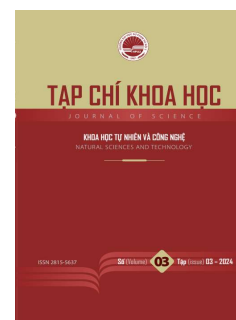




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An investigation into the morphological and anatomical characteristics of three cassava (*Manihot esculenta*) varieties collected from the Northern provinces of Vietnam

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Abstract

Cassava (*Manihot esculenta* Crantz) is a crucial crop that sustains millions of people across tropical and subtropical regions, providing an essential source of carbohydrates and contributing significantly to food security. Morphologically, varieties KM94, 08SA06, and Ruột vàng exhibit distinct traits such as plant form, leaf structure, tuber shape, and starch content, which underline their adaptability and potential for high yield under diverse growing conditions. Anatomically, the stems of these varieties demonstrate unique structural features, including tightly packed epidermal cells with thick cuticle layers, varying layers of collenchyma and parenchyma, robust sclerenchyma rings, and well-organized vascular bundles. These features ensure efficient nutrient and water transport, structural integrity, and resilience against environmental stresses, essential for the growth and development of these cassava varieties. By highlighting these morphological and anatomical traits, this study emphasizes the importance of such detailed analyses for improving cassava cultivation practices.

Keywords: Cassava, morphological characteristic, anatomical feature, Northern provinces of Vietnam, KM94, 08SA06, Ruot vang

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy root vegetable native to South America, specifically the Amazon Basin [1], [2], and is a staple food crop in many tropical regions worldwide. It holds significant importance due to its high carbohydrate content [3], providing a vital energy source

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for millions of people. Cassava is also rich in dietary fiber, vitamin C, and several B vitamins, although it lacks protein and essential micronutrients, necessitating a diverse diet to meet nutritional needs [3], [4]. Cultivation of cassava is relatively straightforward as it thrives in poor soils and can withstand drought conditions [5], making it a resilient crop for food security [6]. In Vietnam, cassava plays a vital role in both the agricultural sector and rural livelihoods, particularly in the Northern provinces where it is cultivated extensively [7]. Introduced during the French colonial era, cassava has adapted well to the local conditions, becoming a staple food and a key economic crop. Several cassava varieties are cultivated in Northern provinces in Vietnam, specifically KM94, 08SA06 and ‘Ruot vang’, each selected for their high yield, disease resistance, and suitability to different environmental conditions. In the Northern provinces, where the climate can be more variable [8], these varieties are particularly valued for their resilience and productivity [7].

Studying the ecological characteristics of specific cassava varieties in the Northern provinces of Vietnam is essential for optimizing agricultural practices and enhancing crop yields. The Northern provinces present unique climatic and soil conditions, including cooler temperatures, higher humidity, and diverse topographies, significantly impacting cassava growth and productivity. Understanding these ecological variables allows for selecting and breeding of cassava varieties that are well-adapted to local conditions, ensuring better resistance to pests, diseases, and environmental stresses [9]. This knowledge also aids in developing tailored cultivation techniques that maximize resource use efficiency, such as appropriate planting times, irrigation practices, and soil management strategies. Furthermore, detailed ecological studies contribute to sustainable agriculture by promoting practices that maintain soil health and biodiversity, ultimately supporting farmers' livelihoods and regional food security [10]. Previous studies have focused on cassava varieties' broad adaptability and resilience to various environmental conditions. For example, the yield potential of cassava varieties KM94 and 08SA06 in Central Vietnam highlights their drought resistance and high starch content. Similarly, a recent study demonstrated cassava varieties' disease resistance and nutrient uptake efficiency across Vietnam, including Northern regions. While these studies provide valuable insights, they lack comprehensive morphological and anatomical analyses specific to the Northern provinces of Vietnam, where unique ecological factors, such as cooler temperatures, higher humidity, and diverse topographies, play a critical role in cassava growth and productivity. Furthermore, these studies often fail to address the limitations of cassava's response to fluctuating microclimates, pest pressures, and soil variations found in Northern Vietnam.

