Medicinal & Aromatic Plants
ISSN: 2167-0412

Osuoha et al., Med Aromat Plants 2015, S1
DOI: 10.4172/2167-0412.S1-005

Research Article Open Access

Stematic Characterization of Six Citrus Species Using Petiole Anatomy

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Abstract

The petiole anatomical characters of six Citrus species showed that *C. sinensis, C. limon, C. aurantifolia, C. paradisi* and *C. maxima* petiole have oval shaped vascular bundles while it is kidney-shaped in *C. reticulata*. The vascular bundle of the node of *C. limon* is oval to bulbose to spherical while others have boat-shaped vascular bundles. The stellar arrangement is pent arch in *C. maxima* while others have hexarch. The results on the petiole anatomy of the six *Citrus* species were used to determine the phylogenetic relationships between the studied species.

Keyword: Petiole, Anatomy, Citrus, Rutaceae, Phylogenetic relationship

Introduction

Rutaceae is a family of flowering plants belonging to the order Sapindales. The family comprises of 150 genera and 1500 species. Mainly distributed in tropical and temperate regions. In West Africa, it is represented by 9 genera and about 30 species. Most species are tree or shrubs rarely herbs. Citrus species are small trees. Sometimes thorns are present which are usually solitary, straight, robust in the axils of the leaves or on the stem. Members have glands yielding essential oils which are responsible for the aromatic smell obvious on their fruits or leaves (except in Ptelea) [1]. Leaves simple, palmate or pinnate compound, alternate or opposite [1,2]. In some genera such as Citrus, *Feronia sp;* the leaves are reduced to spines. Inflorescence Cymose or racemose. Flowers pedicellate, complete (Perfect), bisexual actinomorphic or occasionally Zygomorphic, white or yellow hypogynous. Sepals 4-5, polysepalous represented by disc and green. Petals 4-5, polypetalous, inbricate. Stamen 3-10 or more filaments free. Gynoecium 3-5 carpels, syncarpous. Ovary is 4 or 5-locular or multilocular as in *Citrua.* Each locule contains one or more anatropous ovary. Fruit drupe, Capsule, Samara, or follicle.

Some *Citrus* species have been cultivated in China thousands of years for their Juicy fruits [3]. They are preserved in marmalades, used as a garnish for water, soft drinks or cocktails; they are cultivated for Ornamental value. The oil extracted from lemon (citral) and sweet orange (limonene) are used in perfume making [4]. Citrus has been used for its medicinal purpose than most fruits crops. Leaves of sweet orange are used in treating swollen limbs; juice from lemon use in hookworm treatment while lime juice is use in treating dermatitis [5]. Citrus species can give health remedies such as chewing sticks for oral hygiene; and toothache relief to contraceptive, laxatives; purgetives sedatives and treatment of a wide Varity of common ailments such as diarrhea and vomiting. Citrates in lemon prevent the formation of kidney stones and ease their dissolution [6]. Limonene and petigrain oils from peel or leaves of Citrus can be used as insecticide [7].

Over two centuries, the classification within Citrus has been a matter of controversy [8-11]. For example grape and shaddock have been regarded by some authors as one and the same fruit belonging to an entirely separate species [11]. It is hoped that this type of biosystematics study would provide solution to all the controversy discrepancies, confusion and uncertainties to the taxonomy of Citrus by applying other lines of evidences apart from morphology to delimit the taxa. Hence, the use of anatomical features in systematic delimitation of different taxa has been emphasized by many authors. Researchers such as Okoli [12], in Curcurbitaceae; Edeoga and Osawe [13] in Caesalpinoideae; Heo [14] in Moniniaceae have been able to use anatomic characters to distinguish between species they worked on. Though this genus Citrus is common, not much research have been done on the petiole anatomy of the genus Citrus.

Therefore, the study described the petiole anatomy of six Citrus species and discussed the possibility of utilizing this in determine the phylogenetic relationship between the investigated species.

Materials and Methods

Specimen collections

C. sinensis, C. limon, C. aurantifolia, C. paradisi, C. reticulata and *C. maxima* specimens were collected from Ministry of Agriculture and Natural Resources Nekede, Agricultural Development Programme (ADP) farms, Plant Garden at Aladinma Housing Estate, Homestead Garden at Amakohia Layout, Songhai Farms, Nekede, Imo ADP Egbeada and Homestead, Obazu Mbieri.

