Calcium crystals in the leaves of some species of Moraceae

Chi-Chih Wu and Ling-Long Kuo-Huang

Department of Botany, National Taiwan University, Taipei, Taiwan, Republic of China

(Received September 19, 1996; Accepted December 2, 1996)

Abstract. The type, morphology, and distribution of calcium oxalate and calcium carbonate crystals in mature leaves of nine species (eight genera) of Moraceae were studied. All the studied species contain calcium crystals. Based on types of crystals, these species can be classified into three groups: (a) species with only calcium oxalate: Artocarpus altilis and Cudrania cochinchinensis; (b) species with only calcium carbonate: Fatoua pilosa and Humulus scandens; and, (c) species with both calcium oxalate and calcium carbonate: Broussonetia papyrifera, Ficus elastica, Ficus virgata, Malaisia scandens, and Morus australis. The calcium oxalate crystals were mainly found as druses or prismatic crystals. Druses were located in the crystal cells of both mesophyll and bundle sheath, but prismatic crystals were found only in cells of the bundle sheath. All calcium carbonate cystoliths were located in the epidermal lithocysts, and the types of lithocysts were related to the number of epidermal layers, i.e. hair-like lithocysts in uniseriate epidermis and papillate lithocysts in multiseriate epidermis.

Keywords: Calcium oxalate crystals; Calcium carbonate crystals; Moraceae.

Introduction

In many plant species calcium crystals are commonly formed under ordinary conditions (Arnott and Pautard, 1970). These crystals are structural components in the leaves of many higher plant families. Their type and location are often used in plant taxonomic classification (Solereder, 1908; Hsieh and Huang, 1974; Genua and Hillson, 1985). Calcium oxalate is the most prominently deposited calcium salt (Fahn, 1990). The crystals may occur in different plant organs and in various shapes, e.g. druses, prismatic crystals, raphides, styloides, and crystal sands. However, calcium carbonate crystals are found only in a few families such as Moraceae, Urticaceae, and Acanthaceae. The cystolith (consisting of calcium carbonate) is located in lithocysts. They are in the forms of papillate or hair-like lithocysts and occur mostly in the epidermis of the leaves (Mauseth, 1988).

There is an increasing number of reports on the formation of calcium crystals, but they deal only with either calcium oxalate crystals (Franceschi and Horner, 1980; Borchert, 1986; Doaigey, 1991; Kuo-Huang et al., 1994) or with calcium carbonate crystals (Watt et al., 1987; Yu and Li, 1990; Kuo-Huang and Yen, 1996). Only few reports describe both calcium oxalate and calcium carbonate crystals in a given species (Fahn, 1990). In Taiwan there are 8 genera and 49 endemic species of Moraceae, 39 species of Ficus, two species each of Artocarpus, Broussonetia, and Fatoua, and one species each of Cudrania, Humulus, Malaisia, and Morus (Li et al., 1979).

In a preliminary investigation of the Moraceae, we found both calcium oxalate and carbonate crystals, which encouraged us to study the specific distribution of differently shaped calcium oxalate and carbonate crystals in mature leaves of selected species and genera of the Moraceae.

Materials and Methods

Nine species belonging to eight genera of the Moraceae were selected for study (Table 1). They were collected during 1993–1995 and identified according to Li et al. (1979). The voucher specimens were kept in the Herbarium or Anatomy Laboratory, Department of Botany, National Taiwan University. A few representative mature leaves of each species were decolorized in 95% ethanol and cleared in lactic acid (Sporne, 1948). Photographs of the cleared leaves were prepared with a Nikon Optiphot Microscope and a Leica Diaplan Microscope. Materials for scanning electron microscopy (SEM) were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO4, dehydrated in an ethanol - acetone series, dried with a Hitachi Critical Point Dryer (HCP-1), coated with an IB-2 ion coater (Dawes, 1979), and examined with the Hitachi S-550 SEM. The acid-etching test was used to identify the chemical compositions of crystals (Horner and Wagner, 1992).

