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Phytotomy of ten accessions of *Vigna subterranean* (L.) Verdc

Josephine U. Agogbua *, Gift O. Chuku and Ekeke Chimezie

Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria.

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Abstract

This study aimed at examining the leaf epidermal and anatomical features of the stem and petiole of ten Nigerian accessions (Tvsu30, Tvsu173, Tvsu258, Tvsu278, Tvsu282, Tvsu362, Tvsu596, Tvsu600, Tvsu633 and PHC-001) of *Vigna subterranean* (L.) Verdc. Foliar epidermal peels collected from matured plants were peeled and stained with alcian blue while stem and petiole obtained from matured plants in the field were stained and mounted on slide and observed under the microscope and microphotographs were taken. Results of the epidermal studies revealed paracytic stomata with polygonal cells on the abaxial surface. The ten accession had foliar epidermal traits in common but varied in the number of vascular bundles present in the stem. The stem anatomy revealed 13 collateral vascular bundles for Tvsu633, 14 for Tvsu30, Tvsu362, 15 collateral vascular bundles for Tvsu 597 and 16 collateral vascular bundles for, Tvsu173, Tvsu258, Tvsu278, Tvsu282 and Tvsu600. The petiole revealed a winged petal with 5 collateral vascular bundles for all the accessions. These foliar epidermal and anatomical characteristics provide information for maintaining them as accessions.

Keywords: Vigna Subterranea; Epidermis; Vascular Bundles

1. Introduction

The Bambara groundnut (*Vigna subterranean* [L.] Verdc.) [1] Belongs to the family Leguminosae and sub-family Papilionoideae [2]. It is commonly known as 'nyimo' (Shona) or 'indlubu' (Ndebele), and gujjiya in Hausa language [3]. It is an indigenous African legume whose origin is known from West Africa to extend from Jos in Plateau State and Yola in Adamawa State of Nigeria [4]; to Garua in Cameroon, Central African Republic and Chad [5]. In Southern Africa, Zimbabwe is the centre of production [6]. It can also be found in tropical parts of America, Asia and Australia [7] but the present degree of cultivation outside Africa is basically not considered. It is the third most commonly eaten legume after groundnut and cowpea in Africa [8] *Vigna subterranea* grows well where groundnut does not [9]. Even though the crop is nutritious and is cultivated throughout Africa, it remains largely neglected by the scientific community [10] Nevertheless, empirical evidence and fragmentary research results suggest that it is a crop with great potential [11]. To fight against inadequate nourishment, food legumes have a major role to play [12]. It is important to increase their level of consumption in developing countries [13]. The high carbohydrate (65%) and relatively high protein of 18% content of Bambara nut makes it a whole food [14] It is farmed primarily for its seeds and high nutritional value [15], which are used by human as food [16]. The seeds are used in varieties of food; some of which play a vital part in human diets [17]. Mature, dry seeds are boiled and eaten as a pulse [18]. Seeds can be ground to make flour [6]. Ripe seeds are hard and a longer period of time is required for it to be cooked properly than those of other legumes [19]. Large seeds are more accepted than smaller ones, specifically use as a snack [20]. Smaller seeds are turned into flour for use in different recipes. It is known to be medicinal as it contains antioxidants and antimicrobial agents and also known as nutraceuticals [21] and also possess biological active compounds [22]. This study is aimed at comparing the anatomical features of ten accessions of *Vigna subterranean* (L) Verdc.

* Corresponding author: Josephine U Agogbua
Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria.

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2. Material and methods

2.1. Experimental Site

This study was carried out using the field and Laboratory facilities in the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt.

