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Fruit Wall Anatomy of *Ocotea* (Lauraceae)

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ABSTRACT

The fruit wall anatomy of *Ocotea* was investigated on the basis of 14 species within the genus to contribute to a better understanding of specific relationships and homogeneity of genus. The species have a similar mature fruit wall structure, but differences among the species are found with respect to whether or not sclerenchyma cells are present in the mesocarp, if present, whether or not they are present in particular positions and forms. Comparisons with species studied suggested that at least a few groups of species can be distinguished in *Ocotea*. They are divided into five groups on the basis of anatomical structures, i.e., group 1) *O. atrriensis*, *O. cujumari*, *O. helicterifolia*, *O. rubra*, and *O. schomburgkiana*; group 2) *O. aciphylla*, *O. javitensis*, and *O.* sp. [*Werff et al. 12676*]; group 3) *O. tonduzii*; group 4) *O. foetens*, *O. quixos*, and *O. veraguensis*; and group 5) *O. floribunda* and *O. nitida*. These various variations in *Ocotea* were also discussed to invite its respective systematic revisions. By the comparisons with species, on the other hand, it suggested that the specialized species are evolved from non-specialized species.

Key words : anatomy, fruit wall, Lauraceae, *Ocotea*, systematics.

Introduction

Ocotea Aublet is a large genus comprising about 400 species which are woody plants (Cronquist 1981). Most of them are distributed in tropical and subtropical America, ca. 50 in Madagascar, seven in Africa, and one in the Canary Islands (Rohwer 1993). The genus is characterized by an unicarpellate flowers, superior ovary, valvate anthers, inaperturate pollen grains, exalbuminous seeds, and one-seeded fruits (Cronquist 1988). *Ocotea* is also of great economic importance, providing medicinal sources, aromatic oils, spice, and good timber (Hutchinson 1973).

In spite of such a well-defined genus circumscription and its great economic value, little attention has been paid to systematics and relationships within genus. Indeed, there is no consensus with respect to specific relationships yet (Raj and Werff 1988). Werff (pers. comm.) also divided into seven subgroups in *Ocotea* on the basis of flower and sexual characters. Moreover, new species are described in South America continuously (Gomez 1993).

In this paper, I present a comparative study of the fruit wall anatomy of *Ocotea* (Lauraceae). This study is expected to make great progress in understanding the specific relationships within genus. However, fruit wall anatomy of *Ocotea* has never studied yet.

Materials and Methods

Fourteen species of *Ocotea* were investigated in the study. Species investigated and their collection data are presented in Table 1.

Both mature fruits preserved in F.A.A. (5 parts formalin: 5 parts glacial acetic acid: 90 parts 70% ethanol) and

Table 1. Species studied and collection data of *Ocotea*.

Taxa	Collection data
Ocotea aciphylla (Nees) Mez	Brazil. *Stewart 20150* (MO).
O. atirrensis Mez et J. D. Sm.	Costa Rica. *Utley 3238* (MO).
O. cujumari Mart.	Brazil. *Rabelo 3200* (MO).
O. floribunda (Sw.) Mez	Ecuador. *H. v. d. Werff 13374* (MO).
O. foetens (Aiton) Benth. et Hook. f.	Canary Isl. *D. Bramwell s.n.in 1992* (MO).
O. helicterifolia (Meisn.) Hemsl.	Mexico. South de El molote, *F. Lorea 5542* (MO).
O. javitensis (H. B. K.) Pittier	Ecuador. *H. v. d. Werff 13447* (MO).
O. nitida (Meisn.) Rohwer	Brazil. Espirito Santo, *Hatchbach 58033* (MO).
O. quixos (Lam.) Kosterm.	Ecuador. *no voucher* (MO).
O. rubra Mez	Venezuela. *Holst 2827* (MO).
O. schomburgkiana (Nees) Mez	Venezuela. *Liesner 14059* (MO).
O. tonduzii Standl.	Costa Rica. San jose, *W. A. Haber 10616* (CR).
O. veraguensis (Meisn.) Mez	Panama. *B. E. Hammel 13763* (MO).
O. sp.	Madagascar. Fianarantosa, Ranomafana, cloud forest, *H. v. d. Werff et al. 12676* (MO)

mature fruits collected from herbarium specimens were used for anatomical study. In the case of dry fruits, they were soaked in F.A.A. solution at least for a few days. The whole fruit or its pieces were dehydrated through a *t*-butyl alcohol series and embedded in paraplast with a melting point 56-58℃ for microtoming. Sections cut at 6-10㎛ in thickness were stained with Heidenhain's hematoxylin, safranin, and fastgreen FCF. They were mounted with Entellan. Most of the paraffin-embedded fruits were too hard to be sectioned by standard method. Therefore, after the fruits walls were exposed by microtoming, they were softened with a mixture of glycerol: 10% Aerosol OT: and water (10: 5: 85) for several weeks (Schmid and Turner 1977). Fruits of a few species which have harder walls were embedded in a resin (Technovit 7100, Kulzer & Co., Germany) and sectioned at 2-4㎛ by a rotary microtome with a hard knife.