Results

At least one type of calcium crystal was observed in the mature leaves of all species. The distribution of calcium crystals is summarized in Table 2. The characteristics of the calcium crystals are described below.
Calcium Oxalate Crystals

Calcium oxalate crystals were observed in the leaves of *Artocarpus altilis* (Figure 1C and D), *Broussonetia papyrifera* (Figure 1J), *Cudrania cochinchinensis* (Figure 2C and D), *Ficus elastica* (Figure 2L), *Ficus virgata* (Figure 3D), *Malaisia scandens* (Figure 4D and E), and *Morus australis* (Figure 4I–K). Druses and prismatic crystals were the preponderant types in these species. Druses occurred in all but *Ficus elastica*, and prismatic in all but *Ficus virgata* (Table 2). Where found, prismatic crystals were restricted to the bundle sheath. Druses also tended to be found in the bundle sheath, except in the leaves of *Artocarpus altilis* (Figure 1E), *Cudrania cochinchinensis* (Figure 2E), *Ficus virgata* (Figure 3E), and *Morus australis* (Figure 4K), where they also occurred in the cells of palisade mesophyll layers. Furthermore, druses were observed in the spongy mesophyll layers of *Ficus virgata* and in the adaxial epidermis of *Artocarpus altilis* (Table 2).

Crystal sands were observed in the adaxial epidermis of the leaves of *Cudrania cochinchinensis* and *Ficus virgata* (Table 2). Some needle shaped crystals were also found in the epidermis of the leaves of *Ficus virgata*. No raphides or styloids were found in any of the species studied.

Calcium Carbonate Crystals

Calcium carbonate crystals were observed in the leaves of *Broussonetia papyrifera* (Figure 1H), *Fatoua pilosa* (Figure 2H), *Ficus elastica* (Figure 2K and M), *Ficus virgata* (Figure 3C), *Humulus scandens* (Figure 3H and I), *Malaisia scandens* (Figure 4C), and *Morus australis* (Figure 4H). Some of the calcium carbonate cystoliths had prominent stalks. All cystoliths were located in the epidermal idioblasts (lithocysts). They were distributed along leaf veins or scattered in intercostal areas of leaves (Figure 3J). In the leaves of *Broussonetia papyrifera*, *Ficus elastica*, *Ficus virgata*, and *Humulus scandens*, the lithocysts were observed in both adaxial and abaxial epidermis. However, they were found only in the adaxial epidermis of leaves of *Malaisia scandens*, and the abaxial epidermis of *Fatoua pilosa* (Table 2).

Two types of lithocysts were identified: the hair lithocyst and the papillate lithocyst. The hair lithocysts were found in the uniseriate epidermis of *Broussonetia papyrifera* (Figure 1H and I), *Fatoua pilosa* (Figure 2H), *Humulus scandens* (Figure 3H and I), and *Morus australis* (Figure 4H). In leaves of species the epidermis was uniseriate. The papillate lithocysts were found in the multiseriate (2–5 layers) epidermis of *Ficus elastica* (Figure 2K and M), *Ficus virgata* (Figure 3C), and *Malaisia*

---

**Table 1.** Specimens used in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Collection time</th>
<th>Collection site</th>
<th>Voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artocarpus altilis</em> (Park.) Forberg</td>
<td>1993.9., 1995.3.</td>
<td>NTU, Taipei</td>
<td>wcc-0006</td>
</tr>
<tr>
<td><em>Broussonetia papyrifera</em> (L.) L’Hér.</td>
<td>1993.9., 1995.3.</td>
<td>NTU, Taipei</td>
<td>wcc-0001</td>
</tr>
<tr>
<td><em>Cudrania cochinchinensis</em> (Lour.) Kudo &amp; Masamune var. <em>gerontogaea</em></td>
<td>1994.9.</td>
<td>NTH, Kao-hsiung</td>
<td>wcc-0004</td>
</tr>
<tr>
<td><em>Fatoua pilosa</em> Gaud.</td>
<td>1993.10.</td>
<td>Shou-shan, Kao-hsiung</td>
<td>wcc-0002</td>
</tr>
<tr>
<td><em>Ficus elastica</em> Roxb.</td>
<td>1993.9., 1995.3.</td>
<td>NTU, Taipei</td>
<td>wcc-0007</td>
</tr>
<tr>
<td><em>Ficus virgata</em> Reinw</td>
<td>1993.9., 1995.3.</td>
<td>NTU, Taipei</td>
<td>wcc-0008</td>
</tr>
<tr>
<td><em>Humulus scandens</em> (Lour.) Merr.</td>
<td>1993.10.</td>
<td>Wu-chih-shan, Taipei</td>
<td>wcc-0005</td>
</tr>
<tr>
<td><em>Malaisia scandens</em> (Lour.) Planch</td>
<td>1993.10.</td>
<td>Shou-shan, Kao-hsiung</td>
<td>wcc-0003</td>
</tr>
<tr>
<td><em>Morus australis</em> Poir</td>
<td>1993.9., 1995.3.</td>
<td>NTU, Taipei</td>
<td>wcc-0009</td>
</tr>
</tbody>
</table>

NTU= National Taiwan University.