2.2. Plant Material

Nine bambara accessions (Tvsu 30, Tvsu 173, Tvsu 258, Tvsu 278, Tvsu 282, Tvsu 362, Tvsu 597, Tvsu 600 and Tvsu 633) used in this study were obtained from the Genetic Resource Unit of the International Institute of Tropical Agriculture (IITA), Ibadan while one accession (PHC-001) was obtained from a local market in Port Harcourt City, Rivers State Nigeria. The seeds of Bambara nut were planted in plastic bucket filled with topsoil and watered daily.. The seeds germinated between 5-7 days after planting while flowering commenced five weeks after planting. Samples were collected at the flowering stage.

2.3. Epidermal features

Foliar materials for epidermal studies were collected fresh from matured plants in the field. Peels were stained with 1% alcian blue rinsed with distilled water to remove excess stain and then mounted in a drop of pure glycerol on clean glass slides. A cover glass was placed over the drop and sealed with nail vanish to prevent dehydration (Okoli & Ndukwu, 1992).

2.4. Anatomical Features

Stem and petiole specimens for anatomical evaluation were obtained fresh from matured plants and fixed in formalin acetic acid (FAA) for 24 hours. The samples were stored in bottles containing 70% ethanol and kept at room temperaure until required. The stem and petiole of the ten accessions were hand sectioned using sharp razor blades, stained with 1% methyl blue for 2 minutes and then rinsed with distilled water to get rid of the excess stain, after which the sections were preserved with glycerin on a slide and covered with a cover slip. The prepared slides were viewed under the microscope using x10 magnification and microphotographs were taken with the aid of a digital camera.

3. Results

3.1. Epidermal Characters

The result of the foliar epidermal study revealed uniseriate epidermis with paracyctic stomata and amphistomatic distribution. Trichomes were not observed in all the accessions (Figure 1).

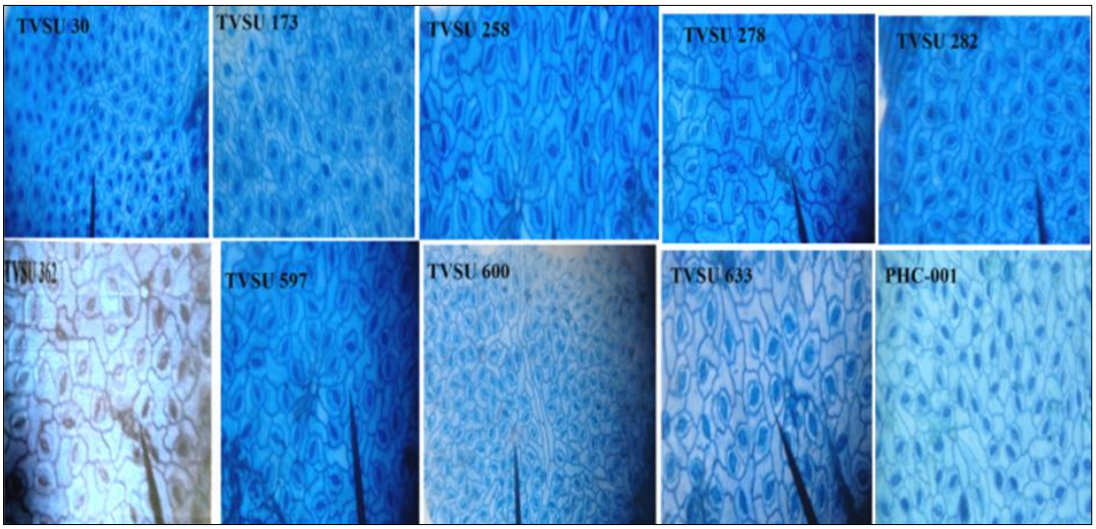

Figure 1 Epidermal Features of the abaxial surface of ten accessions of *Vigna subterranea*

3.2. Stem Anatomy

The stem anatomical investigations revealed that the cortex is made of parenchyma and sclerenchyma cells, the number of vascular bundles varied among accessions, Tvsu 600 and Tvsu 633 had 13 vascular bundles, Tvsu 362 had 14 vascular bundles, Tvsu 597 had vascular bundles arranged in ring form. Vascular tissues alternate with the vascular bundles for all the accessions studied (Figure 2).

