling points.

and were mainly spherical or oval, with few irregular shapes, typical of root and tuber starches which can be compared to starch granules extracted from related tuber crops like sweet potato and yam. Similarly, the scanning electron microscope images of cassava starch recorded by Vasconcelos *et al.* (2017) revealed spherical, oval-shaped, smooth-surfaced and truncated granules across different cassava varieties evaluated.

Based on three sampling points (6, 9 and 12 MAP), we noted similar morphological arrangements of starch granules among the three cassava genotypes as revealed by SEM. At six MAP, the size of the starch granules ranged between 12.64-14.26 ìm, while at nine and twelve MAP, the starch granule size ranged between 20.72-25.14 ìm and 20.78-31.87 ìm respectively.

We could not differentiate the starch granules of the three cassava varieties by any distinct or specific shape. It was observed that the three cultivars had the same type of starch with no significant differences in their conformational structure, suggesting similar botanical origins. The granule size of starches from the cassava varieties in the present study is similar to those observed in earlier studies (13.18 to 32.63 µm) reported by Rolland-Sabate *et al*. (2012). The variation observed in granule size of the cassava starches was suggested to be cultivar-specific and may not have any connection with the different planting seasons.

In the analysis of variance for storage root yield in comparison of the different planting dates (Table 3), there was significant difference between the genotypes (G), Planting dates (P) and the interaction between G and P. The planting dates showed the largest variations in terms of storage root yield. High significant difference at *p*=0.001 was recorded in the rate of storage root yield across the three cassava genotypes at the three planting dates (May, August and November). The peak of storage root yield was recorded at 12 MAP for TME-419 (36.21 t/ha) and H-226 (33.91 t/ha) varieties planted in May.

The variability in root yield can be said to be genotype-related as well as season-related, as TME-419 variety gave the highest yield at final harvest (12 MAP) (36.21 t/ha).

These results correspond to previous investigations reported by Mahakosee *et al.* (2019) on cassava storage root yield and biomass (37.12 t/ha), where they recorded that planting dates had greater effect on the rate of final yield of the cassava storage roots than other studied parameters.

To ascertain the impact of the environment on yield of the cassava storage roots in relation to the planting seasons, the climatic factors were evaluated. Also, the relationship between root yield and the following environmental parameters; relative humidity, minimum and maximum temperatures, photoperiod and rainfall for the period of cassava growth in the field was put into consideration (Fig 3). Variation was observed in the root yield across the growing seasons and this can be attributed to variations in weather conditions during each season. The rate of cassava storage root yield was observed to have a strong positive correlation with maximum temperature and sunshine and negative correlation with relative humidity and rainfall. This finding is in line with previous research report which indicated that the ideal temperature range for cassava to undergo carbohydrate production through photosynthesis and starch biosynthesis falls between 25-35°C (Vongcharoen *et al*., 2018).

Cassava cultivated during the summer and late monsoon experiences higher temperatures, increased solar radiation and longer day lengths during their early growth stages, leading to rapid shoot growth and starch accumulation in the storage roots (Vongcharoen *et al*.,

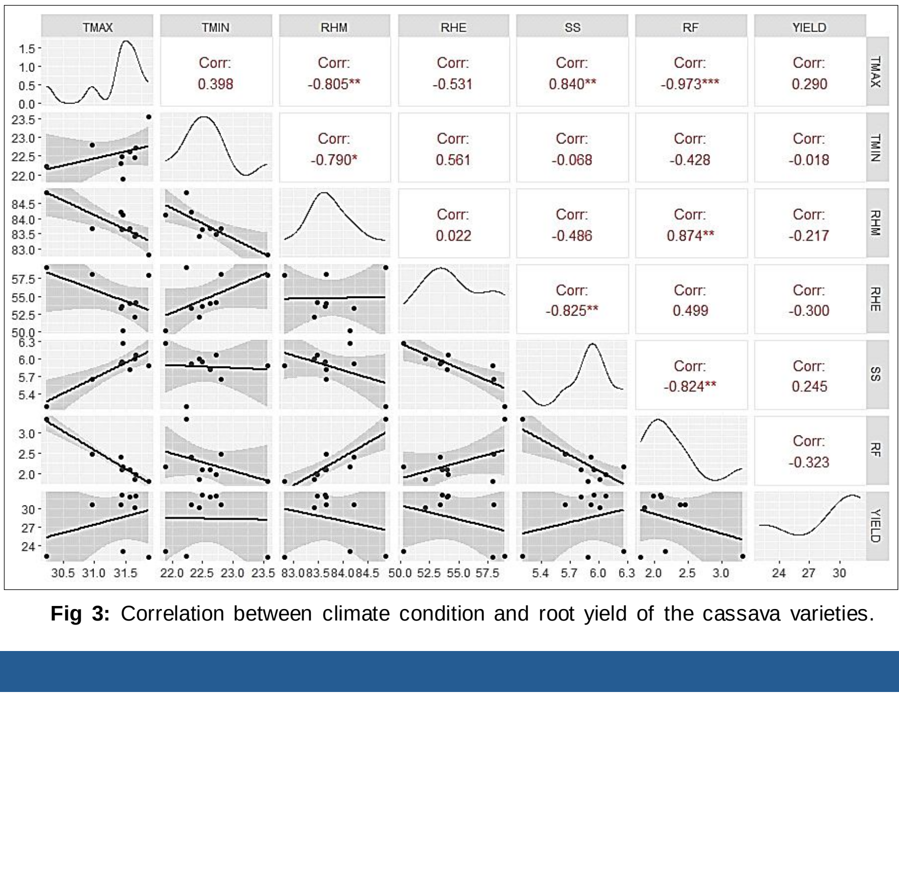


Fig 3: Correlation between climate condition and root yield of the cassava varieties.

Table 3: Analysis of variance for the storage root yield of the three casava genotypes at three different planting seasons.

Source of variance	Df	May	August	November
Genotypes (G)	2	149.70 (32.64)***	120.25 (28.69)***	68.85 (18.19)***
Planting dates (P)	2	268.30 (58.50)***	254.85 (60.80)***	211.15 (55.78)***
G × P	4	20.18 (8.80)***	21.88 (10.44)***	49.28 (26.03)***
Residuals	18	0.028 (0.05)	0.033 (0.07)	0.001 (0.00)

Significant differences are indicated by asterisk, ***Represents significant difference at p=0.001 level. Df= Degree of freedom. Numbers within the parenthesis are the percentage variation of the mean squares.

2018). A study conducted by Byju and Suja (2020) revealed that cassava root expansion is significantly impaired under low light intensity.

CONCLUSION

The study shows that growing seasons played an important role in the significant differences recorded in the storage root yield at the final harvest. The variations obtained in the starch granule morphology, starch content, amylose and amylopectin contents were affected more by genotype. The level of increase in starch content and size of the starch granules during the early growth stages of the cassava planted at the early monsoon was more rapid than those planted during summer and late monsoon, while a speedy increase in starch content and starch granule size was observed during the middle and late stages of growth in those planted in late monsoon and summer. Hence, the knowledge acquired from this study is expected to enhance our understanding of the molecular processes that govern cassava storage root yield and starch physiology concerning the various growing seasons, which should be useful in developing cassava genotypes with improved starch quality and root yield. There is need therefore to implement targeted breeding programs to develop cassava genotypes with improved starch properties and higher root yields.

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Conflict of interest

The authors declare that they have no conflict of interests that may influence the work reported in this paper.

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