The sample specimens as authenticated by expert in taxonomy at Imo State University Herbarium are deposited at Imo State University Herbarium with Herbarium Nos. IMSUH 001-006. The study was conducted at the Plant Science and Biotechnology laboratory, Imo State University, Owerri and Federal University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Mature and fresh petiole of *C. sinensis, C. limon, C. aurantifolia, C. reticulata, C. paradisi* and *C. maxima* were collected. The whole parts were initially fixed in FAA (1:1:18) glacial acetic acid. 40% formaldehyde: 70% ethanol (v/v) for 48-72 h. The fixed materials were thereafter used for anatomical studies petioles. These were washed several times in distilled water (then two changes of 30% ethanol) and dehydrated in the order 30% - 50% - 70% - 95% - absolute alcohol. To infilterate wax into the specimens, they were placed for 3-h in each of the following solutions containing a ratio of absolute alcohol to pure chloroform (v/v: 3.1, 1:1, 1:3), then pure chloroform. At the stage of

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Received February 26, 2015; **Accepted** October 17, 2015; **Published** October 22, 2015

Citation: Osuoha VUN, Mbagwu FN, Inyama CN, Ukpai KU (2015) Stematic Characterization of Six Citrus Species Using Petiole Anatomy. Med Aromat Plants S1: 005. doi:10.4172/2167-0412.S1-005

pure chloroform, wax pellets (60°C melting point) were added and the wax charged with new ones at intervals. The specimen were left in the oven for 2-7 days to remove the chloroform. To embed in wax, the contents of the vials were transferred into moulds and the specimens kept in place with hot needles. As the wax solidified, it was transferred to a cold water bath for hardening and later stored for two days in a refrigerator. For sectioning, a Reichert rotary microtome was used and 10-20 mm thick sections was made. The ribbons were placed on clean slides smeared with thin film of Haupt's albumen, allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate at 40°C for a few minutes to allow the ribbons to expand and were stored over night. The slides were immersed in

Table 1: Petiole Anatomical features of the six *Citrus* species studied.

Characters	*C. sinensis*	*C. limon*	*C. aurantifolia*	*C. reticulata*	*C. paradisi*	*C. maxima*
Epidermal cell layer	Mono layered	Mono layered	Mono layered	Mono layered	Mono layered	Mono layered
Number of cell layer of cortex	21 – 22	23 – 24	15 – 16	16 – 17	23 – 24	16 – 17
Shape of Vascular Bundle	Oval-shaped	Oval-shaped	Oval-shaped	Kidney-shaped	Oval-shaped	Oval-shaped
Type of Vascular Bundle	Collateral	Collateral	Collateral	Collateral	Collateral	Collateral
Nature of Vascular Bundle	Continuously ringed	Continuously ringed	Continuously ringed	Continuously ringed	Continuously ringed	Continuously ringed

