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TAXONOMIC SIGNIFICANCE OF PHYSICAL CHARACTERISTICS OF STARCH GRAINS IN TUBERS OF RIZGA (*Plectranthus esculentus* N.E. Br.) COLLECTED FROM JOS AND MAMBILLA PLATEAUS, NIGERIA.

By

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Abstract

Physical characteristics of starch grains in tubers offer key distinguishing characters amongst plants at higher taxonomic hierarchy. This research was aimed at utilizing micromorphology of tuber starch grains to delineate between populations of *Plectranthus esculentus* (Lamiaceae) which were collected from upper and lower regions of the Jos and Mambilla Plateaus. Images and data were produced microscopically from shapes, relative sizes and configurations of starch grains in representative populations. Binary coded data were subjected to statistical analyses. There was broad similarity among the physical characteristics of tuber starch grains in populations of *Plectranthus esculentus* based on different sampling localities and terrains. Small and large grains of simple and compound types occurred as flat rounded or amorphous particles in the same population, suggesting that the starch physical properties are not specific or peculiar to any of the variety or landrace investigated. However, Starch grains in tubers of the landrace Gwe were over 90% amorphous polyhedral without clear lines of stratification. Hilum was close in the flat simple particles. Significant differences ($P=0.05$) were recorded

in confidence interval (CI) on true means between populations based on sampling locality and terrain. Thus, it is recommended that landrace Isci collected from lower regions of Mambilla Plateau may be explored for desirable starch characteristics such as swelling, viscosity, gelatinization and milling quality.

Keywords: Physical characteristics, starch grains, *Plectranthus esculentus*, taxonomy, micromorphology, Jos, Mambilla, Plateaus, Nigeria

Introduction

Plants manufacture food during photosynthesis and deposit them in semi-crystalline forms in most tissues, but particularly abundantly in storage tissues such as seeds and tubers (Buléon et al., 1998). The food formed granules have starch as the main component while small amounts of lipids, protein, ash, minerals, phosphorus and moisture also occur. However, the semi-crystalline starch granule consists almost entirely of two glucose polymers, amylose and amylopectin molecules.

According to Wilson et al. (2010), these molecules become variable due to influence of environment on the underlying genetic diversity and actions of biosynthetic enzymes. These factors bring about natural variability in amylose and amylopectin molecules, which in turn resulted in diversity in granules morphology. Therefore, starch granules from different botanical origins differ in terms of size, shape and definition of the hilum because of inherent characteristics such as shape, structure, molecular weight, and proportion of the polymers to each other within the granule. This variability has been reported to be associated with differences in functional properties and nutritional qualities in the food and pharmaceutical processing industries (Gallant et al., 1992; Itiola & Odeku, 2005)

The physicochemical properties of starch grains in *P. esculentus* have been determined and its potential for pharmaceutical application evaluated by Muazu et al. (2011). They reported that amylose content was 24%, mean particle size 20.16 μm , true density 1.45 g/ml, and swelling power 20.04%. *P. esculentus* starch was found to compare well with maize starch BP in physicochemical properties (Ochekpe et al., 2013). Its binding and disintegrating ability in Paracetamol tablets was also studied, and it had shown comparable binding and disintegration capacity to potato and maize starch BP.

Reichert (1913) was one of the first to recognise the potential of starch granule morphology as a taxonomic tool, and morphological characteristics of starch granules are being considered as anatomical characters today. Torrence et al. (2004) used measurements obtained interactively from images of starch granules in a multivariate analysis to achieve their classification. They reported that, generally, granule

size may vary from less than 1 μm to more than 110 μm . And that granule of tuber and root starches are oval, although round, spherical, polygonal, and irregular shaped granules also exist. For example, taro, dasheen and parsnip starches consist of very small granules compared to other root and tuber starches. In the cereals, the small granule size fractions of wheat, barley, rye and triticale have a different morphology than their larger counterparts. Granules of bean and pea starches are characterized as thick disks with an incision around the middle or at the ends and an indentation at one end. Starch granules from fruits and nuts vary in shape. Some nut starches have unusual granule morphology of half-spheres, although most are round in shape (Hoover, 2001; Lindeboom et al, 2004).

Thomas and Atwell (1999) reiterated that morphological characteristics of starch granules may be studied through microscopic analysis using light or scanning electron microscopy (S.E.M.). Light microscopy has been utilised in this research to identify type and configuration of starch grains, as well as general size, shape and architecture of granules. Knowledge of physical and chemical characteristics of starch grains would assist greatly in the selection of starch with desirable application properties, as well as for the identification of the source plant for necessary breeding program. The aim of this research is to discriminate between physical characteristics of tuber starch granules of populations of *P. esculentus* gathered from Jos and Mambilla Plateaus.