**Table 2.** The leaf characters and the distribution of calcium oxalate and carbonate crystals in the leaves of nine species of Moraceae.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Layers of epidermis (x, y)</th>
<th>Types of trichomes</th>
<th>Calcium oxalate</th>
<th>Calcium carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artocarpus altilis</em> (Park.) Forberg</td>
<td>(1, 1)</td>
<td>PT, HT</td>
<td>D D – D, P –</td>
<td>– – –</td>
</tr>
<tr>
<td><em>Cudrania cochinchinensis</em> (Lour.) Kudo &amp; Masamune var. <em>gerontogaea</em></td>
<td>(1, 1)</td>
<td>–</td>
<td>– D D, P – CS</td>
<td>– – –</td>
</tr>
<tr>
<td><em>Fatoua pilosa</em> Gaud.</td>
<td>(1, 1)</td>
<td>GT, HT</td>
<td>– – D D, P –</td>
<td>– – –</td>
</tr>
<tr>
<td><em>Humulus scandens</em> (Lour.) Merr.</td>
<td>(1, 1)</td>
<td>GT, HT, PT</td>
<td>– – D D, P –</td>
<td>– – –</td>
</tr>
<tr>
<td><em>Broussonetia papyrifera</em> (L.) L’Hér.</td>
<td>(1, 1)</td>
<td>GT, HT</td>
<td>– – D D, P –</td>
<td>– – –</td>
</tr>
<tr>
<td><em>Ficus elastica</em> Roxb.</td>
<td>(5, 3)</td>
<td>–</td>
<td>– P –</td>
<td>PL WS WS</td>
</tr>
<tr>
<td><em>Ficus virgata</em> Reinw</td>
<td>(2, 1)</td>
<td>T</td>
<td>D D D, P</td>
<td>PL WS WS</td>
</tr>
<tr>
<td><em>Malaisia scandens</em> (Lour.) Planch</td>
<td>(3, 1)</td>
<td>HT</td>
<td>– D – D, P</td>
<td>PL NS</td>
</tr>
<tr>
<td><em>Morus australis</em> Poir</td>
<td>(1, 1)</td>
<td>GT</td>
<td>– D – D, P</td>
<td>HL WS NS</td>
</tr>
</tbody>
</table>

AdE: adaxial epidermis; AbE: abaxial epidermis; PM: palisade mesophyll; SM: spongy mesophyll; BS: bundle sheath; GT: glandular trichome; HT: hair trichome; PT: peltate trichome; “-“: no crystal; D: druses; P: prismatics; CS: crystal sands; T: needles; WS: with stalk; NS: without stalk; L: lithocyst; HL: hair lithocyst; PL: papillate lithocyst.

(x, y): x- layers of adaxial epidermis; y- layers of abaxial epidermis.
**Figure 1.** A–E, *Artocarpus altillis*. A, SEM photograph of adaxial leaf surface showing the peltate trichome (arrow) and hair trichome (arrowhead). Bar=100 µm. B, Abaxial leaf surface showing the peltate trichome (arrow), hair trichome (arrowhead), and stomata (S). Bar=100 µm. C, A cross section of the leaf showing the prismatic (P) in the cell of bundle sheath. Bar=5 µm. D, A cross section of the leaf showing the druses (D) in the cell of bundle sheath. Bar=5 µm. E, LM photograph under polarizing light showing calcium crystals (arrows) in the cells of palisade mesophyll and bundle sheath. Bar=160 µm. 

