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The Development of Lithocysts in the Leaves and Sepals of *Justicia procumbens* L.

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ABSTRACT: During the development of the leaves and sepals of *Justicia procumbens*, the formations of lithocysts, trichomes, and diacytic stomata in both adaxial and abaxial epidermises exhibited the regular distribution patterns. The means of densities and lengths of the lithocysts in the leaves were higher and larger in the adaxial epidermis than in the abaxial epidermis, in the central area than the marginal area, and in the basal area than the leaf apex respectively. The mean densities of lithocysts increased during the early developmental stages of leaf, but in the later stages, because the enlargement of the lithocysts and the neighboring ordinary cells, they decreased obviously. The lithocysts associated with the midrib and the primary or secondary veins of the leaf were elongated along the vein axis. Elsewhere in the leaf, the axes of the lithocysts were parallel to the leaf margin or oblique relative to the midrib and showed a correlation between the leaf development and the lithocyst elongation. In the sepals the lithocysts were found only in the abaxial epidermis. They were all parallel to the midrib. The mean densities and lengths of lithocysts were higher and larger in the older sepals than those in the younger sepals. On the other hand, the densities of lithocysts in the sepal were higher than those in the leaves.

KEY WORDS: Lithocyst, Leaves, Sepals, *Justicia*.

INTRODUCTION

The calcium carbonate crystals are formed frequently in the algae, or the other aquatic plants. They are deposited generally on the plant outer surface or in the intercellular spaces (Borowitzka, 1984). But in the land plants the most prominent calcium carbonate deposition is associated with the cystolith formation. Cystoliths are enclosed in the idioblastic cells known as lithocysts. In contrast to the formation of calcium oxalate crystals in many families of angiosperms (Franceschi and Horner, 1980), the cystolits have been found only in the species of a few dicotyledon families (Fahn, 1990). They are found in various organs and tissues, nevertheless most of them are located in the epidermal cells of the leaves. The morphology and distribution of lithocysts in the plants vary among the different taxa, therefore, they have been used for the plant systematic study, especially on the familic, generic, or speciesic level (Pireyre, 1961; Smith, 1982; Okazaki *et al.*, 1986). There is an increasing number of reports concerning with the lithocysts in the plants of Moraceae and Urticaceae (Arnott and Pauart, 1970; Arnott,1980; Smith and Watt, 1986), but in these studies little attempt was made to compare the formation of lithocysts in the vegetative organ with that in the reproductive organ.

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In Acanthaceae the lithocyst is one of the most obvious characters and is valuable for the recognition of the genera (Hsieh and Huang, 1974). In *Justicia procumbens* the lithocysts are occurred frequently in the epidermis. Their shapes and distributions are unique between organs. In this investigation, the morphology and distribution of lithocysts and trichomes in the epidermis of the developing and mature leaves and sepals were studied.

MATERIALS AND METHODS

The leaves and sepals in various developing stages of *Justicea procumbens* L. were collected from the campus or the greenhouse of Department of Botany, National Taiwan University. The materials for SEM were fixed for 2 h in 2.5% glutaraldehyde followed by 1% OsO_4 for 2 h, and dehydrated, dried, and coated as routinely processes, and then examined with the hitachi S-550 SEM (Kuo-Huang, 1990). Some materials were bleached in 95% ethanol, cleared for 30 min in pure lactic acid kept at 100 ˚C in a boiling water-bath, and then storaged or mounted in lactic acid (Sporne, 1948). The cleared samples were investigated and photographed with Leica Diaplan light-microscope under polarized light. The drawings of veins in the leaves and sepals were made by means of a camera lucida. Quantitative results of the densities and lengths of lithocysts in the epidermises were based on the measurements or counts from five cleared leaves or sepals in each size category. The maximal areas of the ordinary epidermal cells or the lithocysts in the young and old sepals were measured by the PC meter image analysis.

