Phenotypic traits of three local cassava (*Manihot esculenta* Crantz) genotypes cultivated by farmers in Rivers State, Nigeria

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Abstract

The study aimed to elucidate the morphological and anatomical traits for varietal delimitation and identification of three local cassava (*Manihot esculenta* var. aguregbu, var. cagbo and var. ekagon) varieties cultivated by farmers in Obio Akpor local government area of Rivers state. Twenty (20) qualitative and six (6) quantitative traits from the descriptors for cassava germplasm was used for morpho-characterization. The results obtained revealed phenotypic similarity for 15 qualitative traits among the three varieties. Trait differences was observed in the petiole color (red and green), prominence of foliar scars, and shape of stem, level of branching, root shape and constrictions. The distance between leaf scars was classed as short for all varieties but the metric value ranged from 1.6 cm for ekagon to 4.0 cm for aguregbu and cagbo. Other quantitative variation observed include petiole length (11.1 to 15.0 cm) and storage root length which was classified as medium (≤30 cm) and long (>30 cm). The number of leaf lobes was seven for all varieties. The epidermal traits showed a uniseriate epidermis with hypostomatic distribution. The stem, petiole, and midrib anatomy was similar in terms of tissue type and arrangement. However, the number of vascular bundles in the petiole was more in aguregbu (10) than in cagbo (8) and ekagon (8). The variable traits identified in this study provide diagnostic features that could aid in the identification of the three cassava genotypes.

Keywords: *Manihot esculenta* var Aguregbu; Cagbo; Ekagon; Petiole; Phenotypic traits

1. Introduction

*Manihot esculenta* Crantz commonly called cassava is major staple in many developing countries of Africa, South and Central America, India and Southeast Asia [1]. It is known for its unique ability to grow and produce well in low fertility soil, withstand attack by locusts and other insect pests, and tolerate drought conditions. Cassava serves as an important staple food and can be used directly or as protein source in the concentrate mixture in animal feed [2]. Naturally fermented cassava starch is used in bread-making [3]. In Nigeria and other African countries, tapioca and fufu are made from starchy cassava root flour while garri is made from fermentation of root [4]. The seeds contains 90% unsaturated oil which might possibly be used as a fuel for pre-combustion diesel engines [5]. The production and growth of cassava is severely limited in certain semi-arid areas such as north-eastern Brazil and Northern Nigeria, due to drought conditions [6]. The bitter variety of Manihot root is used to treat diarrhoea and malaria [7]. The leaves are used to treat hypertension, headache, and pain [8]. Cubans commonly use cassava to treat irritable bowel syndrome, the paste is eaten in excess during treatment [9]. It has been reported that cassava may have anti-cancer properties [7]. Genes isolated from the plant have been already used to eradicate brain tumours in laboratory rats [10].

Anatomical traits in foliar epidermis, stem and petiole which are highly conserved and not affected by the environment have been extensively utilized for identification of plants and elucidation of genetic relationships. Stem [11] and petiole [12] anatomy have been used for taxonomic delimitation of wild cassava genotypes, cultivars and hybrids. The objective
of this research was to determine the relationship of three local varieties of *Manihot esculenta* var. aguregbu, cagbo and ekagon cultivated by farmers in Rivers State, Nigeria using phenotypic markers.

### 2. Material and methods

#### 2.1. Sample collection

The three local varieties of *Manihot esculenta* used in this study (*Manihot esculenta* var. aguregbu, *Manihot esculenta* var. cagbo, *Manihot esculenta* var. Ekagon) were collected from a farmers field in Rumuosi, in Rivers State, with GPS location of Latitude 04˚55’14.8” North, longitude 006˚55’07.7” East.

#### 2.2. Morphological traits

Standard morphological and agronomic descriptors for characterization of cassava germplasm [13] was used to score vegetative traits in the field. Overall morphology of the plants were recorded by photography. The quantitative traits measured were petiole length, leaf width, leaf length, number of lobes, distance between leaf scars and storage root length.

#### 2.3. Anatomical traits

Fresh specimen of leaves, midrib and stems were collected from each of the variety in the field and used for the anatomical studies.

- **2.3.1. Foliar Epidermis**
  
  The upper and lower epidermal layers of fresh matured leaves were peeled and stained with 1% safranin or alcian blue, rinsed with distilled water to remove excess stain and then mounted in a drop of pure glycerol on clean glass slides and viewed using a light microscope. Photomicrographs of both adaxial and abaxial surfaces were taken with the aid of a digital camera.

- **2.3.2. Stem, Petiole and midrib**

  Stem, petiole and midrib were collected and hand sectioned using the method of [14]. Sections were placed in a Petri dish that contained water and subsequently collected using a camel brush, and placed on a clean glass slide and then stained with 1% safranin or alcian blue and then rinsed with the distilled water. The sections were then mounted on slides with a drop of glycerin, viewed under the microscope and photographed.

