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RESEARCH ARTICLE

Taxonomic Importance of Radial longitudinal Section in the Stem Characters of Six *Citrus* Species of Southeastern Nigeria

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ABSTRACT

Radial longitudinal sections (RLS) of the stems of six species of *Citrus* present in Southeastern Nigeria were investigated. *Citrus* is a genus belonging to the family Rutaceae. The results revealed that they have the same and advanced characters. The vessels were not occluded by tyloses and the rays were procumbent ray cells (homocellular rays). The implication of this convincing evidence, proposed that there was a close affinity among *Citrus aurantifolia, C. grandis, C. limon, C. paradisii, C. reticulata* and *C. sinensis*; and that they were all advanced. In addition, it indicated that there was extremely hybrid breakdown in these species of *Citrus*.

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INTRODUCTION

The genus, *Citrus* belongs to the family Rutaceae (Nyananyo, 2006). The family is commonly known as *Citrus* or Rue family with 162 genera and 1, 650 species (Singh, 2004). In Southeastern Nigeria, the commonly available ones are *Citrus aurantifolia* (Christm.) Swingle (Lime), *C. grandis* Osbeck (Shaddock/Pummelo), *C. limon* (L.) Burm.f. (Lemon), *C. paradisii* Macf. (Grapefruit), *C. reticulata* Blanco (Mandarin/Tangerine), and *C. sinensis* (L.) Osbeck (Sweet orange). Geographically, Southeastern Nigeria extends from latitudes 4° 40' to 7° 20' north latitude, and 6° 00' to 8° 20' east longitude; it covers area of about 50 000 km^2 of Nigeria's total area of 923 768 km^2 (Okeke *et al.*, 2009). There are two well marked seasons: the dry season, lasting from November to March and the rainy season, lasting from April to October (Okoye and Daramola, 1999).

Anatomy can be of great help in learning the differences and similarities between various plants, which are important part of plant taxonomy. Two plants may appear very similar on the surface but when their sections are viewed, they would be radically different and vice versa. *Citrus* taxonomy has been a problem for a long time (Barrett and Rhodes, 1976); and it is still current (Abkenar and Isshiki, 2003; Araujo *et al*., 2003). Researchers are therefore making great effort to find a lasting solution to it. This implied that a proper understanding of the affinities existing among the species of *Citrus* is required.

The objective of this research, therefore, was to study the radial longitudinal section (RLS) of the stem of these *Citrus* species and compare them with the view of understanding the distinctions and affinities existing among them. This will shed light on the species phylogenetic relationship and in addition, provide information for determination of hybrid breakdown, if any, and to what extent, in these species of *Citrus* under study.

MATERIALS AND METHODS

Sources of Materials

Twigs of *Citrus aurantifolia, C. grandis, C. limon, C. paradisii, C. reticulata* and *C. sinensis* were collected from Agricultural and Natural Resources Department Market Garden, Amawbia, Awka South Local Government Area, Anambra State, in the months of May-June at optimum maturity.

The *Citrus* species were authenticated by Prof. CU Okeke, a plant taxonomist, Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State; where the voucher specimens were deposited.

Anatomical Analysis

The twigs of *Citrus aurantifolia, C. grandis, C. limon, C. reticulata, C. paradisii* and *C. sinensis* were collected in vials containing formaldehyde, glacial acetic acid and alcohol in the ratio of 1:1:18, respectively. The specimens were dehydrated in ethanol series (30, 50, 70 and 95%) each for 2 h. Complete dehydration of specimens was effected by storing the specimens in absolute (99.6%) ethanol overnight. The specimens were then cleared in 3:1, 1:1 and 1:3 ethanol/ chloroform each for 3 h. Wax was melted at 70^0C in an oven. The cleared specimens were then put in molten anatomical wax and alcohol and allowed to stay at 70^0C for 24 h for effective infiltration of wax into the specimen to replace the chloroform which was gradually lost by evaporation.

Embedding was carried out after infiltration. This was done by smearing the inside of clean molds with glycerin, pouring the molten wax into the molds with appropriate orientation in a position suitable for the type of section to be cut. The wax in the mould was allowed to cool into a block. The wax blocks were freed from the mould, stuck on a wooden holder and labeled, pending sectioning.

Wax blocks already freed from the holder and trimmed were affixed on the sledge microtome and sectioned at 15-20 microns thickness. The thin sections were fixed on clean slides already smeared with a thin film of egg albumen. They were stretched by passing them over hot plate. This also made them to become attached to the slides. Slides bearing sections were arranged in a slide rack and placed in an oven at 70^0C to melt off wax from the sections. This lasted for 12 h.