Materials and Methods

Collection of Tuber Samples

Tuber samples were collected from areas of domestication within upper and lower (terrains) regions of the Jos and Mambilla (localities) Plateaus. Preferential sampling technique was applied for on-farm assessment, sorting and collection with the aid of the naked eyes and hand lens (Merck et al., 2011). Local names of the varieties and landraces whose populations were collected included Bebot, Long'at and Riyum (Berom tribe, Longitude 9.515, Latitude 8.699 and Elevation 1031 m), Cma (Goemai tribe, Longitude 8.699, Latitude 8.626 and Elevation 222.5 m), Fina (Rindre tribe, Longitude 8.927, Latitude 8.626 and Elevation 399.5), Gwe (Mambilla tribe, Longitude 7.079, Latitude 11.092 and 1862.76 m), Isci (Kuteb tribe, Longitude 7.184, Latitude 10.152 and Elevation 230.98 m) and Nanjol (Tarok tribe, Longitude 8.524, Latitude 9.851 and Elevation 147.8 m). Tubers of 46 accessions from 23 populations were sampled, bulked, place in perforated Malina envelops for transportation to the Laboratory of Structural Botany/Plant Anatomy Laboratory, University of Ibadan, Nigeria for analyses.

Preparation of Starch Powder from Tuber

Rizga (*P. esculentus*) flour was prepared from selected tubers of accessions of each population. The tubers were aseptically peeled and sliced longitudinally so as to expose more surface area to evaporation. These were separately wrapped in foil, labelled accordingly before displaying to dry in the oven set at 80°C. The sliced pieces were not certified dry until the pellets become easily powdery when crushed between the finger, after which few pellets were gently ground to powder on sterilised glass surface.

Viewing of Slides and Photomicrography

In order to study the morphology of starch granules, a pinhead load of the tuber powder was collected and then suspended in a drop of 20% glycerol contained on a microscope slide. Caution was observed strictly so as not to mix the powders while labels were maintained to ensure correct identification (Jayeola and Akinsebikan, 2013). Starch granules were studied with the aid of a Technico™ binocular light microscope under the X4, X10 and X40 objectives for photomicrography and characterization. Shape, size, configuration and stratification of granules were recorded. A Celestron Digital Microscope Suite 2.0 Ocular Imager was attached to the light microscope for use to capture starch grains images on an HP Pavilion dm4 Laptop.

Data Collection and Analyses

Microscopic observations were made on twelve (12) starch granule physical characteristics in populations of *P. esculentus* collected from the upper and lower regions of Jos and Mambilla Plateaus. Morphoclines in the attributes were coded in binary form thus; hilum visibility- visible= 0, faintly visible= 1; hilum arrangement: eccentric = 0, eccentric + concentric = 1; shape: polyhedral- absent= 0, present= 1; irregular- absent= 0, present= 1; half-sphere- present= 0, absent= 1; conical- present= 0, absent= 1; jug+cap- present= 0, absent= 1; star- present= 0, absent= 1; large grains with central traumatic canal- present= 0, absent= 1; grains with traumatic canal from the sides- present= 0, absent= 1; size: small only= 0, small+large= 1; amorphous grains- present=0. absent=1. Apomorphy were counted and presented in histograms while variations in terms of analysis of variance (ANOVA), confidence intervals (CI) on true means and coefficients of variations (CV) in starch grain micromorphological characters among

accessions of 23 populations collected were calculated based on the different terrains (upper and lower regions) and localities (Jos and Mambilla Plateaus).

Results

Physical Characteristics/Micromorphology of Starch Grains in P. esculentus

Photomicrographs of starch grains observed in representative accessions of *P. esculentus* collected from the upper and lower regions of Jos and Mambilla Plateaus are as shown in Figs 1 to 4. Simple semi-crystalline rounded and compound amorphous particles were seen across the populations studied. Careful observations revealed that physical characteristics of starch grains in all accessions of populations of the landraces studied were a mixed configuration containing compound grains, bimodal simple grains and uniform simple grains in the same accession. The compound grains were mainly trimodal and quadrimodal, at least until they cleave to become simple amorphous grains. The different pieces may be of equal or unequal sizes (Figs 1A, 1C, 2A, 3A and 4E and F). The bimodal simple grains have 2 pieces, 1 of the pieces may be more elongate and each with a hilum (Figs 1C, 3A, B, and 4E). The simple grains occurred singly under the microscope and were flat, dome shaped/ovotriangular or hemispherical.

Starch grains that are shaped round, oval, dome-shaped, irregular, half-sphere, conical and pot-shape abound in flour of all accessions studied. Landrace Isci collected from the lower Mambilla Plateau, had starch grains that were quadrimodal, with each piece fitted in an interlock, at least until they cleave, in their fours (Fig 4F). However, the occurrence of starch grains with star shape seem to be as a result of trauma canals emanating from the periphery of the grain, or the hilum splitting in different directions. Starch grains of such structure are prevalent across accessions of the varieties and landraces studied particular accessions of landrace Isci (Figs 4A and B).