### 3. Results

#### 3.1. Morphological studies

The qualitative and quantitative descriptors scored for all morphological traits in the three varieties are on table 1 and figure 1.

- **3.1.1. Manihot esculenta var. Aguregbu**

  The plant type is cylindrical, it has a one level branching system, it has a dark green color of fully expanded leaf, the shape of the central lobe is lanceolate, it has a light green leaf vein color, the petiole color is totally pigmented with a pink color, the stem is dark brown, the storage root shape is conical-cylindrical, it has prominent leaf scars, storage root constrictions is present with a storage root length of more than 30 cm.

- **3.1.2. Manihot esculenta var. Ekagon**

  The plant type is open, it has a three branching level type, the first fully expanded leaf is light green in color, the leaf shape is lanceolate, the leaf vein color is light green, the petiole color is green without pigmentation, the stem color is dark brown, the root storage system is dark brown, it has a fusiform storage root shape, there is presence of root constrictions, it has little leaf scar prominence, the storage root length is long more than 30 cm.

- **3.1.3. Manihot esculenta var. Cagbo**

  The plant type is open, it has a one level branching system, the first fully expanded leaf is dark green, it has a lanceolate leaf shape, the petiole color is dark green, it has a dark brown stem color, there is absence of root constrictions, it has
moderately prominent leaf scars, the storage root length is medium with a length of 20-30 cm, the storage root shape is conical.

**Table 1** Morphological characters of three local varieties of cassava evaluated in the farmer's field

| Variety   | Aguregbu | Cagbo     | Ekagon  |
|-----------|----------|-----------|---------|
| **Qualitative Traits** |           |           |         |
| Plant shape | Open                 | Cylindrical | Open |
| Color of apical leaf | Light green | Light green | Light green |
| Pubescence on apical leaf | Absent | Absent | Absent |
| Shape of central leaflet | Lanceolate | Lanceolate | Lanceolate |
| Leaf color | Dark green | Light green | Dark green |
| Petiole color | Red | Green | Green |
| Lobe margin | Smooth | Smooth | Smooth |
| Color of leaf vein | Green | Green | Green |
| Orientation of petioles | Horizontal | Horizontal | Horizontal |
| Prominence of foliar scars | Prominent | Semi prominent | Semi prominent |
| Color of stem exterior | Dark brown | Dark brown | Dark brown |
| Color of stem cortex | Dark green | Light green | Light green |
| Color of stem epidermis | Dark brown | Light brown | Light brown |
| Growth habit of stem | Straight | Straight | Straight |
| Color of end branches | Green-purple | Green | Green |
| Shape of stem | Cylindrical | Open | Open |
| Branching habit | Dichotomous | Dichotomous | Dichotomous |
| Level of branching | One | One | Three |
| Root constrictions | Present | Absent | Present |
| Root shape | Conical-cylindrical | Conical | Fusiform |
| **Quantitative Traits** |           |           |         |
| No. of leaf lobes | 7       | 7         | 7       |
| Leaf length (cm) | 12.9 | 11.1 | 14.1 |
| Leaf width (cm) | 18.5 | 20.7 | 23.4 |
| Petiole length (cm) | 11.1 | 14.9 | 15 |
| Distance between Leaf scars (cm) | 4.0 Short | 3.9 Short | 1.6 Short |
| Storage root length (cm) | >30 long | ≤30 medium | >30 long |
Table 2 Epidermal traits of the upper and lower leaf surface of three local cassava varieties

| DESCRIPTORS          | Variety         |
|----------------------|-----------------|
|                      | Aguregbu        |
|                      | Cagbo           |
|                      | Ekagon          |
|                      | Upper Lower     |
|                      | Upper Lower     |
|                      | Upper Lower     |

| Foliar Epidermal Traits | Regular | Irregular | Regular | Irregular | Regular | Irregular |
|-------------------------|---------|-----------|---------|-----------|---------|-----------|
| Shape of epidermal cells|          |           |         |           |         |           |
| Stomata distribution    |          |           |         |           |         |           |
| Trichomes               |          |           |         |           |         |           |

Figure 1 Habit of three local cassava varieties - aguregbu, cagbo and ekagon

3.2. Anatomical Studies

Epidermal anatomy

This is divided into two the adaxial which is the upper part of the leaf, and abaxial which is the lower part of the leaf.

- Leaf-Adaxial
  
  The result revealed a glabrous uniseriate adaxial epidermis with regular shaped cells for the three varieties. No stomata was observed in the upper epidermis (Figure 2).

- The Leaf-Abaxial
  
  The adaxial epidermis revealed a glabrous uniseriate epidermis with irregular shaped cells. The stomata is mostly anomocytic with a stomatal index of 13.02. The guard cells are elliptical with mostly 5–6 subsidiary cells (Figure 2).