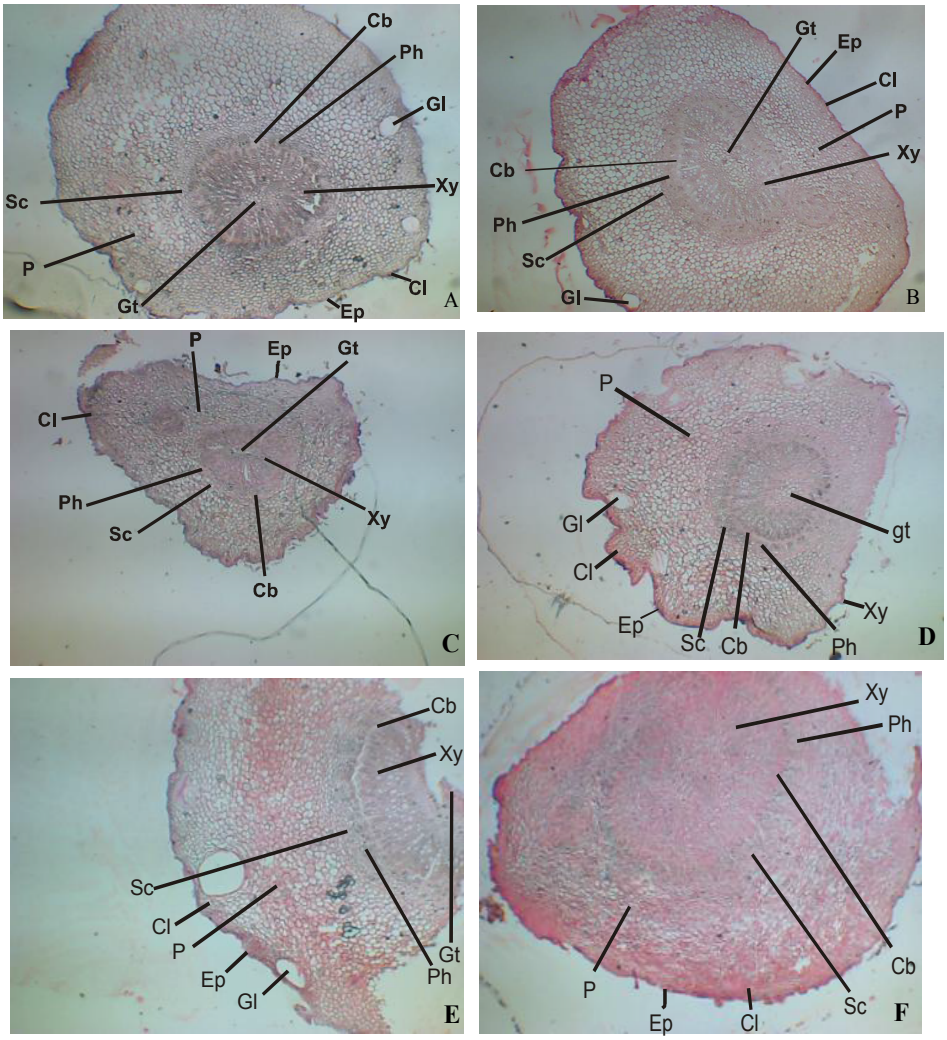

Plate 1: T/S of Petiole: A=*C. sinensis;* B= *C. limon;* C= *C. aurantifolia;* D=*C. reticulate;* E= *C. paradise;* and F=*C. maxima.* (ep-epidermis, cl- collenchyma, p- parenchyma, sc- sclerenchuma, ph- phloem, xy- xylem, cb- cambium, gt- ground tissue, gl- gland duct) x40.

pure xylene for 2-5 minutes in a solution of xylene and absolute alcohol with 1:1 ratio (v/v) for few minutes. The slides were then transferred to another solution of xylene and alcohol in the ratio 1:3 (v/v) for a few minutes, to 95%, 90%, 70% and 50% alcohol. Drops of alcian blue were added on the specimens for five minutes, washed off with water and counter-stained with safranin in 50%, 70%, 80%, 90% alcohol, xylene/alcohol (i.e., 1:3 and 1:1 v/v) and pure xylene at intervals of few seconds and mounted in Canada balsam. The slides were dried on a hot plate at 30°C. All these follow the method of Hoe [14] with slight modifications. Photomicrographs of the specimens were taken from the permanent slides using a Leitz Wetzler Ortholux microscope fitted with vivitar-v-335 camera (Plate 1).

Results

The petiole anatomy of the six Citrus species studied is presented in Plate 1(A-F). The petiole being an extension of the leaf structure has attained certain level of modification from the leaf structure, and shares affinity with the stem. The epidermis of the six Citrus species studied is one layer thick (mono-layered). Below the epidermis, is a multi-layered hypodermis which consists of collenchyma cells, parenchyma and caps of sclerenchyma cells around each of the bundles, constituting the cortex of the petiole. The number of these cortical cells varies enormously ranging from 21-22 in *C. sinensis*, 23-24 in *C. limon* and *C. paradisi,* 15-16 in *C. aurantifolia*, and 16-17 in *C. reticulata* and *C. maxima*. The shape of the vascular bundle varies among the six species studied. The shape is oval in *C. sinensis, C. limon, C. aurantifolia, C. paradisi* and *C. maxima*, while it is kidney-shaped in *C. reticulata*. All the species have collateral type of vascular bundle and their bundles are continuously ringed or radially arranged (Table 1).

Discussion

In the petiole anatomy, the cortical cells are heavily multilayer with variations which are comparable and systematically important. The number of cell layer of cortex showed that *C. sinensis, C. limon* and *C. paradisi* appear closely related but distinct from *C. aurantifolia, C. reticulata* and *C. maxima* showing that they are distinguishable by this attribute. The shape of the vascular bundle also vary considerably indicating that *C. sinensis, C. limon, C. aurantifolia, C. paradisi* and *C. maxima* share a common feature of being oval in shape, different from *C. reticulata* with kidney-shaped vascular bundle (Plate 1).

Thus *C. reticulata* can be delinated from the other five species based on their vascular bundle shape.

Conclusion

The differences in structures are not enough for re-classification of the species studied, but the similarities in structures among the investigated taxa showed interspecific relationships of the individual species.

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This article was originally published in a special issue, **Natural Medicinal Products and Secondary Metabolites** handled by Editor(s). Dr. Chandan S Chanotiya, CSIR-Central Institute of Medicinal and Aromatic Plants, India