The photomicrograph in Fig 3C showed that landrace Gwe has about 90% amorphous and hemispherical starch grains with concave or pressure surfaces. Starch grains of this shape were also seen in flours of accessions of the varieties and landraces studied including the irregularly shaped concave surfaced grains in landraces Riyum collected from Bachit and Nanjol sourced from the lower Jos plateau (Figs 1D and 2D). Figs 1A, 2B and 3B are photomicrographs of accessions of the variety Bebot, landraces Cema and Gwe respectively, showing predominantly small sized starch grains of mixed

shapes. The sizes of grains in variety Bebot are subequal, with the largest being between 20 to 30 times as large as the smallest. The various shapes of starch grains come in different sizes even within the same sample, although some fragments appear to be broken pieces of compound grains. Photomicrographs of flour of Gwe and Isci shown on Figs 3D and 4D respectively show trimodal compound starch grains of different sizes.

The hilum appears elongated in starch grains of variety Bebot, landrace Nanjol and landrace Isci, forming radiating canal-like partitions (Figs 1A, 2D and 4E), while the photomicrographs of starch grains in landraces Gwe and Isci collected from Mambilla plateaus shown in Figs 3C and 4F, the hilum is not clear. The starch grains whose hilums are not visible also do not show lamellations. Lines of stratification are more visible on round shaped grains in flours of landrace Isci than they are in grains of variety Riyum. The round grains showed concentric lines of stratification even as a bimodal grain type (Fig 4D), while the compound grains show more or less eccentric stratification.

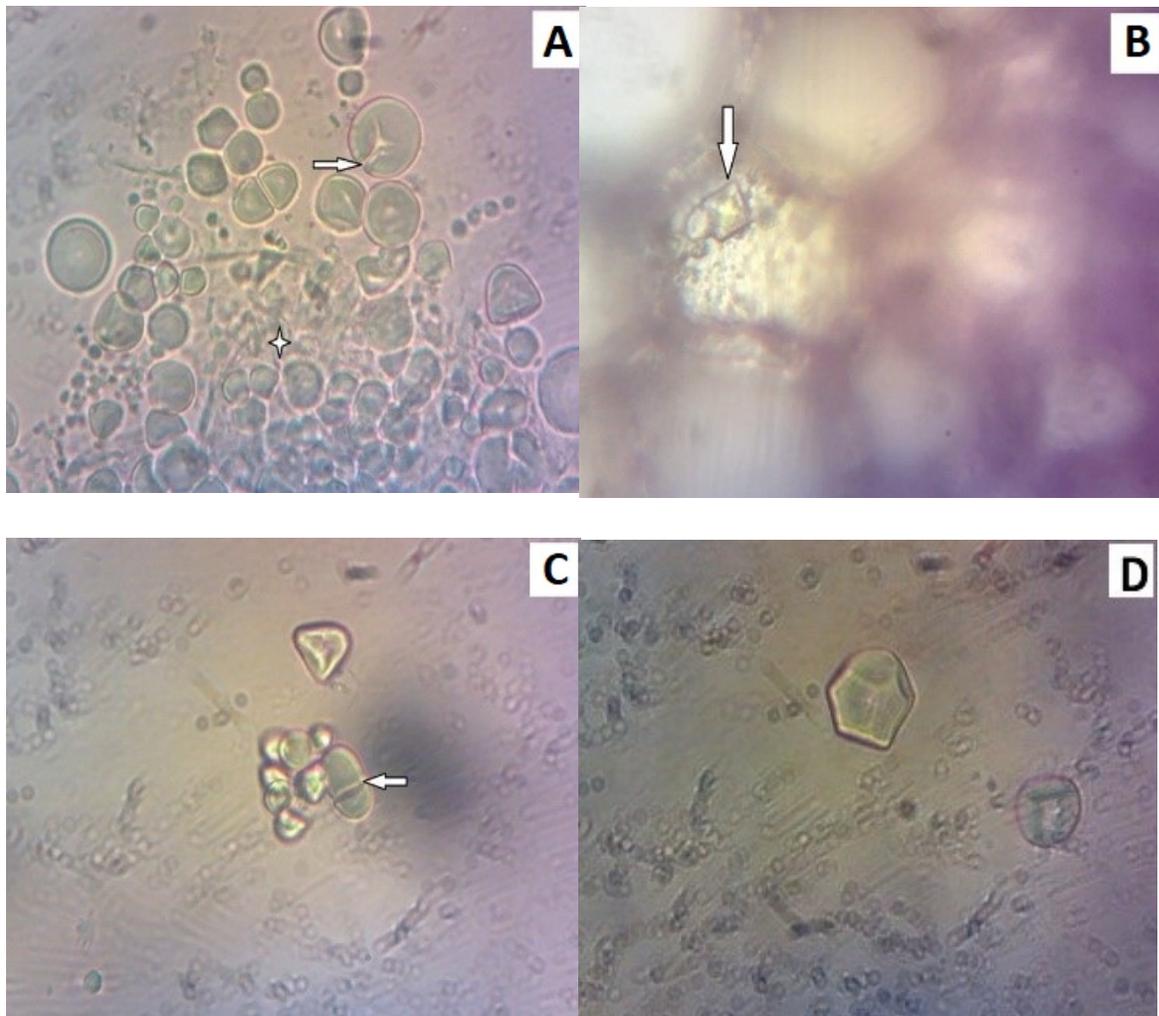


Fig 1: Light Micrograph of Starch Grains in Representative Accessions of *P. esculentus* collected from Upper region of Jos Plateau- A= round, bimodal and dome shapes, hilum visible in variety BebotNRCRIKP1. Arrow indicates trauma/radiating canals B= pith cell in variety Long'atNRCRIKP1 containing square shaped starch grain (arrow). C= landrace Long'atVMKVP2 showing ovotriangular and unequal bimodal grains (arrow). D= Irregular-shaped grain with concave pressured surfaces in RiyumBCHRYMP1