Sections were stained with a 0.5% Toluidine Blue O. In the case of the hard mesocarp structure, it was also observed by scanning electron microscopy. The pieces of fruits wall were dehydrated through an ethanol series and dried with a critical point dryer. After being coated with platinum, they were observed with a Hitachi S-2050 scanning electron microscope.

Comparisons among the species were basically made on the basis of transverse sections of mature fruits walls. As far as they were available, plural fruits per species were investigated.

For anatomical descriptions of the fruits wall structure, the terms exocarp, mesocarp, and endocarp were used. As in the other families such as Coriariaceae (Tobe *et al.*, 1992) and Lamiaceae (Ryding 1994), the exocarp is a developed outer epidermis of an ovary wall, the mesocarp a developed mesophyll, and the endocarp a developed inner epidermis (for details on terminology see Fahn 1990).

Results

General morphology of mature fruits

Fruits of *Ocotea* are indehiscent, exalbuminous one-seeded, and berry or drupe with a cupule. Mature fruits of

Table 2. Shape, size, and thickness of mature fruits used in the study.

Taxa	Shape	No. of fruits measured	Size(㎜) length x diameter	Thickness of pericarp	
				total (㎛)	endocarp
O. aciphylla	ellipsoid	1	26.7×13.9	600-700	140
O. atirrensis	ellipsoid	2	26.4-27.6×10.5-11.3	150-180	30
O. cujumari	ellipsoid	1	13.7×7.7	480-500	60
O. floribunda	ellipsoid	2	32.4-32.8×25.4-26.2	1000-1100	80
O. foetens	ellipsoid	3	22.7-25.2×11.6-12.5	1500-1800	220
O. helicterifolia	ellipsoid	3	13.2-18.1×8.7-12.4	1150-1230	60
O. javitensis	ellipsoid	5	32.1-36.2×21.7-25.1	1450-1500	100
O. nitida	ellipsoid	2	18.5-19.8×11.1-11.4	650-700	90
O. quixos	broadly ellipsoid	4	32.1-39.6×28.2-32.6	2250-2400	390
O. rubra	NA	0	NA	450-500	80
O. schomburgkiana	NA	0	NA	600-660	100
O. tonduzii	ellipsoid	14	12.2-18.9×9.4-12.7	1800-2000	110
O. veraguensis	ellipsoid	1	13.4×7.9	500-600	80
O. sp. (*Werff 12676*)	ellipsoid	1	14.8×9.6	1000-1100	50

NA, Fruits size could not be measured because fruits were broken.

Ocotea are varied in size; the smallest fruit is that of *O. cujumari*, the largest one is that of *O. quixos* (Table 2). The colors are black, dark brown, orange, purple, or red (Hyland 1989). The shapes are broadly ellipsoid, ellipsoid, and their transverse sections are almost circular with an exalbuminous seed.

The mature fruit wall of *Ocotea* usually has a thick wall, ranging from 150 to 2400㎛ (Table 2) or 30 to 60 cell-layers. Early in development, however, the fruit wall of the youngest fruit has a relatively thin wall, but in later stages of development the mesocarp is usually rapidly thickened by cell divisions. Both the one cell-layered exocarp and the one cell-layered endocarp develop as they were, and the number of cell-layers in the fruit wall does not increase. In any of the species studied, none of the cell-layers are destroyed throughout the development of the fruit wall.

Pericarps with the palisade endocarp

The palisade endocarp is found in all species investigated within genus (Figs. 1-11). Only difference lies on the thickness of palisade endocarp. The thickness of endocarp ranges from 30㎛ to 390㎛ in most species, but in Madagascan species (*O.* sp., *Werff et al. 12676*) the palisade endocarp is very thin compare to other species relatively (see Table 2). Some species have a crystal cells in the endocarp (Fig. 8).