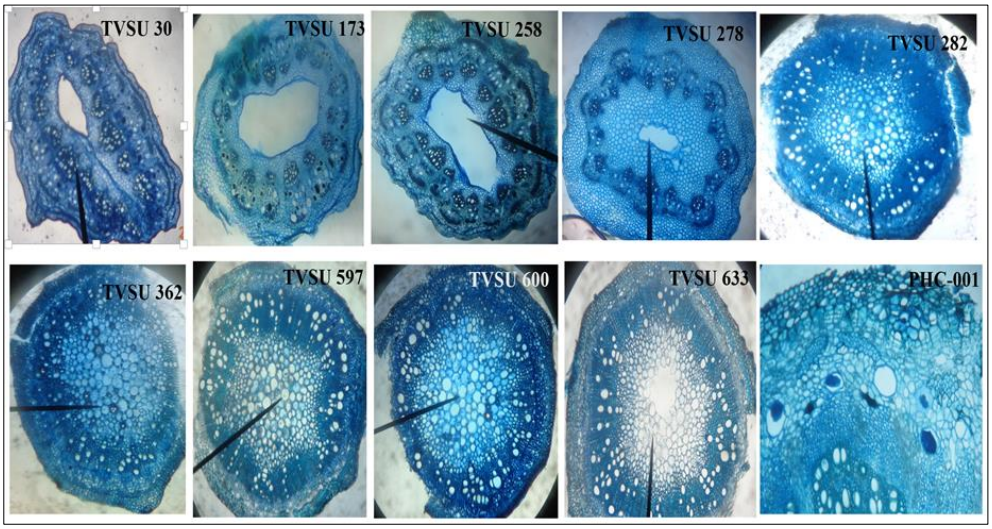

Figure 2 Transverse Section of the Stem of ten accessions of *Vigna subterranea*

3.3. Petiole Anatomy

Petiole anatomical investigations revealed the cortex made of vascular bundles evenly arranged with intercalary spaces, some with well differentiated pith; the vascular bundles are surrounded by parenchyma and sclerenchyma cells. Vascular bundles varied among accessions, Tvsu 30, Tvsu 597 and Tvsu 633 had six vascular bundles while Tvsu 173, Tvsu 258, Tvsu 282, Tvsu 362 and Tvsu 600 had five vascular bundles.

Tvsu 30 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, three to five layers of parenchyma cells, one epidermal layer and the petiole had six vascular bundles, petiole has three to seven layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 173 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, five to seven layers of parenchyma cells, one epidermal layer and the petiole had five vascular bundles, petiole has four to six layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 258 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, five to eight layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 278 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, seven to nine layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 362 has a paracytic stomata with polygonal cells, stem anatomy showed fourteen vascular bundles, three to six layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 282 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, three to five layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 597 has a paracytic stomata with polygonal cells, stem anatomy showed fifteen vascular bundles, three to seven layers of parenchyma cells, one epidermal layer and petiole had six vascular bundles, petiole has three to five layers of parenchyma cells, one to four layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 600 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, three to five layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 278 has a paracytic stomata with polygonal cells, stem anatomy showed sixteen vascular bundles, seven to nine layers of parenchyma cells, one epidermal layer and petiole had five vascular bundles, petiole has three to five layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

Tvsu 633 has a paracytic stomata with polygonal cells, stem anatomy showed thirteen vascular bundles, two to five layers of parenchyma cells, one epidermal layer and petiole had six vascular bundles, petiole has three to seven layers of parenchyma cells, one to three layers of sclerenchyma and the vascular bundles are arranged in ring form.