F–J, *Broussonetia papyrifera*. F, Adaxial leaf surface showing the glandular trichomes (arrow) and hair lithocysts (arrowhead). Bar=100 µm. G, Abaxial leaf surface showing many long and short hair trichomes (arrows) and hair lithocysts (arrowhead). Bar=100 µm. H, A cross section of the leaf showing the hair lithocyst (HL) in the cell of adaxial epidermis (AdE). Bar=5 µm. I, A cross section of the leaf showing the druses (D) in the cells of bundle sheath. Bar=5 µm. J, Calcium crystals (arrows) in the cells of bundle sheath. Bar=160 µm.
Figure 2. A–E, Cudrania cochinchinensis. A, SEM photograph of adaxial leaf surface showing the linear epicuticular wax. Bar=20 µm. B, Abaxial leaf surface showing the epicuticular wax and stomata (S). Bar=20 µm. C, A cross section of the leaf showing the druses (D) in the cell of bundle sheath. Bar=5 µm. D, A cross section of the leaf showing the prisms (P) in the cell of bundle sheath. Bar=5 µm. E, LM photograph under polarizing light showing calcium crystals (arrows) in the cells of palisade mesophyll and bundle sheath. Bar=200 µm. F–H, Fatoua pilosa. F, Adaxial leaf surface showing the glandular trichomes (arrow) and hair trichomes (arrowhead). Bar=50 µm. G, Abaxial leaf surface showing the glandular trichomes (arrow) and the hair lithocysts (arrowhead). Bar=20 µm. H, A cross section of the leaf showing the hair lithocyst (HL) in the cell of abaxial epidermis (AbE). Bar=20 µm. I–M, Ficus elastica. I, Adaxial leaf surface showing the epicuticular wax. Bar=100 µm. J, Abaxial leaf surface showing the epicuticular wax and stomata (S). Bar=100 µm. K, A cross section of the leaf showing the cystolith (C) with a prominent stalk (arrow) in the cell of multiseriate adaxial epidermis. Bar=20 µm. L, A cross section of the leaf showing the prisms (P) in the cell of bundle sheath. Bar=5 µm. M, A cross section of the leaf showing the papillate lithocyst (PL) in the cells of the multiseriate adaxial epidermis (arrow) and the multiseriate abaxial epidermis (arrowhead). Bar=100 µm.
Figure 3. A–E, Ficus virgata. A, SEM photograph of adaxial leaf surface showing the papillate lithocyst (arrow). Bar=50 µm. B, Abaxial leaf surface showing the papillate lithocysts (arrow) and stomata (S). Bar=50 µm. C, A cross section of the leaf showing the cystolith (C) with a prominent stalk (arrow) in the cell of multiseriate adaxial epidermis (AdE). Bar=10 µm. D, A cross section of the leaf showing druses (D) in the cell of bundle sheath. Bar=5 µm. E, LM photograph under polarizing light showing calcium crystals (arrows) in the cells of palisade mesophyll and bundle sheath. Bar=160 µm. F–J, Humulus scandens. F, Adaxial leaf surface showing the glandular trichomes (small arrow), hair lithocysts (large arrow), and hair trichome (arrowhead). Bar=100 µm. G, Abaxial leaf surface showing many glandular trichomes (small arrow), hair trichomes (large arrow), peltate trichomes (small arrowhead), and hair lithocysts (large arrowhead). Bar=100 µm. H, A cross section of the leaf showing hair lithocyst (HL) in the cell of adaxial epidermis (AdE). Bar=10 µm. I, A cross section of the leaf showing the hair lithocyst (HL) in the cell of abaxial epidermis (AbE). Bar=10 µm. J, The cystoliths (arrows) sporadically distributed in the cells of adaxial epidermis. Bar=40 µm.
Figure 4. A–E, *Malaisia scandens*. A, SEM photograph of adaxial leaf surface showing the papillate lithocysts (arrow). Bar=10 µm. B, Abaxial leaf surface showing the hair trichome (arrow) and stomata (S). Bar=50 µm. C, A cross section of the leaf showing the cystolith (C) without prominent stalk in the papillate lithocyst of the cell of multiseriate adaxial epidermis (AdE). Bar=10 µm. D, A cross section of the leaf showing druses (D) in the cell of bundle sheath. Bar=5 µm. E, LM photograph under polarizing light showing calcium crystals (arrows) in the cells of palisade mesophyll and bundle sheath. Bar=200 µm. F–K, *Morus australis*. F, Adaxial leaf surface showing the hair lithocysts (arrows). Bar=100 µm. G, Abaxial leaf surface showing the glandular trichomes (arrows) and hair lithocysts (arrowheads) distributed along the leaf veins. Bar=50 µm. H, A cross section of the leaf showing the uniseriate adaxial and abaxial epidermis and a hair lithocyst without a prominent stalk in the cell of adaxial epidermis (AdE). Bar=10 µm. I, A cross section of the leaf showing druses (D) in the cells of bundle sheath. Bar=10 µm. J, A cross section of the leaf showing the prisms (P) in the cells of bundle sheath. Bar=5 µm. K, Calcium crystals in the cells of palisade mesophyll (arrow) and bundle sheath (arrowhead). Bar=120 µm.
scandens (Figure 4C). However, in the multiseriate abaxial epidermis of Ficus elastica or in the uniseriate abaxial epidermis of Ficus virgata, the lithocysts were all papillate.