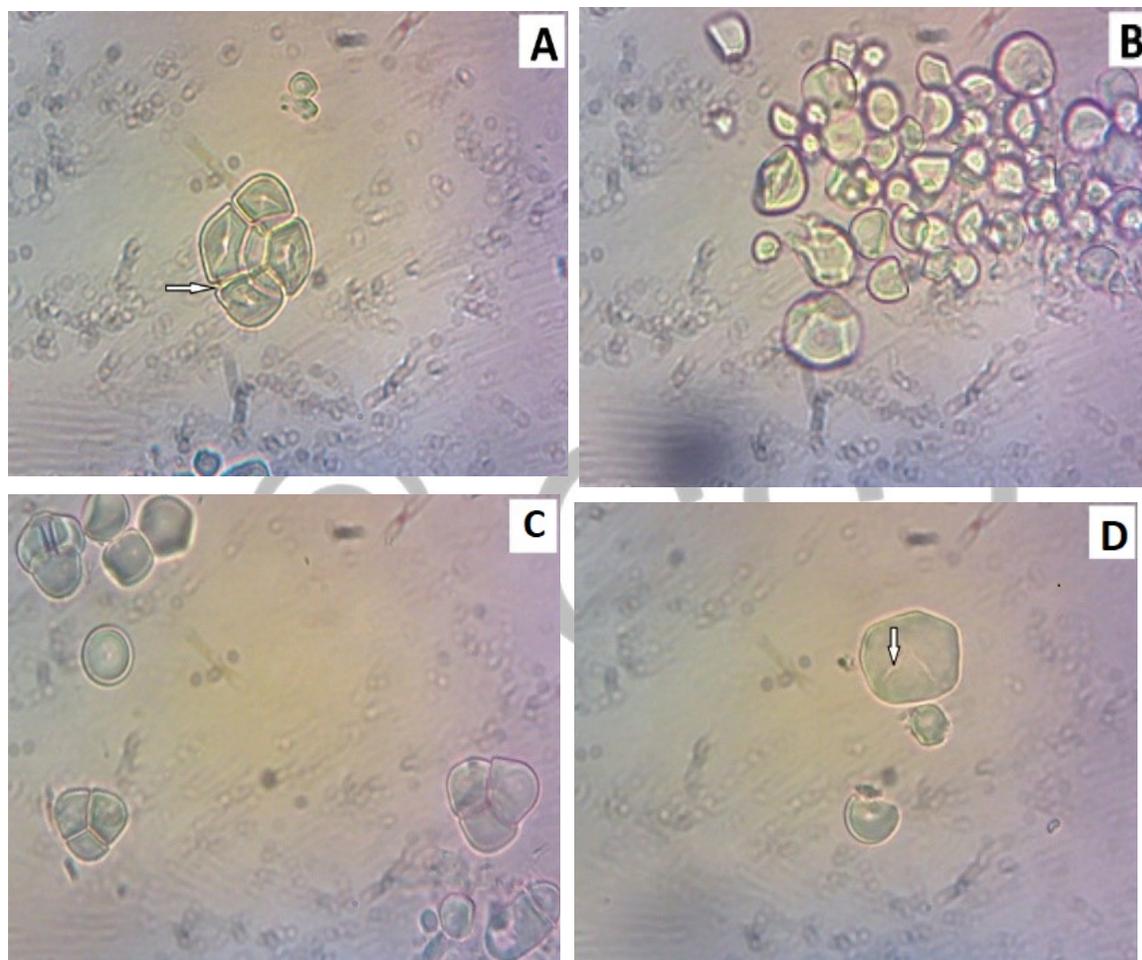


Fig 2: Light Micrograph of Starch Grains in Representative Accessions of *P. esculentus* collected from Lower region of the Jos Plateau-A= split quadrimodal grain in landrace CemaGKWP1. Arrow shows line of cleavage B= accession of CemaGKWP1 showing grains of different shapes. C= landrace FinaWNBP1 showing trimodal, round and other shapes. D= irregular shape with elongated hilum (arrow) in landrace NanjoLLNGGNGP1