RESULTS

Lithocysts in leaves

The shoot apical meristem of *Justicia procumbens* was surrounded by the opposite arranged leaf primordia (Fig. 1a). The leaves of a third to fifth leaf pair were 0.1-2 mm in length. These young leaves had a midrib and 2-4 pairs of lateral veins arising near the tip of midrib and being continuous to the leaf margin (Fig. 2a). The midrib developed outward from the base of primordium acropetally. However, the midribs, the lateral veins and veinlets tended to mature earliest in the distal regions. At this stage the differentiations of the epidermal cells in both leaf surfaces have taken place. Many uniseriate multicellular nonglandular trichomes originated basipetally from the cells on the leaf edge, and then the epidermal cells of the midrib on the abaxial surface and the lower part of the midrib on the adaxial side (Figs. 1b-e). The glandular trichomes, with a flat circular head of 2-4 cells, were found scatteredly on both surfaces. There was no hydathode, but near the leaf apex some lithocyst initials were originated basipetally from the adaxial epidermal cells (Figs. 1d-f). The lithocyst initials became elongated between the ordinary epidermal cells and somtimes between the epidermis and the palisade tissue. Above lithocysts neither stoma nor trichome was observed.

In the leaves 4-8 mm in length, the midrib and the lateral veins were connected and with the minor veins from the tip to the base (Fig. 2b). The lamina expansion did not show uniform over the whole developing blades. It was obviously that the leaves grew fastest and longest in both basal and central regions. Many lithocysts were formed basipetally and