No trichome was found in mature leaves of Cudrania cochinchinensis (Figure 2A and B), Ficus elastica (Figure 21 and J) or Ficus virgata (Figure 3A and B). But there were many glandular, paltate, or hair trichomes (Table 2) in the epidermis of leaves of Artocarpus altitlis (Figure 1A and B), Broussonetia papyrifera (Figure 1F and G), Fatoua pilosa (Figure 2F and G), Humulus scandens (Figure 3F and G), Malaisia scandens (Figure 4B), and Morus australis (Figure 4F and G). No calcium crystal was found in these trichomes.

Discussion

Calcium crystals were observed in all plants investigated (Tables 1 and 2). Based on the kind of calcium crystals, these species can be classified into three groups: (a) species with only calcium oxalate: Artocarpus altitlis and Cudrania cochinchinensis; (b) species with only calcium carbonate: Fatoua pilosa and Humulus scandens; and (c) species with both calcium oxalate and calcium carbonate: Broussonetia papyrifera, Ficus elastica, Ficus virgata, Malaisia scandens, and Morus australis.

Two types of calcium oxalate crystals were common. The prismatic crystals occurred only in crystal cells of the bundle sheath, and druses were located in crystal cells of both mesophyll and bundle sheath. Daoiegy (1991) observed various shaped calcium oxalate crystals in individual plants of Datura, Nerium, and Rumex. In this study, crystals with different shapes were found in adjacent cells of the same plant (Figure 11). Impurities present in crystal cells may have been a factor in the formation of different types of calcium oxalate crystals (Scurfield et al., 1973).

The idioblasts of calcium oxalate crystals are located mostly in the bundle sheath surrounding the vascular bundles (Table 2), which supports the suggestion that the formation of calcium oxalate crystals in plants may also be linked to the evaporation of water (Franceschi and Horner, 1980; Kuo-Huang, 1990).

The morphology of cystolith and the distribution of lithocyst are genera and species specific in the Acanthaceae (Hsieh and Huang, 1974). Furthermore, the types of lithocytes are related to the number of layers of epidermis of leaves, i.e., hair-like lithocysts in the uniseriate epidermis as found in Broussonetia papyrifera, Fatoua pilosa, Humulus scandens, Morus australis, and the papillate lithocysts of multiseriate epidermis in Ficus elastica, Ficus virgata, and Malaisia scandens.

It is interesting to note that, among the plants studied, calcium oxalate crystals were associated with woody species and not herbaceous species such as Fatoua pilosa and Humulus scandens (Table 2). However, there is a shortage of information in the literature on this particular relationship. The presence of crystals is certainly not detrimental to the plant. Physical and chemical conditions, such as temperature, pressure, pH, and ion concentration, may affect crystal growth, habit, and properties (Franceschi and Horner, 1980), but the precise controlling mechanism for crystal formation in plants is still unknown.

Crystals in Moraceae are commonly described in taxonomic literature. There are 8 genera and 49 species of Moraceae in Taiwan (Li et al., 1979). Although the examination of crystals in 9 of 49 species is inadequate for generalization regarding the taxonomic significance of crystals in this family, those observed in a species were consistently found in specific locations within the plant and even only of specific shapes and compositions.

The formation of crystal idioblasts is a complex process involving changes of cell development and formation of specific crystal structures. Factors which control oxalate synthesis and cellular calcium uptake and mobility may affect crystal induction and formation (Scott, 1941; Frank, 1972; Borchert, 1986; Franceschi, 1987). The presence or absence of crystals is one of the important characters for understanding the evolutionary relationships of plant species (Franceschi and Horner, 1980).

Acknowledgements. This research was partly supported by the National Science Council of Taiwan, R.O.C. under the grant NSC-83-0211-B002-266.

Literature Cited


Frank, E. 1972. The formation of crystal idioblasts in Cana-valia ensiformis DC. at different levels of calcium supply. Z. Pflanzenphysiol. 67: 350–358