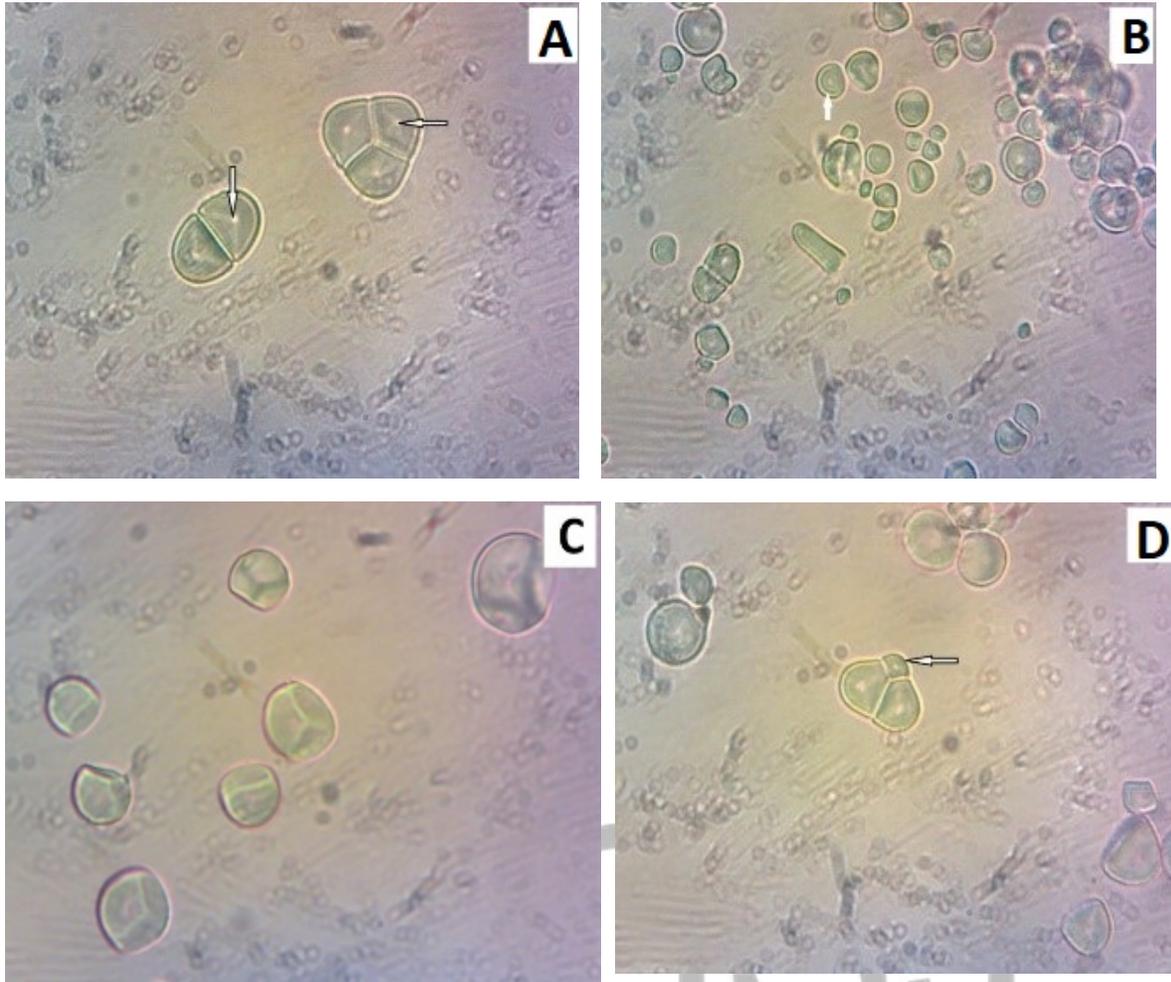


Fig 3: Light Micrograph of Starch Grains in Representative Accessions of *P. esculentus* collected from Upper regions of the Mambilla Plateaus-A=Bimodal and trimodal compound grains in landrace GweSDNYNP3. Arrows reveal open hilum. B=landrace GweSDNNBP2 showing a combination of different shapes and sizes. Arrow shows round grain with stratification. C=Hemispherical shapes with concave pressure surfaces in landrace GweSDNYNP1. D= arrow showing budding piece in a compound grain and other shapes in landrace GweSDNYNP3.