This study aimed to evaluate the ecological characteristics of three specific cassava varieties in the Northern provinces of Vietnam. Particularly, the morphological descriptors of each cassava variety were obtained. Then, we analyzed the primary and secondary anatomical characteristics of the stems and leaves of cassava plants.

2. Materials and Methods

2.1. Materials

Three specific cassava varieties in the Northern provinces of Vietnam, including KM94, 08SA06, and ‘Ruot vang’, were utilized as materials in this study. These homozygous stems were obtained from the Root Crop Research and Development Center, Field Crops Research Institute, Vietnam Academy of Agricultural Sciences (Thanh Tri, Hanoi City, Vietnam).

2.2. Experiment design and methods

2.2.1. Cultivation of cassava plants

Stems of each cassava variety were cultivated based on the local experience combined with previous studies [11]. Briefly, cultivating cassava plants by stems involves selecting healthy, disease-free cassava stems, preferably from 8-12 months old plants. Cut the stems into sections, each about 20-30 cm long, ensuring each section has at least 4-6 nodes. Plant these stem cuttings at a slight angle in well-drained soil, leaving one or two nodes above the ground. Space the cuttings about 1 meter apart to allow sufficient room for growth. Water the cuttings regularly, especially during the first few weeks, to ensure they establish roots. Cassava thrives in full sunlight and requires minimal maintenance once established, making it a suitable crop for various climates and soil types. All experiments were conducted in Xuan Hoa, Vinh Yen City, Vinh Phuc province.

2.2.2. Estimation of morphological descriptors of cassava plants

Available descriptor lists from the International Union for the Protection of New Varieties of Plants and the International Institute of Tropical Agriculture were analyzed to describe the morphology of cassava plants [12]. Each morphological descriptor was observed on 3 different plants of the same variety, repeated 3 times, with a total of 9 randomly selected plants describing the morphological characteristics [13]. Assess each plant for specific morphological characteristics such as leaf shape, stem color, root size, and tuberous root configuration. Each characteristic should be observed on three different plants of the same variety and repeated three times to ensure accuracy. Document these observations systematically using standardized descriptors from relevant guidelines or databases. Using ImageJ software [14], measure quantitative traits, like root length and diameter, and record qualitative traits, such as color and shape, through visual inspection. Compile and analyze the data statistically to identify patterns and variations within and between varieties, providing a comprehensive understanding of the morphological traits of cassava plants.

2.2.3. Determination of dry matter content in cassava tubers

Fresh tubers are harvested and thoroughly cleaned to determine the dry matter content to remove soil or debris. After cleaning, the tubers are peeled, and a representative sample is taken from each tuber. These samples are weighed immediately to record the fresh weight. The tuber samples are cut into thin slices or cubes for uniform drying. The samples are dried in an oven at 60 - 70°C for 48 to 72 hours or until a constant weight is achieved. It is crucial to monitor the samples periodically to ensure complete drying without burning or degrading the material. Once the drying process is complete, the dried samples are weighed to obtain the dry weight. The dry matter content is calculated using the formula:

$$\text{Dry matter content (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

2.2.4. Determination of HCN content in cassava

Samples are typically taken from various parts of the cassava plant to measure the HCN content, including tubers, leaves, and stems. The method begins with finely chopping or grinding the cassava samples to homogenize them. The homogenized samples are then subjected to acid hydrolysis, which simulates the enzymatic breakdown of cyanogenic glycosides. This process releases free HCN, which is captured in a distillation apparatus. The HCN released is absorbed in NaOH, forming a stable cyanide complex. A colorimetric assay is typically used to quantify the cyanide content. In this assay,

the cyanide complex is reacted with a reagent such as chloramine-T in the presence of pyridine and barbituric acid, forming a colored compound. The intensity of the color is measured using a spectrophotometer at a specific wavelength (typically around 580 nm). The HCN content is then calculated by comparing the absorbance of the sample to a standard curve generated using known concentrations of cyanide. The results are typically expressed in milligrams of HCN per kilogram of fresh weight (mg HCN/kg).