For staining, sections were dehydrated by passing slides across xylene and xylene/absolute ethanol series (3:1, 1:1 and 1:3 v/v), absolute ethanol, 95, 70, 50 and 30% ethanol. Slides were briefly immersed in water, then stained with 0.1% alcian blue and counter stained with 1% safranin. Stained slides were rinsed briefly in tap water, dehydrated through the ethanol series and cleared across the xylene/absolute ethanol series.

Mounting was carried out by placing one drop of Canada balsam on a clean slide and carefully covering the sections with the coverslip in such a way that the Canada balsam spread and covered the specimen sections overlaid by the coverslip. They were studied and photo-micrographs taken with a photomicroscope (2.0 model; China).

RESULTS

The results of the Radial longitudinal section were shown in Plates 1- 6.

Plate 1: Radial longitudinal section of stem wood of *Citrus aurantifolia*.x100; the vessels were not occluded by tyloses. The rays were procumbent ray cells (homocellular rays). Plate 2: Radial longitudinal section of stem wood of *Citrus grandis*.x100; the vessels were not occluded by tyloses. The rays were procumbent ray cells (homocellular rays). Plate 3: Radial longitudinal section of stem wood of *Citrus limon*.x100; the vessels were not occluded by tyloses. The rays were procumbent ray cells (homocellular rays). Plate 4: Radial longitudinal section of stem wood of *Citrus paradisii*.x100; the vessels were

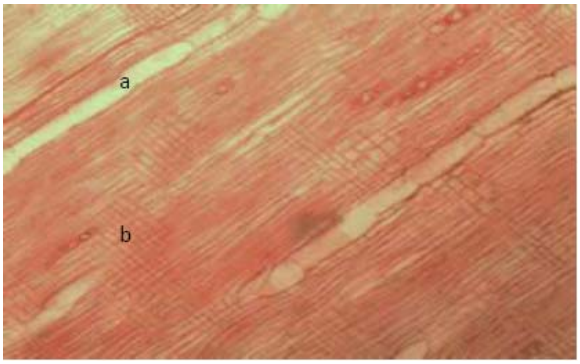

Plate 1: Photomicrograph of radial longitudinal section of stem of *Citrus aurantifolia*.x100

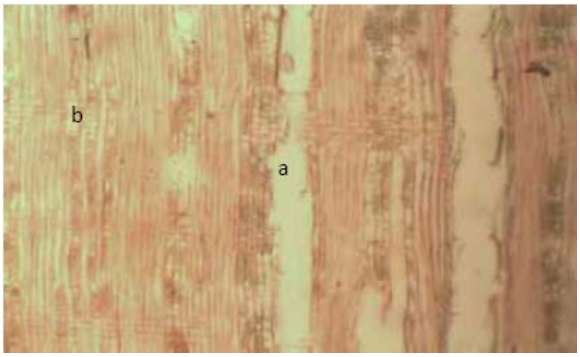

Plate 2: Photomicrograph of radial longitudinal section of stem of *Citrus grandis*.x100

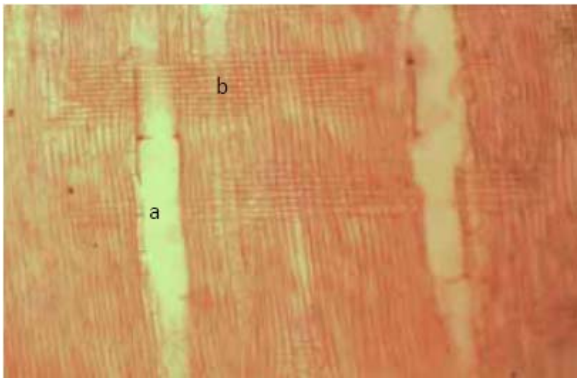

Plate 3: Photomicrograph of radial longitudinal section of stem of *Citrus limon*.x100

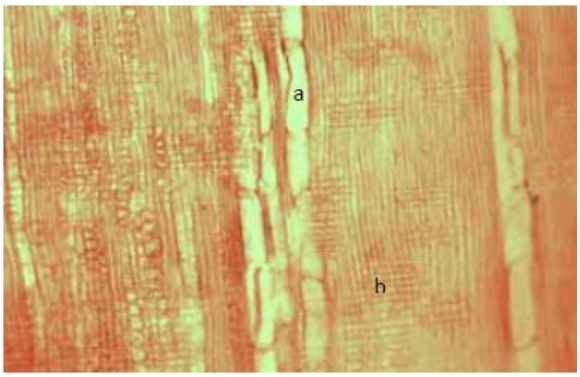

Plate 4: Photomicrograph of radial longitudinal section of stem of *Citrus paradisii*.x100