Stem anatomy

The stem is pentagonal in cross or transverse section, having 5 ridges and 5 furrows. There is presence of epidermal layer, with the absence of trichomes. Endodermis is found to enclose the vascular bundles. The vasculature of the stem shows the arrangement of the vascular bundles with respect to number of furrows and ridges present. The vascular bundles are collateral having phloem and xylem linked by the cambium. Pith is distinct with a well-developed parenchymatous cells. These cells interspersed with air spaces to form a characteristic pattern of pith tissues. The stem cross section is seen in figure 3.

Petiole Anatomy

The outline of the petiole was observed to be circular in shape. The epidermal cells are uniseriate, with the absence of trichomes. Endodermis is found to surround the vasculatures in which the vascular bundles are arranged. The number of vasculatures present varies among the varieties and this variation ranges from 8 to 10. The arrangement of vascular...
bundle is circular in nature. The vascular bundles vary in shape and are collateral, having phloem and xylem linked by cambium. Pith is also present and consists of well-developed parenchymatous cells which are irregularly arranged. The number of vascular bundles in the petiole of aguregbu was more (10 vascular bundles) than var. cagbo and var. ekagon which had 8 vascular bundles (Figure 3). Trichomes were not observed in any of the varieties.

Midrib anatomy

Figure 2 Foliar epidermis of aguregbu, cagbo and ekagon cassava varieties

Figure 3 Transverse section of Stem, Petiole and Midrib of aguregbu, cagbo and ekagon cassava varieties

The midrib is highly conspicuous and U-shape in cross section with angular collenchyma cells on both upper and lower surfaces. The shape varies among the varieties. The vascular bundles are collateral and are arranged in U shape. The
bundle sheaths are mainly parenchymatous and are also arranged in u shape. The parenchyma cells filled the space between the collenchyma and the vascular bundles. The vascular bundles in the midrib is collateral with the phloem towards the lower leaf surface. Trichomes were absent (Figure 3).

4. Discussion

This present study provides information on the morphological and anatomical studies of three varieties of cassava. The results obtained from the morphological studies, showed that the variety aguregbu exhibited the highest leaf number and are also branched. This indicates that the variety will exhibit higher tendency of carrying out photosynthetic activities, which may also lead to an increase in yield (15).

The morphology of the cassava varieties provided diagnostic features (16). Some of the varieties like aguregbu consists of petiole that are highly pigmented (17) while varieties like ekagon and cagbo consist of petioles that are not pigmented but are greenish in color. This variation in petiole pigmentation distinguishes Aguregbu from the other two varieties. Most Manihot species have long petioles (18). The petioles and tissues contain the same arrangement of those present in the stem of Manihot (19). The petiole of dicotyledons often have similar provision stem (20). Trichomes were not observed in this study. The presence of trichomes, thick cuticles, crystals and anthocyanins help to minimize water loss in dry environment [21]. The existence of trichomes is related to the process of speciation that favor the trichomes in low humidity environment (22). The presence of anthocyanins leads to photoinhibition and can accumulate on the stem of the plant in response to stress induced by cold or heat [23].

Morphological and Anatomical features, together with other sources of evidence such as pigmentation, stem leaf scars, branching system and stem root constrictions, shows that the varieties of cassava (Manihot esculenta Crantz) worked on, are mesophytes and upright [24], with highly evolving characteristics of a dicot. The hypodermis plays an important role in drought resistance, and probably functions as a water storage tissue in such species [19]. These anatomical changes may occur in plants as an adaptation to drought stress [25].

All the varieties studied, expressed an identical stem color [26]. Results obtained from the anatomical studies on the three varieties, showed a useful characteristic with respect to the presence of branching system and arrangement of the vascular bundles in both petiole and midrib [27]. In the stem anatomy, the anatomical evidence reveals the relatedness of these. Some specific variations were observed in the midrib and petiole. These variations are of great taxonomic importance [28].

In petiole anatomy, variety aguregbu expressed the highest number of vascular bundles (10) and are branched, followed by Cagbo and Ekagon which both had 8 vascular bundles each. A continuous layer of collenchyma was observed (which is involved in mechanical support) of variable thickness in the cortex below the chlorenchyma. Pericyclic fibers in the cortex forms two to three continuous layers [29]. Plants under drought stress, the fibers have the same distribution, and the number of layers increases. Sclerenchyma helps to prevents water loss, which explains the adaptability of species in tropical regions, such as Manihot, to drought stress [19]. Laticifers were observed in the cortex, phloematic tissue, xylem, and on the periphery of the pith, and is a common characteristic of the Euphorbiaceae.

5. Conclusion

In this study, the distinguishing traits among the three varieties were petiole color, petiole length, level of branching, storage root constrictions and number of vascular bundles in petiole. These variable traits identified in this study provide diagnostic features that could aid in the identification of the three cassava genotypes.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.
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