2.2.5. Analysis of anatomical characteristics of cassava plants

Analyzing the anatomical characteristics of cassava stem and leaf tissues involves collecting fresh samples from healthy plants, preferably from different growth stages. For stem analysis, cross-sections should be taken using a sharp razor blade or microtome to ensure thin, even slices [15]. For leaves, cut transverse sections from the midrib and petiole. Stain the sections with appropriate dyes, such as safranin and fast green, to differentiate cellular structures. Mount the stained sections on microscope slides with a cover slip. Observe under a light microscope, focusing on key anatomical features like vascular bundles, xylem, phloem, and epidermal cells in stems, and stomata, mesophyll, and vascular tissues in leaves [16]. Document the observations with detailed notes and microphotographs, comparing the structures to standard anatomical references to identify any unique or distinguishing features of the cassava plant tissues.

3. Results and Discussion

3.1. Analysis of the morphological descriptors of three specific cassava varieties in the Northern provinces of Vietnam

A list of morphological characteristics of the cassava plant was explored to authenticate three specific cassava varieties in the Northern provinces of Vietnam. As expected, the morphology of these varieties was described.

The cassava variety KM94 exhibits distinct morphological characteristics contributing to its agricultural viability. The plant has an open shape with 2-3 stems per plant and semi-prominent foliar scars. Its stems exhibit a brownish-purple growth habit, and the apical leaves lack coloration. The apical leaves are lanceolate and lack pubescence, while the central leaflet is reddish-green, with a reddish-green petiole orientation. The leaves are long, with the vein also being long, and typically have 7-9 lobes per leaf, with a total of 16-20 lobes per plant. Leaf lobes are 4.1-5.0 cm in length and green-brown. The exterior of the stem is rated at 270 in terms of color. The plant height is horizontal, and it features a conical tuberization pattern. The roots are light brown-cream in shape and color, with the external storage root color matching the root shape. The root cortex is white-cream, and the root pulp has a difficult ease of peeling. Each plant produces about 12 storage roots, with a total of 25.3 storage roots per plant, containing 25% starch content. These morphological traits underline the variety's adaptability and potential for high yield, making it suitable for diverse growing conditions.

The cassava variety 08SA06 exhibits various morphological characteristics crucial for its evaluation and cultivation. The plant has a cultivation period of 9-11 months, showing no ability to branch, with an average plant height of 250 cm. It scores 5 on a scale of 1-5 for plant form, indicating excellent morphology. The base diameter of the stem is 3.0 cm. Each plant produces 11.7 tubers, with an average tuber length of 29.0 cm. The average fresh yield ranges from 35 to 43 tons per hectare, and the harvest index is 0.6. The dry matter content of the tubers is 41.1%, with a starch content of 30.7%. The HCN content in various parts of the plant is measured as follows: 43.8 mg/100g fresh weight in

the peel, 11.9 mg/100g in the flesh, and 44.5 mg/100g in the leaves. The variety scores 1 for sensory quality, indicating suitability for processing. These morphological traits suggest that 08SA06 is a robust variety with high yield potential, good dry matter and starch content, and suitable for various processing applications.



Figure 1. Morphological descriptors of the Ruot Vang cassava variety.

Finally, the cassava variety “Ruot vang” demonstrates distinctive morphological traits significant for its cultivation and classification. The inner bark of the stem is orange, while the outer bark is yellow, and the skin is cream-colored. This variety has an upright growth habit and a branching capability classified as level 1 (see Figure 1). The plant morphology is described as compact. The apical leaves exhibit a light purple color with no pubescence. The central leaf lobes are shaped like a narrow triangle, and the petiole is red. The leaves themselves are dark green, with five lobes per leaf, and the petiole color is green. Leaf scars are prominent, with a length of 4 mm or more. The leaf petiole related to the stem is semi-upright. Root formation is stalked, and the tubers are cylindrical, with a dark brown skin surface. The flesh of the tuber is dark yellow, while the peel is white/cream. These morphological characteristics highlight the variety's compact structure, distinct coloration, and robust root formation, making it suitable for specific cultivation conditions and potentially beneficial for certain processing applications.