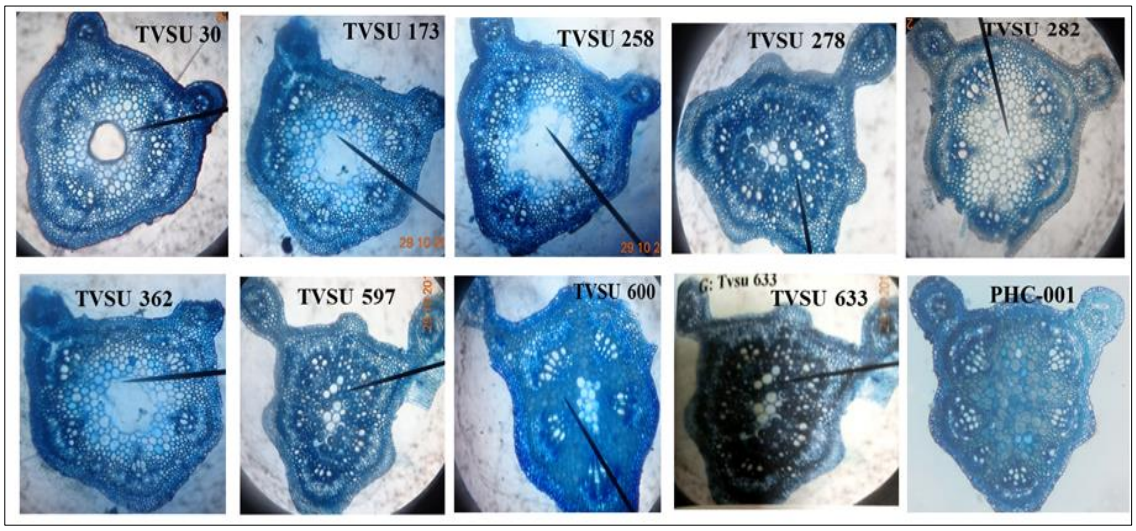

Figure 3 Transverse Section of the Petiole of ten accessions of *Vigna subterranea*

4. Discussion

Epidermal evaluation of the ten accessions of *Vigna subterranea* revealed uniseriate epidermis with paracyctic stomata [24]. The epidermal features of some accessions revealed some characteristics that can be used for taxonomic decisions [25]. The accessions studied all had paracytic stomata and polygonal cell type [26] Paracytic stomata were referenced to Fabaceae family by [23]. The epidermal features of *Vigna radiata* L. revealed paracytic stomata [27] which is in line with the stomata type found in the ten accessions studied. The number of stomata increased as observed on the abaxial surface of the accessions. This increase might be an adaptation to water loss [28]. In this study, the stem anatomical investigations revealed that the cortex is made of parenchyma and sclerenchyma cells, vascular bundles varied among accessions. Also Vascular tissues alternate with the vascular bundles for all the accessions studied considering the type of vascular bundles found in the stem and petiole of the ten accessions studied (collateral vascular bundles), the same were found in *Erythrina velutina* of Fabaceae family [29].

Petiole anatomical investigations shows the cortex is made of vascular bundles evenly arranged with intercalary spaces [30] some with well differentiated pith [31]; the vascular bundles are engulfed by parenchyma and sclerenchyma cells [32]. The number of vascular bundles varied among accessions.

The results obtained from the study revealed that the anatomical features of stem and petiole had stele which consist of collateral vascular bundles arranged in a ring that is separated by interfascicular cambium [27].

5. Conclusion

The stem and petiole anatomy, constitute diagnostic characters for the ten accessions studied. The characterization allows for proper identification of the taxa and provides additional information for maintaining them as accessions.

Compliance with ethical standards

Acknowledgments

All individuals who have contributed to this work have been listed as authors.

Disclosure of conflict of interest

No potential conflict of interest reported by the authors.

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Author's Short Biography

Josephine Udunma Agogbua is a University Lecturer with experience in plant breeding using wild plant species with desirable traits. She utilizes her skills in plant tissue culture techniques for in vitro polyploidization and conservation of crop wild relatives. Her current research is on domestication of Gnetum africanum using classical plant breeding and tissue culture techniques.