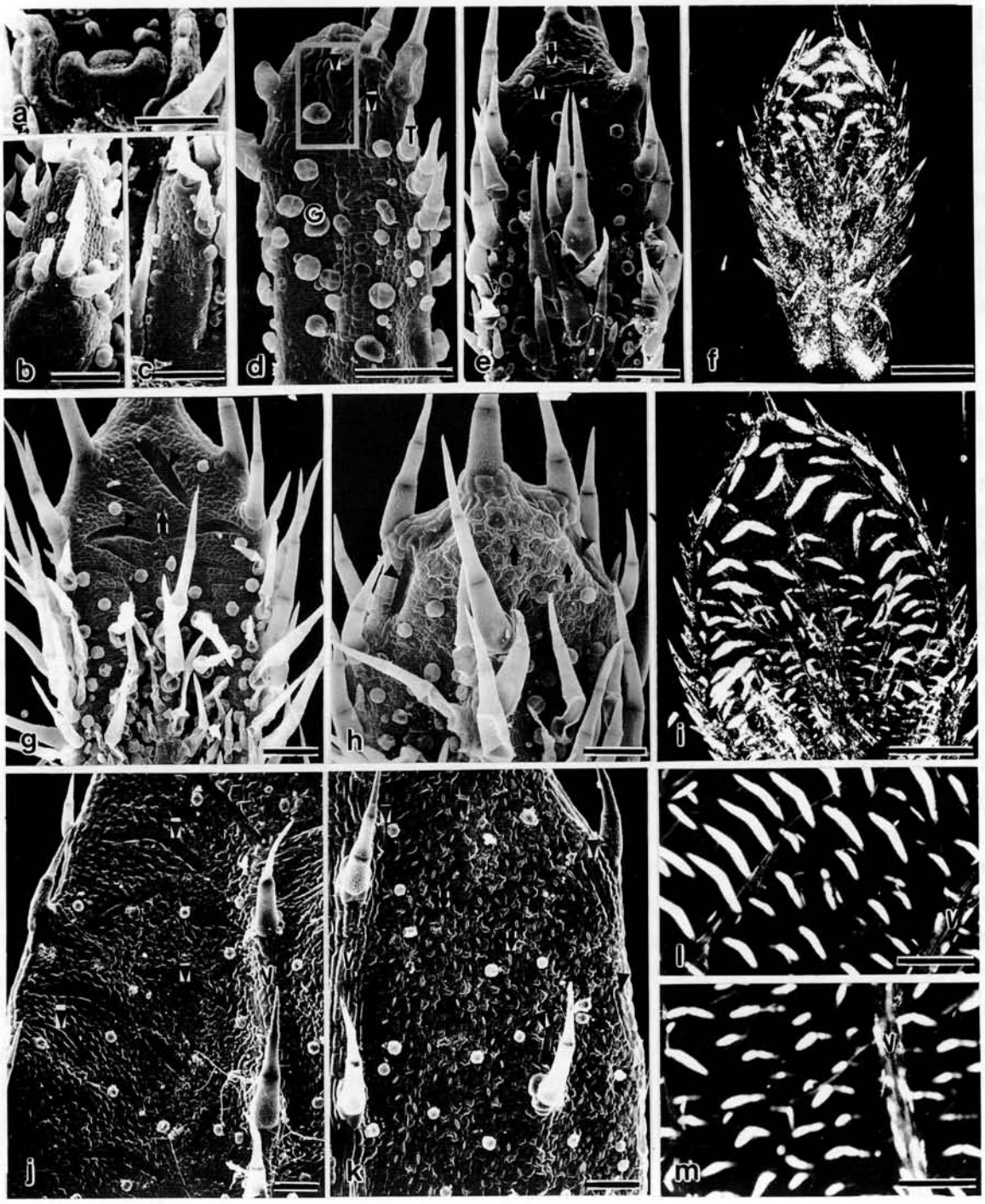

Fig. 1. SEM photographs and polarized light photographs of the leaves at various developmental stages showing the distributions of glandular and non-glandualr trichomes, stomata, and lithocysts in the epidermis. a: shoot apex surrounded by the opposite arranged leaf primordia (bar=100 μm). b: abaxial surface of a 0.1 mm leaf (bar=30 μm). c: adaxial surface of a 0.2 mm leaf (bar=100 μm). d: adaxial surface of a 0.4 mm leaf (bar=100 μm). e: adaxial surface of a 0.8 mm leaf (bar=100 μm). f: polarized light photograph of a cleared 2cm leaf (bar=0.5 mm). g: adaxial surface of a 3 cm leaf (bar=100 μm). h: abaxial surface of a 3 cm leaf (bar=100 μm). i: polarized light photograph of a cleared 3 cm leaf (bar=0.5 mm). j: adaxial surface of a mature leaf (bar=100 μm). k: abaxial surface of a mature leaf (bar=100 μm). l: polaried light photograph of a cleared mature leaf near the margin (bar=0.2 mm). m: polarized light photograph of a cleared mature leaf near the center (bar=0.2 mm). (arrow: stoma; arrow head: lithocyst; V: vein; G: glandular trichrome; T: nonglandular trichome.)

centripetally on the both leaf surfaces (Figs. 1g, h). Most of them appeared scatteredly on the adaxial surface. On the abaxial epidermis the lithocysts were arranged only near the leaf margin, however many stomata were scatteredly located on this surface. The stomata were diacytic type and the lithocysts were spindle- or eyebrow- shaped. In the central area of adaxial surface of the leaves 12-15 mm in length much more lithocysts were seen (Figs. 1i-k), besides, on the central area of the abaxial epidermis some lithocysts were also investigated. They located almostly along the leaf veins. The distribution pattern of vascular bundles and lithocysts (Figs. 1 l, m, 2c) in the mature leaves (20-25 mm) was regular and identical with that found in 12-15 mm leaves, despite the fact that the length of lithocysts on both surfaces have continued to increase. The orientational correlation between the lithocysts and the veins were recognizable at about the time of lithocysts elongation. In the epidermis on or near the midrib, primary lateral veins and leaf margin the lithocysts were orientated longitudinally along the vein axes or the leaf margin. But elsewhere in the adaxial or the abaxial surfaces of the leaf the orientations were predominantly oblique (Figs. 1 f-m).