In a recent study, 102 cassava accessions in Sierra Leone were collected to focus on 20 morphological traits, including plant height, number of leaves, stem diameter, and tuber size [17]. Significant variation was observed among the varieties, with plant height ranging from 80 cm to 280 cm, stem diameter varying between 1.5 cm and 5.5 cm, and the number of leaves per plant ranging from 30 to 150 [17]. Tuber morphology also varied greatly, with some varieties producing long, large tubers while others had short, round tubers [17]. Previously, the morphological characteristics of 29 cassava genotypes in Indonesia were assessed [18]. Generally, the leaf characteristics included light green apical leaves, no pubescence on apical leaves, average leaf retention, lanceolate and elliptic-lanceolate shapes of central leaflets, light green leaf color, and green leaf veins. The color of apical leaves was predominantly light green (65.5%), with some genotypes showing purplish (6.9%) or dark

green (27.6%) colors [18]. None of the genotypes exhibited pubescence on apical leaves [18]. Leaf retention was mostly average (72.4%), with some having better-than-average retention (27.6%) [18]. The shapes of the central leaflets were primarily lanceolate (48.3%), elliptic-lanceolate (37.9%), and obovate-lanceolate (13.8%) [18]. Leaf colors were mostly light green (65.5%), with dark green (17.3%) and purple-green (17.2%) also observed. Leaf vein color was mainly green (93.1%), with a few genotypes showing reddish-green in less than half of the lobe (3.4%) or entirely red (3.4%) [18].

3.2. Analysis of the anatomical characteristics of the stems of three specific cassava varieties in the Northern provinces of Vietnam

The anatomical features of the stems of three cassava varieties reveal distinct structural characteristics observed in transverse sections. The outermost layer is protected by a plate-shaped epidermal cell layer, tightly packed without intercellular spaces. The outer wall of the epidermal cells is thicker than the lateral and inner walls, and the outermost surface is covered by a relatively thick cuticle layer that reduces water loss and protects the plant from microbial invasion. Some epidermal cells extend to form protective hairs that safeguard internal tissues and limit transpiration. The collenchyma tissue is directly beneath or a few layers away from the epidermis. The KM94 variety has 5-7 layers of collenchyma, while the 08SA06 and Ruot vang varieties have 7-13 layers. The cell walls of the collenchyma are impregnated with cellulose but are not uniformly thickened. Following the collenchyma are 7-10 layers of larger parenchyma cells, which are loosely arranged with intercellular spaces and primarily serve as storage tissue. Surrounding the stem is a continuous ring of 1-2 layers of sclerenchyma, forming a closed loop around the stem and providing additional structural support.