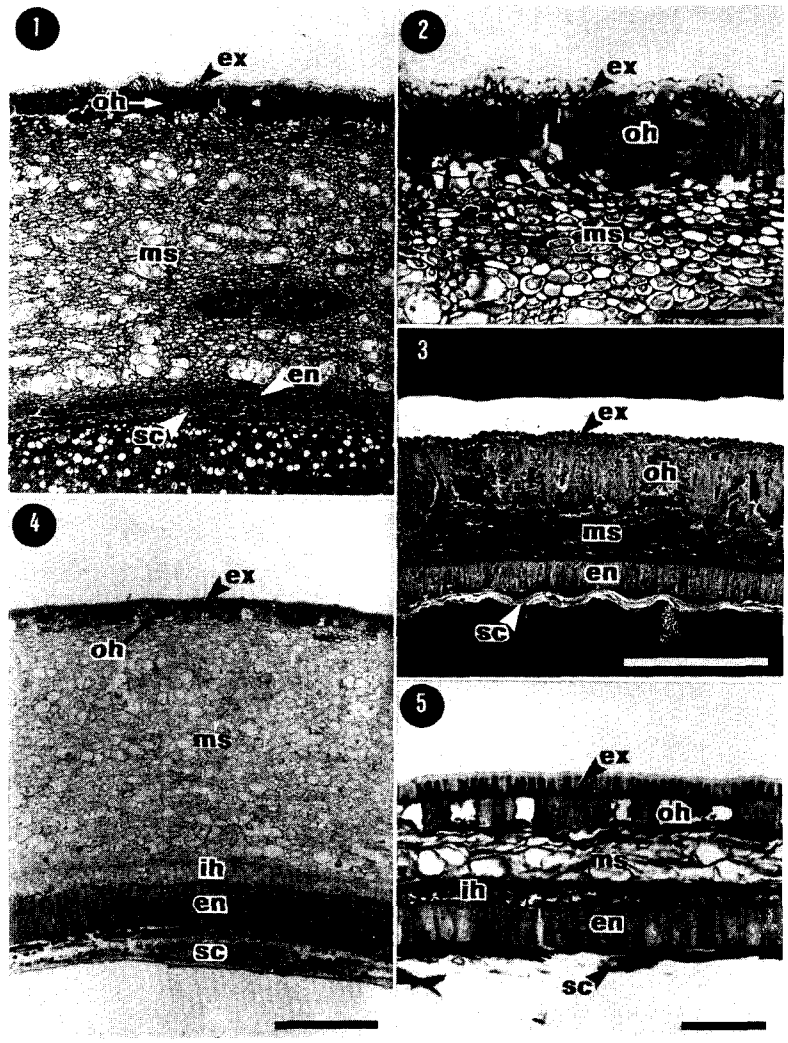
Pericarps with sclerotic cells in the mesocarp

Besides of non-specialized species, remain species have sclenchyma cells in various positions and in various forms in the mesocarp.

Five species of the genus investigated have sclenchyma cells under the exocarp, i.e., outer hypodermal cell layers (Figs. 1-5). The sclenchyma cells are usually elongated and thus palisade shape together cuticle layer (Fig. 2). They are found

in *Ocotea aciphylla* (Fig.3), *O. foetens* (Fig. 4), *O. javitensis* (Fig. 1), *O. quixos*, *O. veraguensis* (Fig. 5), and *O.* sp. [*Werff et al. 12676*]. On the other hand, this structure was also found in other genera of Lauraceae as in *Aniba, Kubitzkia, Licaria*, and *Paraia* (Heo 1996, Ph.D. dissertation)