The means of the densities and lengths of the lithocysts were higher and larger in the adaxial epidermis than in the abaxial surface (Fig. 4). Besides, they were higher in the central area than the marginal area and also higher in the basal area than the leaf apex (Figs. 3a-c). Nevertheless the mean lithocyst densities increased during the early developmental stages, whereas they decreased obviously in the later growth stages of leaves (Figs. 3a-c, 4).

Lithocysts in sepals

The calyx of *Justicia procumbens* was pentamerous and united at the base. A well expanded sepal was linear-lanceolate in shape. The vascular system of the young or mature sepal consisted of only the midrib (Figs. 2d, e). Along the abaxial side of the midrib there was a keel (Figs. 2m). The lamella of the sepal was scarious and composed of only 2-3 layers of cells. Many multicellular nonglandular trichomes were located along the margin and on the abaxial keel, but the glandular trichomes were occurred scatteredly on both surfaces (Figs. 2, k-m). In the sepals 0.5-1 mm in length, the lithocysts were originated basipetally from the abaxial epidermal cells (Figs. 2 f, g). Their shapes were elongated form with one end pointed. The axes of all lithocysts and their enclosing cystoliths were parallel to the midrib with their pointed ends downwards (Fig. 2h). In the sepals 1.5-2.5 mm long many lithocysts were found in the lamella between the keel and the margin of the sepals (Figs. 2 i-l). All lithocysts were along the midribs of the sepals. In the older sepals 3.5-4.5 mm long the distribution pattern of the lithocysts were like the younger sepals (Fig, 2m), but both of the mean density and the length of lithocyst were higher and larger in the older sepals than that of younger sepals (Fig. 5a). Besides, the mean area of the lithocysts was 1184 $x10^3$ μm^2 in young sepal, and 2738 $x10^3$ μm^2 in older sepal (Fig. 5b), respectively. The ordinary epidermal cells were about the same size (2150 μm^2) at these two developmental stages (Fig. 5b). On the adaxial surface stomata located scatteredly mainly in the central area (Fig. 2k), on the other hand, they were found in the area between the keel and the lithocyst area of the abaxial epidermis (Fig. 2l, m).