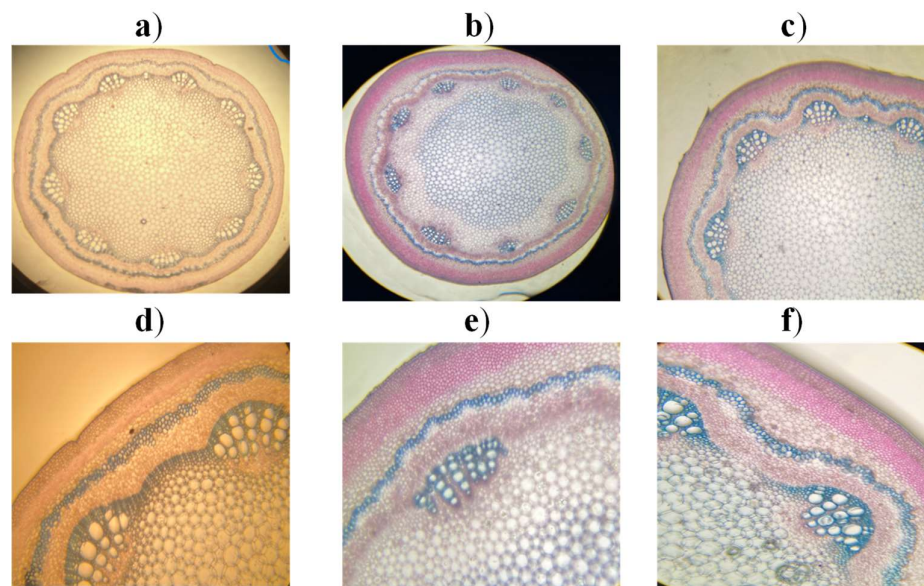


Figure 2. Anatomical characteristics of stems of KM94 (a, d), Ruot Vang (b, e), and 08SA06 (c, f) cassava varieties shown at different magnifications.

Next, the anatomical features of the stems of three cassava varieties exhibit distinct characteristics in their central columns, which occupy three-quarters of the transverse stem section. Surrounding the central column is the cortex, consisting of 2-3 layers of small, polygonal cells. The vascular system in the stem is organized into superimposed bundles, with the phloem located on the outside and the xylem on the inside. Smaller vessels are positioned inwardly within the xylem bundles, and larger vessels (7-10 in number) are outward. This differentiation is centrifugal, in contrast to the centripetal

differentiation in roots. The vascular bundles are interconnected by a sclerenchyma ring, forming a continuous wavy ring. The KM94 and Ruot vang varieties have an average of 9 vascular bundles, while the 08SA06 variety has 9-10 bundles. This detailed anatomical arrangement provides structural integrity and efficient nutrient and water transport within the stem, which is essential for the growth and development of these cassava varieties. The anatomical features of the stems of three cassava varieties include a prominent pith that occupies approximately 70% of the central column's cross-sectional area. This pith consists of large, polygonal parenchyma cells, which are larger in the center and gradually decrease in size towards the periphery. The primary function of the pith is to store nutrients. In the primary stem, leaf traces are present, connecting the vascular system of the stem with the petiole's vascular system at the nodes. As the leaf traces extend into the central column, the vascular bundles of the central column separate at the point where they meet the leaf traces, forming leaf gaps filled with parenchyma tissue. These leaf gaps ensure the continuity of nutrient and water transport between the stem and leaves, playing a crucial role in the overall physiology of the plant.

Additionally, we get insight into the secondary anatomical characteristics of the stems of three cassava varieties. As a result, the outermost layer of the stem is protected by an epidermis composed of tightly packed, plate-shaped cells with no intercellular spaces. The outer wall of the epidermal cells is thicker than the lateral and inner walls. A relatively thick cuticle layer covers it, reducing water loss and protecting the plant from microbial invasion. Some epidermal cells extend to form protective hairs, which help shield the inner tissues and reduce transpiration. The collenchyma tissue is directly beneath the epidermis or separated by a few layers of parenchyma cells. The KM94 variety has 5-7 layers of collenchyma, while the 08SA06 and Ruot Vang varieties have 7-13 layers. These cells have cellulose-impregnated walls that are unevenly thickened. Next are 7-10 layers of larger parenchyma cells in the cortex, which are loosely arranged, leaving intercellular spaces, and primarily serve as storage tissue. Surrounding the stem is a continuous ring of 1-2 layers of sclerenchyma, forming a closed loop around the stem. The central column occupies three-quarters of the stem's cross-sectional area and is surrounded by a small cortex with 2-3 layers of small, polygonal cells. The vascular system is organized into superimposed bundles, with phloem on the outside and xylem on the inside. Smaller vessels are positioned inwardly within the xylem bundles, and larger vessels (7-10 in number) are outward. This differentiation is centrifugal, opposite to the centripetal differentiation in roots. The vascular bundles are interconnected by a sclerenchyma ring, forming a continuous wavy ring. The KM94 and Ruot Vang varieties have an average of 9 vascular bundles, while the 08SA06 variety has 9-10 bundles. These anatomical features ensure efficient nutrient and water transport, structural integrity, and adaptability to various environmental conditions for these cassava varieties.