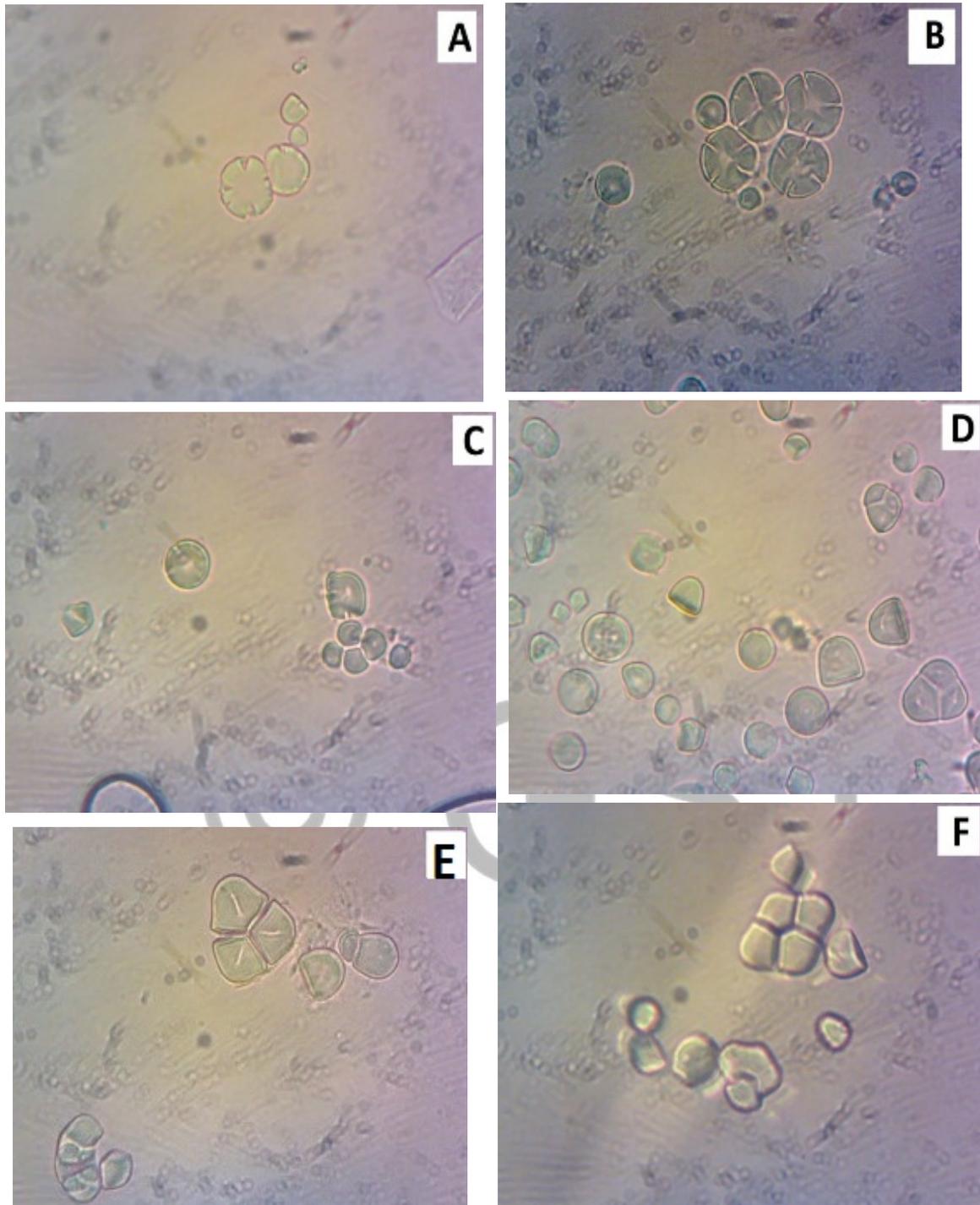


Plate 4: Light Micrograph of Starch Grains in Representative Accessions of *P. esculentus* collected from Lower regions of the Mambilla Plateaus A= flattened starch star-shaped grains in landrace IsciTKBBP3. B= star-shaped grains in landrace IsciUSKWSTP1. C= starch grains of landrace IsciTKTMYP5 with concentric rings, round and quadrimodal shapes. D= landrace IsciTKBLP1 with mixed shapes particularly bimodal round, hemispherical and dome E= trimodal, elongated bimodal and dome shapes in landrace IsciUSLSMP2. F= cleaved quadrimodal compound, irregular shapes in landrace IsciUSLSMP2.

Results of Statistical Analyses

Apomorphy of starch grain characters were counted and the histogram in Figure 1 reveals that populations collected from the lower regions of Jos and Mambilla Plateaus (CemaGKWP1, NanjoLLNGGNGP1 and IsciUSKWSTP1) had starch granules with a greater number of advanced characters including occurrence of both small and large grains in the same individual, presence of rounded grains as well as formation of trauma canals. However, Table 1 shows that these differences were not significant ($P=0.05$) within the localities and terrains. The populations sourced from the upper Jos and Mambilla Plateaus recorded similar number of advanced starch grain physical characters (60.41 and 60.45 respectively). Samples collected from lower regions of the two localities had greater number of apomorphous starch micromorphological features (64.09) although the difference between the two terrains was not significant ($P=0.05$). A significant difference ($P=0.05$) was however recorded based on occurrence of the 12 characters observed in the entire populations of landraces of *P. esculentus* studied (Table 1).

Confidence intervals (CI) on true means shown in Table 2 indicates significant differences ($P=0.05$) in the number of starch grains apomorphous micromorphological characters between accessions of populations sourced from the lower regions of Jos plateau (14.000) and the upper regions of Jos and Mambilla plateaus (11.556 and 11.333 respectively). However, the same table shows that samples of populations of *P. esculentus* collected from the lower region of Jos Plateau recorded the least variations with the CV of 10, while samples sourced from lower region of the Mambilla Plateau were statistically more varied (CV= 17).