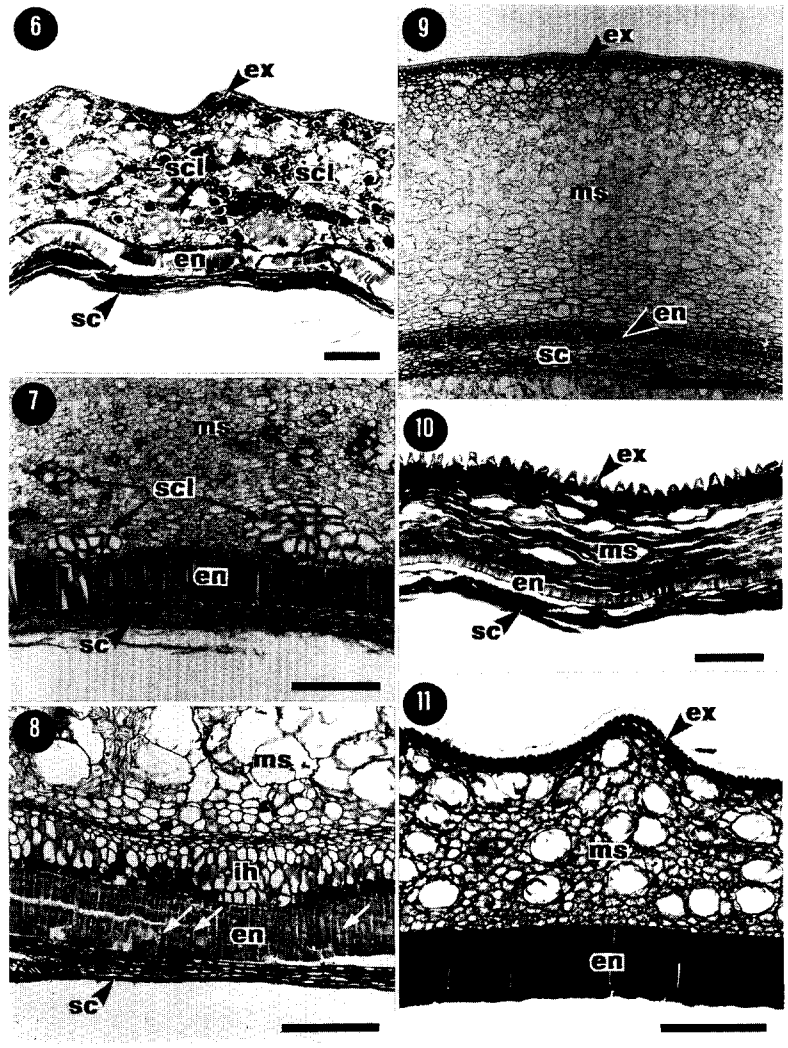
Also, in two species of the genus studied, cells of the inner hypodermal cells are more or less sclerotic. Such structure occurs in *Ocotea foetens* (Fig. 4), *O. quixos, O. tonduzii* (Fig. 8), and *O. veraguensis* (Fig. 5) but not present in *O. aciphylla* (Fig. 3), *O. atirrensis* (Fig. 10), *O. cujumari, O. floribunda* (Fig. 7), *O. helicterifolia* (Fig. 9), *O. javitensis* (Fig. 1), *O. nitida* (Fig. 6), *O. rubra, O. schomburgkiana* (Fig. 1), and *O.* sp. [*Werff et al. 12676*]. The sclenchyma inner hypodermal cells are not so conspicuous, but are still easily distinguishable as representing specialized structures.



Figs. 1-5. Transverse sections of mature fruits wall in *Ocotea*. Fig. 1. *O. javitensis*. Fig. 2. *O. javitensis* showing the sclenchyma outer hypodermal cells. Fig. 3. Scanning electron micrograph of mature fruit wall of *O. acipfylla*. Fig. 4. *O. foetens*. Fig. 5. *O. veraguensis*. Abbreviations: en, endocarp; ex, exocarp; ih, inner hypodermal cells; ms, mesocarp; oh, outer hypodermal cells; sc, seed coat. Scale bars equal 100㎛ in Fig. 5; 200㎛ in Fig. 2; 500㎛ in Figs. 1, 3, and 4.

Distinctively, two species of the genus studied sclenchyma cells are scattered or evenly dispersed in the mesocarp, in other words, the sclenchyma cells are present neither in partcular position nor in particular form. Such sclenchyma cells are found in *Ocotea nitida* (Fig. 6) and *O. floribunda* (Fig. 7).

The exocarp of the mature fruits is mostly glossy black, and contains tanniniferous cells with cuticle layer (Fig. 2).



Figs. 6-11. Transverse sections of mature fruits wall in *Ocotea*. Fig. 6. *O. nitida*. Fig. 7. *O. floribunda* showing the scattered sclenchyma cells in the mesocarp. Fig. 8. *O. tonduzii* showing the specialized inner hypodermal cells; arrows indicate crystal cells. Fig. 9. *O. helicterifolia*. Fig. 10. *O. atirrensis*. Fig. 11. *O. schomburgkiana*. Abbreviations: en, endocarp; ex, exocarp; ih, inner hypodermal cells; ms, mesocarp; oh, outer hypodermal cells; sc, seed coat; scl, sclenchyma cells. Scale bars equal 100㎛ in Figs. 7, 10, and 11; 200㎛ in Figs. 6 and 8; 500㎛ in Fig. 9.