The secondary structure of the cassava stem exhibits several distinct anatomical features. The periderm forms as the plant transitions to secondary growth, developing a cork layer composed of rectangular cells with relatively uniform size and thin cutin-covered outer walls. This layer is followed by an active phellogen, which produces 5-7 layers of cork cells externally and a green cortex of living cells internally, containing chlorophyll and thin cellulose walls. Collenchyma, consisting of 4-6 layers of polygonal cells with varying sizes, surrounds the stem, primarily serving a supportive function. The cortex parenchyma, comprising 3-4 layers of oval cells with thin walls and irregular arrangement, contains latex and intercellular spaces with light brown crystals. A continuous ring of sclerenchyma, made up of dead cells with lignified thick walls that stain blue with methylene blue, provides additional support. The vascular cambium, situated between the secondary xylem and phloem, differentiates into secondary phloem externally, composed of parenchyma and phloem fibers, and secondary xylem internally, with thick-walled vessel elements and wide lumens. The vascular bundles

are arranged in a superimposed manner, with 9-10 vessels per bundle. Due to uneven differentiation, the cambium produces more xylem cells than phloem cells, resulting in annual rings of secondary growth. Old phloem layers are compressed by new phloem. The pith consists of large, thin-walled, oval, or polygonal parenchyma cells, which are largest in the center and gradually decrease in size outward. Secondary medullary rays from cambium cells facilitate nutrient exchange between the stem's outer and inner regions. The xylem vessels are round and irregularly arranged within the xylem parenchyma, which includes polygonal, round, or oval cells aligned radially. The primary xylem, located innermost, has smaller vessel elements, while the secondary xylem features larger vessels and displays centrifugal differentiation. The phloem comprises fibers and parenchyma, alternating and forming a continuous ring. The narrow medullary rays, usually one to two cell layers thick, play a crucial role in nutrient transport between the pith and peripheral regions. Parenchyma cells in the pith are almost round and large, with thin walls, and contain scattered calcium oxalate crystals.

Previously, *M. glaziovii* stems exhibited dense prismatic and druse crystals within the cortical parenchyma, along with numerous gelatinous fibers [19]. The pericycle fibers also had notably thicker walls. In contrast, clone UnB 99 lacked crystals but contained abundant starch [19]. *M. glaziovii* also showed a high presence of tyloses in vessel elements, which were scarce in clones UnB 99 and UnB 110 [19]. The wild species had larger vascular vessels and minimal starch in the secondary xylem, unlike the starch-rich UnB 99 and UnB 110 clones [19]. Clone UnB 110 had starch in the cortical region and medulla, with gelatinous fibers in both the pericycle and secondary xylem [19]. Additionally, brown stem color was associated with drought tolerance [19]. Additionally, anatomical variations among *Manihot* species and varieties were observed in the shape and wall thickness of epidermal and ectodermal cells, the composition of cortical parenchyma, and the number of xylem poles [20]. While the wall thickness of the epidermis and exodermis in the tap root was consistent across all species, differences were noted in the cell shape and wall thickness in the lateral root. Thick-walled epidermal cells were found in all species' tap roots and cassava varieties' lateral roots, a trait linked to drought and disease tolerance. The number of xylem poles varied more in cassava varieties (4-8) than in wild species (4-6), suggesting a hybrid origin for cassava [20].

4. Conclusion

In conclusion, analyzing the morphological and anatomical features of three popular cassava varieties in the Northern provinces of Vietnam provides valuable insights into their distinctive characteristics and adaptability to local conditions. Morphologically, varieties such as KM94, 08SA06, and Ruot vang display typical traits, including plant form, leaf structure, tuber shape, and starch content, highlighting their potential for high yield and suitability for diverse agricultural practices. Anatomically, the detailed study of their stems reveals crucial structural attributes like tightly packed epidermal cells, varying layers of collenchyma and parenchyma, robust sclerenchyma rings, and well-organized vascular bundles. These features ensure efficient nutrient and water transport, structural integrity, and resilience against environmental stresses. Understanding these traits not only aids in classifying and cultivating these varieties but also contributes to improving cassava farming practices, ultimately enhancing food security and economic stability in the region.

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