DISCUSSION

The development of leaves or the related organs is, in general, an orderly and regulated

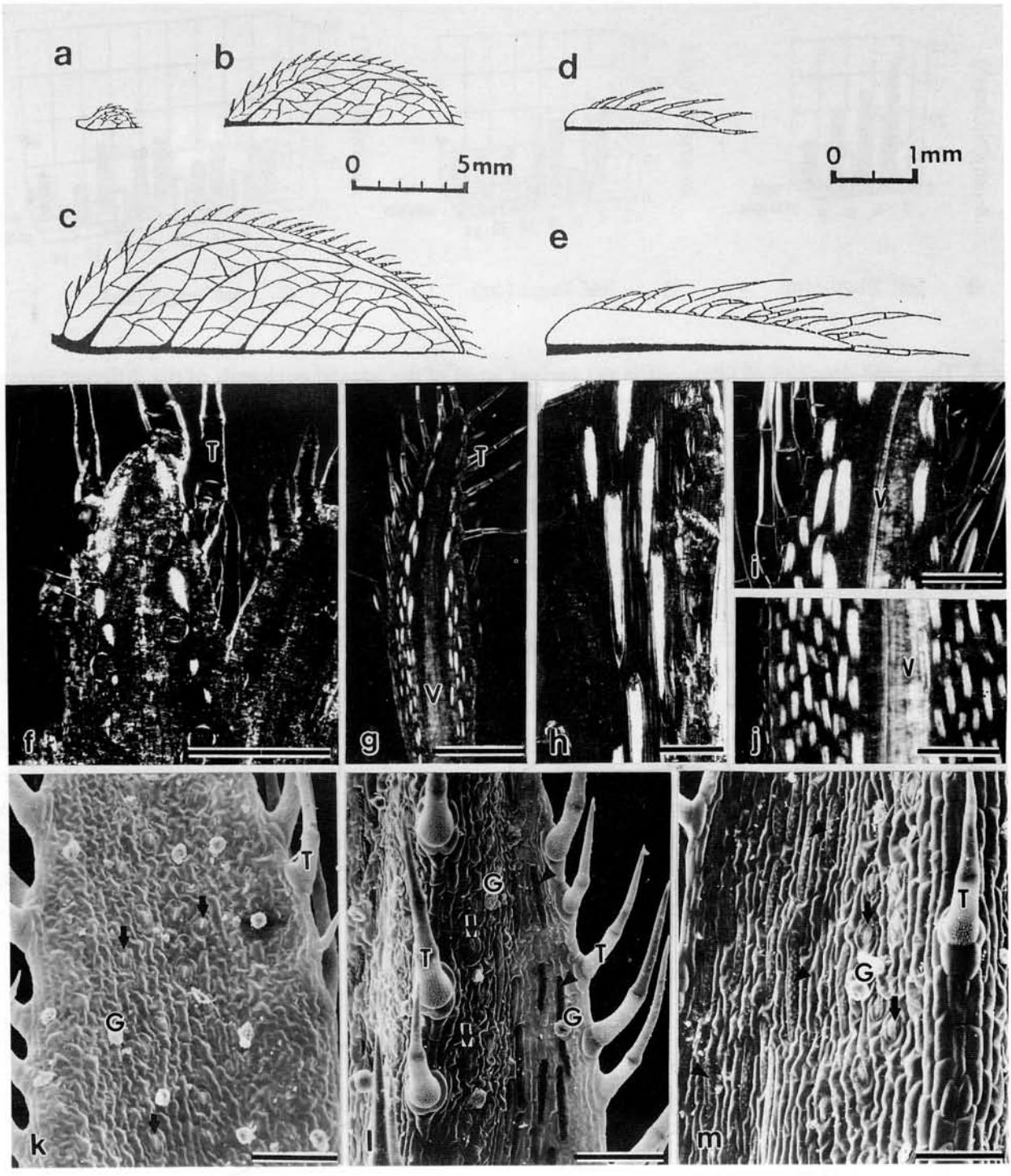

Fig. 2. a-e: Representative diagrams of the half leaves (a, 2-3 mm; b, 8-12 mm; c, 15-20 mm) and half sepals (d, 1.5-2.5 mm; e, 3.5-4.5 mm) from the size groups used in this investigation, showing development of the venation system. f-m: SEM photographs and polarized light photographs of the sepals at various developmental stages showing the distributions of glandular and non-glandualr trichomes, stomata, and lithocysts in the epidermis. f: 0.5 mm (bar=0.2 mm). g: 1.5 cm (bar=0.5 mm). h: enlargement of g (bar=50 mm). i: apex of a mature sepal (bar=0.2 mm). j: middle of a mature sepal (bar=0.2 mm). k: adaxial surface of a 2 cm sepal showing the non-glandular trichome along the margin but stomata and glandular trichome scattered on the sepal (bar=100 μm). l: abaxial surface of a 2 cm sepal showing the non-glandular trichome along the margin and on the midrib but stomata acummulated near the midrib and the lithocysts near the margin (bar=100 μm). m: abaxial surface of a 3.5 mm mature sepal showing the distribution of various epidermal cells (bar=100 μm). (arrow: stoma; arrow head: lithocyst; V: vein; G: glandular trichrome; T: nonglandular trichome.)

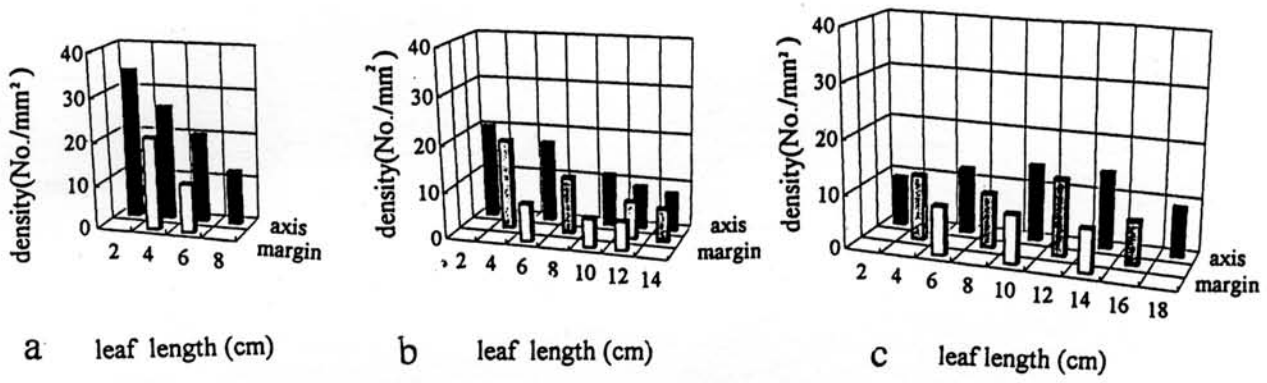

Fig. 3. The mean densities of lithocysts in the various areas of the adaxial epidermis of the different groups of leaves. a: 7-8 mm leaf group, b: 13-14 mm leaf group, c: 17-18 mm leaf group.

process. The results obtained from this investigation suggest that in *Justicia procumbens* the morphology and distribution of lithocysts in the epidermis of leaves and sepals are specific, however, their origin and development are correlated with the developmental patterns of the located organs. There is a gradient of origin or maturation of the lithocysts along the leaf or sepal from the tip to the base, and from the margin to the midrib. Besides, the lithocyst initial cells inhibite the origin of the same or some other kind of cells (stoma, trichome). It may be controlled by the spacing patterns by the mutual incompatibility between the initial cells of the same or different types (Buenning, 1965; Smith and Watt, 1986).