Discussion

The preference for the use of starch grains micromorphological characters prepared from tubers to differentiate between populations of *P. esculentus* is in agreement with results of previous studies which have shown that the morphology of many starch granules, especially those derived from storage organs, is distinctive to a specific plant taxon (Torrence et al., 2004). Photomicrographs shown and values obtained indicate that physical characteristics of tuber starch granules in varieties and landraces of *P. esculentus* studied within the guinea savanna zone of Nigeria are generally the same in terms of type, shape, size and configuration. Population of the landrace Isci, which was sourced from the lower regions of Mambilla Plateau varied significantly in terms of these properties including the prevalence of compound

grains of rounded shapes, presence of small and large size granules, as well as the formation of traumatic or pressure canals in the same individual (Verwimp et al., 2004). Experts in the field including Czaja (1978) who carried out extensive and prolonged microscopic identification of powdered plant materials, asserted that starch grains from different sources show variations in structural and chemical compositions at the family level and above. This author opined that due to lack of analytical key to adequately classify the families Casaurinaceae, Nymphaeaceae and Cyclanthaceae, starch grains characteristics could offer one or more characters which can be used to group families of plants into higher categories. In contrast, findings in this research agree with reports by Jane et al. (1994) and Torrence et al. (2004) that starch granules from multiple plant sources showed considerable similarities in morphologies between and within species. However, Malomo and Jayeola (2009), and Jayeola and Akinsebikan (2013), reiterated the limitations of variations in starch grains micromorphology as a tool for intraspecific delineation when they studied several cultivars of *Dioscorea alata* L. (White yam) and observed that morphologically similar granules occurred within class and between locations.

It may be inferred from the 95% statistically significant difference existing in this research between populations of *P. esculentus* collected from the lower regions of Jos Plateau and the upper Jos and Mambilla Plateaus that difference in topography could impart on deposition and interactions of amylose, lipids, amylopectin and phosphorylated residues during formation of the starch grains. However, this line of reasoning is being limited by the fact that there is dearth of systematic research information on starch grains micromorphology in *P. esculentus* and related taxa. This implies that relevant reference collections are not available for comparison between micromorphologies of starch granules in population of plants of the same species but which have been collected from different geographical gradients (Perez and Bertoft, 2010; Tester et al., 2004; Cornejo-Ramírez et al., 2018).

The square shaped crystalline starchy particle seen in pith cell of population of variety Long'atNRCRIKP1 could be grains of transitional starch that can be observed in all stroma of chloroplasts and cytoplasm. In addition to this, a wide range of shapes of tuber starch grains were seen in samples of *P. esculentus* studied, ranging from regular disc, oval/spherical, elongated, rounded, ovotriangular/cone shape to compound grains with two to four parts as well as other irregular shapes. They appeared flat or polyhedral amorphous without clearly defined lines of stratification. The hilum is mainly positioned concentrically but a few eccentric types observed were either elongated, central, open or closed. Hilum

appeared elongated in starch grains of variety Bebot, landrace Nanjol and landrace Isci, forming radiating canal-like partitions which Fatokun (2019) labelled as reversed and asymmetric fissures. Size of the grains observed ranged between 50 μm to 150 μm with landrace Gwe sourced from the upper parts of Mambilla having the largest amorphous grains although small grains also occurred. Zeeman et al. (1910), Gaines et al. (2000), Duran et al. (2001), Peroni et al. (2006), Le Corre et al. (2010) and Cornejo-Ramírez et al. (2018) reported that there were significant overlaps in the sizes of starch grains in the same and different species and thus, starch grain size may not be useful for classification at specific level. The prevalence of small and large starch grains with trauma canals or pressure fissures in landraces collected from the lower regions of the plateaus are distinguishing features. These authors also emphasized that smaller grains have higher superficial area and thus, higher hydration rates which increases their swelling, viscosity and gelatinization capacity. These are desirable characteristics in starch granules, and the abundant small and large size grains with pressure canals in landraces collected from the lower regions of the Jos and Mambilla plateaus may make them more acceptable for specific end use (Gallant et al., 1997; Itiola and Odeku, 2005; Vermeylen et al., 2005; Copeland et al., 2009; Ocheke et al., 2013; Cia et al., 2014).

In conclusion, tuber starch grains physical characteristics in varieties and landraces of *P. esculentus* studied have the distinctive compound grain configuration in addition to grains of other shapes and sizes. With this broad similarities, it has become permissible to assert the origin of this plant species from a related ancestral member of the family Lamiaceae, or some other levels of taxonomic classification such as the taxon *Solenostemon rotundifolius* (Poir) J.K. Morotn (Bertoft, 2017). However, identification of the ancestral plant through comparison of physical characteristics and micromorphological features of starch grains has remained problematic due to lack of a suitable database of reference images. Hopefully, the photomicrographs provided in this research will be alluded to towards taxonomic elucidation of starch grains, particularly in neglected minor tuber crops of Africa since knowledge of these characteristics would assist in informed selection of the most appropriate variety and landrace for a specific end use. Based on findings in this research, it may be recommended that the varieties and landraces of *P. esculentus* studied, especially those collected from the lower regions of Jos and Mambilla Plateaus, may be harnessed for properties such as swelling, viscosity, gelatinization and milling quality.