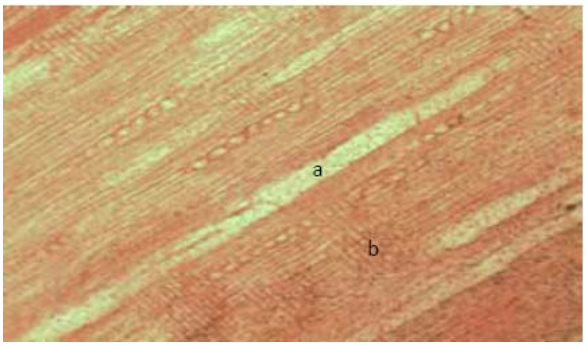

Plate 5: Photomicrograph of radial longitudinal section of stem of *Citrus reticulata*.x100

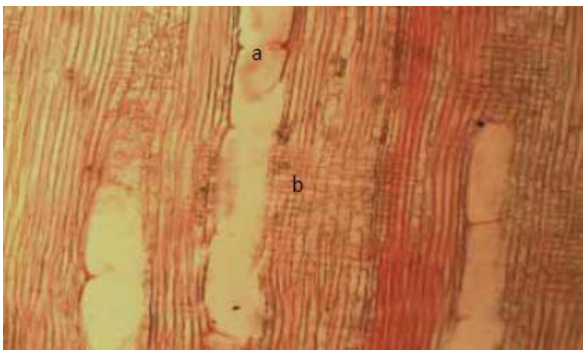

Plate 6: Photomicrograph of radial longitudinal section of stem of *Citrus sinensis*.x100; a = Vessel; b = Procumbent ray

not occluded by tyloses. The rays were procumbent ray cells (homocellular rays). Plate 5: Radial longitudinal section of stem wood of *Citrus reticulata*.x100; the vessels were not occluded by tyloses. The rays were procumbent ray cells (homocellular rays). Plate 6: Radial longitudinal section of stem wood of *Citrus sinensis*.x100; the vessels were not occluded by tyloses. The rays were procumbent ray cells (homocellular rays).

DISCUSSION

The investigations showed that the vessels were not occluded by tyloses (Plates 1, 2, 3, 4, 5 and 6). In addition, the rays were procumbent ray cells (homocellular rays) (Plates 1, 2, 3, 4, 5 and 6). The possession of the same characters indicated that they have very close affinity. Sharma (1993) reported that anatomical characters are most useful in determining relationship among taxonomic categories. This therefore, suggested that they are closely related and probably contributed to their existence in one genus *Citrus*.

The homocellular (procumbent rays only) is more specialized and advanced than heterogenous rays (Pandey, 1981). This suggested that *Citrus aurantifolia*, *C. grandis*, *C. limon*, *C. paradisii*, *C. reticulata* and *C. sinensis* are all advanced because they possessed an advanced character. It further suggested that they probably evolved at the same time, therefore explaining the relative advancement of these characters.

However, *Citrus grandis* and *C. reticulata* were reported to be among the three only true species of *Citrus;* and that *C. aurantifolia*, *C. limon*, *C. paradisii* and *C. sinensis* are hybrids (Barrett and Rhodes, 1976; Mabberley, 1997).It is probable that the close affinity and relationship between these species of *Citrus* resulted from series of crosses between the true species and the hybrids; and also within the hybrids. Moreover, it could be as a result of being cultivated for a long period of time. They were reported to have been internationally introduced to the worldwide tropics and subtropics over a span of more than 1,000 years (USDA-FDA, 2006); and are cultivated throughout the worldwide tropics 0-1600m and subtropics 0-750m (Pandey, 1981; Manner *et al*., 2006). It then implied that long period of existence and cultivation of these *Citrus* species might have lead to hybrid breakdown thereby resulted in the similarity of their internal features.

Conclusion

There was close affinity among *Citrus aurantifolia*, *C. grandis*, *C. limon*, *C. paradisii*, *C. reticulata* and *C. sinensis.* In addition, they possessed procumbent ray cell which is a specialized and advanced character. This provided evidence which might be of some importance in the elucidation of the phylogenetic relationship of the genus *Citrus*.

Furthermore, considering the fact that *C. grandis* and *C. reticulata* were believed to be among the three only true species of *Citrus* which then suggested that others were hybrids; the similarity of the characters revealed that there was extremely hybrid breakdown in these species of *Citrus*.

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