In *Justicia procumbens* the cystoliths bearing lithocyst initials were firstly observed in the young leaves about 0.5 mm in length. Accompanied with the development of the leaves, whose final lengths were about 20mm, the lithocysts matured and continued to originate basipetally in the submarginal areas of the epidermis. In *Pilea cadierei* the lithocysts initials were firstly recognizable in the abaxial epidermis of the leaves, which were 4 mm in length. In these lithocysts the cystoliths were not yet formed (Smith and Watt, 1986). In the 8-10

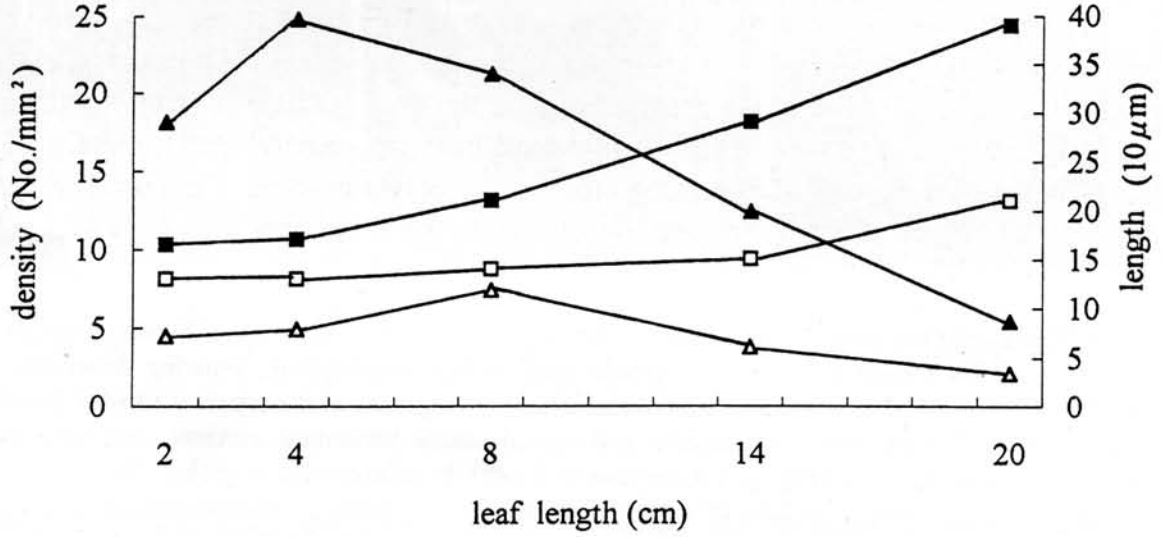

Fig. 4. Mean densities and mean lengths of lithocysts in the adaxial and abaxial epidermises of the leaves in different developmental stages. (▲, mean densities of lithocysts in the adaxial surface; △, mean densities of lithocysts in the abaxial surface; ■, mean lengths of lithocysts in the adaxial surface; □, mean lengths of lithocysts in the abaxial surface).

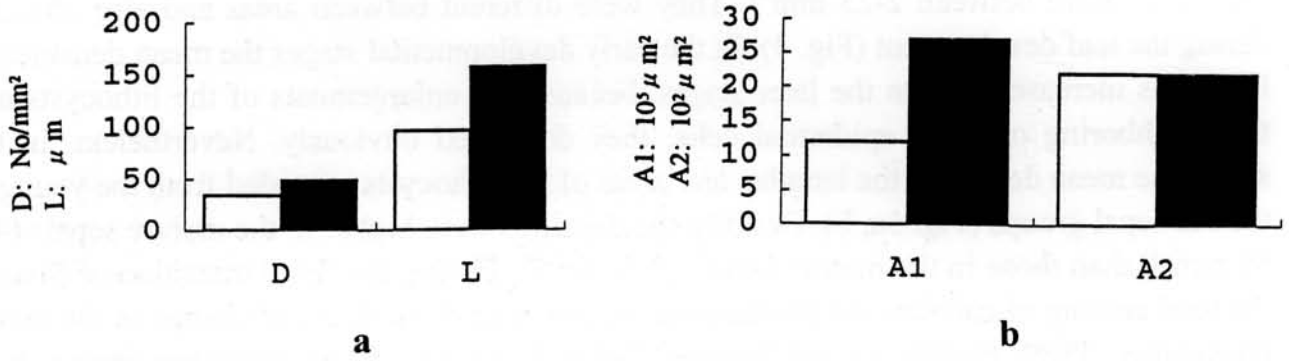

Fig. 5. a: Mean densities (D) and mean lengths (L) of the lithocysts in the young (□,1.5-2.5 mm) and old (■, 3.5-4.5 mm) sepals. b: Mean areas of lithocysts (A1) and the ordinary epidermal cells (A2) in the young (□, 1.5-2.5 mm) and old (■, 3.5-4.5 mm) sepals.

mm developing leaves or the 12-15 mm mature leaves of *Pilea* lithocysts were found in both of the adaxial and abaxial epidermises. They contained cystoliths and all of them were approximately at the same stage of development. It is probably that the origin and maturation of the lithocysts are genetically controled, but the accumulations of the calcium carbonate crystals in the formed of cystolith in the cells are mostly depended on the calcium ion supply (Zindler-Frank, 1980; Wu, 1995).