Discussion

Fourteen species of *Ocotea* examined showed the remarkable differences in fruits wall structure within the genus. The fruits wall anatomical features of them are summarized five groups as follows; 1) non-specialized species (*O. helicterifolia, O. atirrensis, O. cujumari, O. rubra, and O. schomburgkiana*), 2) sclenchyma cells developed only in outer hypodermal cells (*O. javitensis, O. aciphylla*, and *O.* sp. [*Werff et al. 12676*]), 3) sclenchyma cells developed only in inner hypodermal cells (*O. tonduzii*), 4) sclenchyma cells developed both inner and outer hypodermal cells (*O. foetens, O. quixos*, and *O. veraguensis*), and 5) sclenchyma cells scattered and dispersed in the mesocarp (*O. nitida* and *O. floribunda*).

Although the present study covers only 14 of the about 400 species, it seems to show what sorts of structural diversity may be found in the fruits wall of unstudied species, and the results of the present study seem to represent well the diversity of the genus. In other words, the specialized fruit wall structures may be indicative of specific relationships within the genus and useful to assess a homogeneity of individual species.

Ocotea has its center in tropical America, but it is also distributed in Africa, Madagascar, Canary Islands and the Mascarenes. It was often cited as an example of a "Gondwana" distribution, but upon closer examination its distribution seems to be more recent (Rohwer 1986). *Ocotea foetens*, only endemic species in the Canary Islands, is well-developed both inner and outer hypodermal sclenchyma cells as well as South American species, but it remains geographically isolated.

Concerning *Ocotea rubra*, although this species has no specialized features in the fruit wall structure, it differences from other species with respect to embryological characters (Heo 1996, Ph.D. dissertation). Therefore, I suggest that *Ocotea rubra* must be separated from *Ocotea*, and then place in more advanced systematic position than *Ocotea*.

According to the position of anther cells, Kostermans (1957) divided *Ocotea* into three subgenera, i.e., *Nectandra, Ocotea*, and *Pleurothyrium*. However, this character is certainly not of generic value. Some species of *Ocotea*, especially species with non-specialized fruits wall structure, are very similar to *Nectandra* (Heo 1996, Ph.D. dissertation).

On the other hand, Werff (pers. comm.) suspected that *Ocotea* is to be divided into seven subgroups, i.e., a) Malagasy species; b) African species; c) Canary Islands species (*O. foetens*); d) species with hermaphrodite flowers and papillate stamens; e) other species with hermaphrodite flowers; f) species with unisexual flowers; g) South American species (*O. rubra*). It is considered that these previous studies and communication have already suggested diversity of species in the *Ocotea*.

In order to evaluate evolutionary implications of characters, on the other hand, comparisons within species have been considered. Comparisons with those species suggest that the specialized species may be evolved from non-specialized species. Therefore, these specialized features are apomorphic characters. As a result of the present study, *Ocotea* is found to be variously inconsistent with respect to the fruits wall structure.

Therefore, considering the occurrence of so great diversity in *Ocotea*, it seems very likely that the genus is heterogeneous and needs critical systematic revision. Attention may have to be paid to this genus in the near future as to its evolutionary position in the Lauraceae.

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摘 要

種間 類緣關係 및 屬의 이해를 돕기 위하여 *Ocotea* 屬의 과실 解剖形態의 硏究가 14種에 대하여 수행되었다. 調査된 種들간의 과실해부형태는 거의 비슷했으나 種들간 差異는 中果皮내에 후벽細胞의 有無에 있었고, 만일 中果皮내에 후벽細胞가 存在한다면 어느部位에, 또는 어떠한 形態로 存在하는가에 달려있다. 調査된 種들을 가지고 比較한 結果, *Ocotea*屬은 적어도 5개의 group으로 나눌 수 있었다. 즉, 1) 후벽細胞가 發達하지 않은 種 *O. atriensis, O. cujumari, O. helicterifolia, O. rubra and O. schomburgkiana*; 2) 후벽細胞가 外下皮에만 발달한 種 *O. aciphylla, O. javitensis*, and *O.* sp. [*Werff et al. 12676*]; 3) 후벽細胞가 內下皮에만 발달한 種 *O. tonduzii*; 4) 후벽細胞가 外下皮와 內下皮에 모두 발달한 種 *O. foetens, O. quixos*, and *O. veraguensis*; 과 5) 후벽細胞가 中果皮의 여기저기에 發達한 種 *O. floribunda* and *O. nitida*으로 나눌 수 있었다.

아울러, *Ocotea*屬에 있어서 果皮 解剖形態의 多樣性이 논의되었으며, 屬에 대한 分類學的 재인식이 要求되었다. 한편, 果實의 解剖形態 形質의 進化傾向은 中果皮내에 후벽細胞가 發達하지 않은 種으로부터 후벽細胞가 發達한 種으로 進化했을것으로 추정되었다.

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