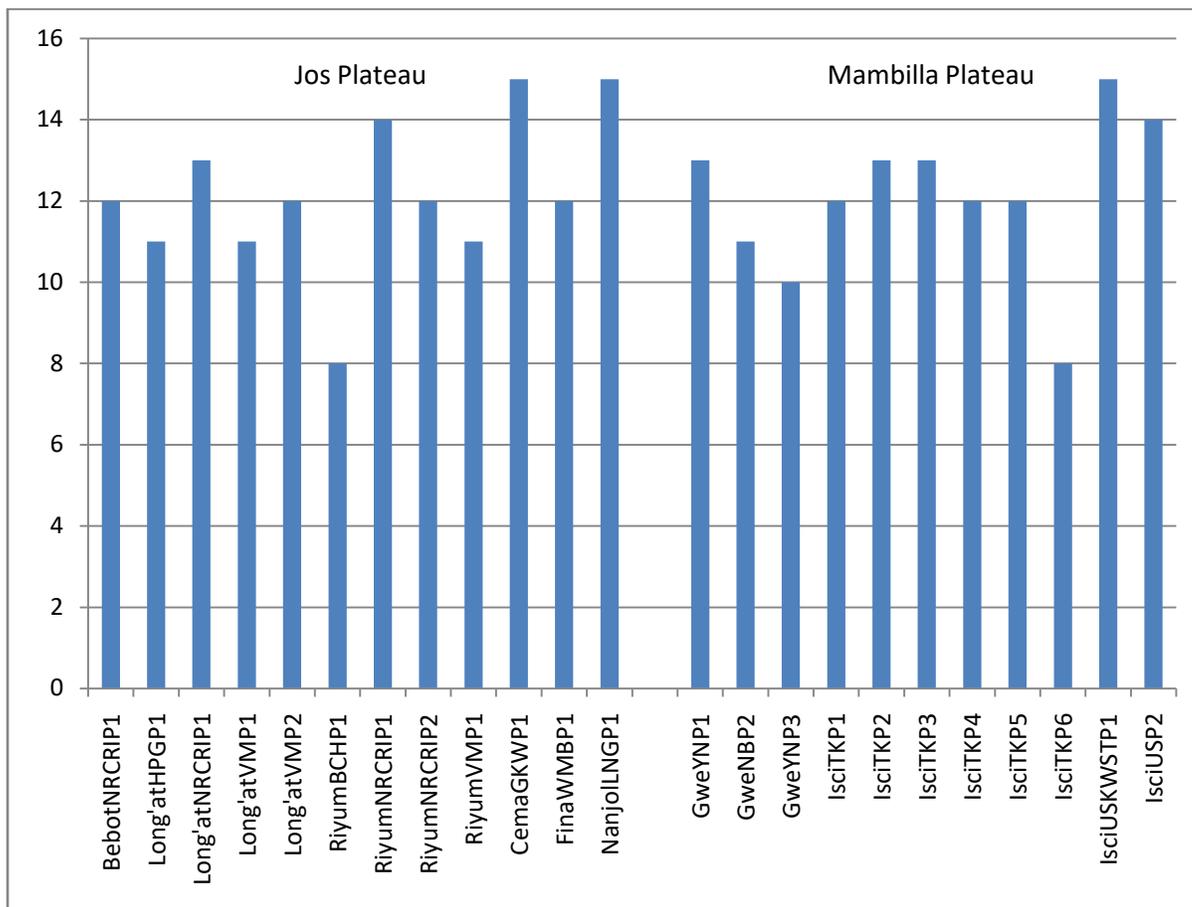


Fig 1: Number of Apomorphous Characters Diagnosed in Tuber Starch micromorphology of 23 Populations of *P. esculentus* collected from Upper and Lower terrains of the Jos and Mambilla Plateaus, Nigeria.

Table 1: Difference in Number of Apomorphous Characters Diagnosed in Organs and Starch grains of 23 Populations of *P. esculentus* collected based on Locality and Terrain.

Starch micromorphology	
LOCALITY	
Jos	60.41
Mambilla	60.45
F-ratio	NS
TERRAIN	
Upper Plateau	57.08
Lower Plateau	64.09

F-ratio NS

CHARACTER

F-ratio *

*= Significant (P=1.0, P<0.05)

Table 2: Mean Number of Apomorphous Characters of various types Diagnosed in Organs of Accessions of 23 Populations of *P. esculentus* collected from Upper and Lower terrains of the Jos and Mambilla Plateaus, Nigeria.

CHARACTER	LOCATION	TERRAIN			
		Upper plateau	CV(%)	Lower plateau	CV(%)
STARCH	Jos	11.556 ±0.46	14	14.000 ±0.58	10
MICROMORPHOLOGY	Mambilla	11.333 ±0.63	13	12.375 ±0.65	17

NOTE: a) Values are means ± Confidence interval (CI)

b) Means whose confidence limits (CL) overlap are not significantly different (P>0.05) for each character set using the t-test.

c) CV= Coefficient of variation

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