There were many trichomes on the both epidermises of leaves and sepals of *Justicia procumbens*, but in these trichomes no cystolith could be found. In the leaves of *Pilea* (Urticaceae) the trichomes contain also no cystolith (Smith and Watt, 1986). Nevertheless in the leaves of *Morus*, *Broussonetia*, *Humulus*, *Fatoua* (Moraceae) cystoliths are located only in the trichomes (Yu and Li, 1990; Wu, 1995), in another words, they are formed in the hair-like lithocysts. Crystal cells are almostly distributed in a specific region of a tissue (Fahn, 1990). The fate of a trichome in the leaf primordium, whether destined to be deposited with cystolith, or to become a ordinary glandular or non-glandular trichome, may be already determined.

In *Justicia procumbens* the leaves contain more lithocysts in the adaxial epidermis and less in the abaxial epidermis, but in sepals they were found only in the abaxial surface. In a preliminary observation by the present work in the cotyledons of *Justicia procumbens*, it is interesting to note that before the germination the mature cotyledons contain no lithocysts. Nevertheless, during the germinating process the cotyledons were exposed to the light and then, as the ordinary leaves, formed the lithocysts mostly on the adaxial surface. Franceschi and Horner (1980) reported that various physical and chemical parameters such as light, pressure, pH, and ion concentration affect the crystal growth and habit. Okazaki et al. (1986) supposed that the deposition of calcium carbonate crystals in the lithocysts of the epidermis can be related with the photosynthesis. The different ratios of light intensities between the adaxial and abaxial surfaces in the leaves, sepals, and cotyledons of *Justicia procumbens* may play a role in the lithocysts formation.

The number of lithocysts per unit area of leaf was varying from species to species. It was estimated to be from 10 to 39 mm^{-2} by Okazaki et al. (1986) from the microradiographys. In the lamella of the mature leaves of *Pilea cadierei* the estimated mean densities were about 6.5 mm^{-2} (Smith and Watt, 1986). In *Justicia procumbens* the mean densities of lithocysts in

the leaves were between 2-25 mm^{-2}. They were different between areas and also changed during the leaf development (Fig. 4). In the early developmental stages the mean densities of lithocysts increased, but in the later stages, because the enlargements of the lithocysts and the neighboring ordinary epidermal cells, they decreased obviously. Nevertheless, in the sepals the mean densities, the lengths, and areas of the lithocysts ascended from the young to the old sepal groups (Fig. 5a, b). Besides, the densities were higher in the mature sepals (40-50 mm^{-2}) than those in the mature leaves (2-5 mm^{-2}). During the floral transition of *Sinapis* the total amount of calcium ion increases in the apical bud but does not change in the leaves (Havelange, 1989; Havelange and Bernier, 1993). The value of cystoliths and lithocysts in the normal plant growth and development is speculative. However, a possible role of cystolith is proposed that it serves as calcium and CO_2 reservoir.

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爵床葉部與花萼之石胞的發育

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摘　　要

爵床（*Justicia procumbens*）葉部與花萼發育過程中，石胞、毛茸與氣孔的形成均顯示有一規則性的分布型式。葉片向軸面石胞之密度與長度均較背軸面者為大，並且在葉片中央及基部之石胞的密度與長度亦較大；然而隨著葉部的發育，石胞的密度在初期增加但後期則明顯減少。接近中肋與支脈之石胞的主軸與葉脈平行，其他部位則與葉緣平行或與葉脈呈歪斜分布，石胞的排列可顯示石胞延長與葉部發育的關係。在花萼石胞只形成於背軸面表皮中，並且其主軸均平行於花萼的中肋。隨著花萼的發育，石胞的密度與長度均明顯增加，並且花萼之石胞的密度較葉部者為大。

關鍵詞：石胞，葉片，花萼，爵床。

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