Observation on the Epidermal Stomata of a Cup-Like Gall of *Litsea acuminata* (Lauraceae)

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ABSTRACT

Scanning electronic microscopy was used to observe the morphology and structure of the epidermis of a cup-like gall of *Litsea acuminata* (Bl.) Kurata leaf. No regular stomata were found in the epidermis of galls at any growth stages and the epidermis of the gall is smoother than that of the leaf. At the young stage, a series of little cracks, with the size of approximate 16.5×6.7 μm^2-23.3×20.0 μm^2, line up along an axis and no underneath hole could be seen in the cracks. As the gall grows older, the little cracks further rip open wider and deeper, with a size of 20.2×21.0 μm^2-24.9×17.1 μm^2, and an underneath tunnel-like network was exposed. It is unknown whether or not the tunnel-like network plays any role in the function of air exchange for photosynthesis or insect breath.

Key words: stomata, epidermis, cup-like gall, *Litsea acuminate*, crack, tunnel-like network

Introduction

Stomata, located in the lower and/or upper epidermis depending on species, support higher plant photosynthesis by allowing the exchange of carbon dioxide and oxygen between the outside air and the leaf interior. Stomata are also the major avenues by which water exits the leaves by evaporation. Each typical stoma is flanked by a pair of guard cells which are kidney-shaped in dicots and dumbbell-shaped in monocots. The guard cells are suspended by their epidermal neighbor cells over an air chamber, leading to a honeycomb of air spaces (Campbell and Reece, 2002).

In addition to the functions mentioned above, stomata also play a role in insect infection on leaf. The stomata opening is used by agrobacterium to circumvent the physical barrier of the cutile (Escudero and Hohn, 1997). Females of *Cystiphora sonchi* (Bremi), a gall-inducing species, oviposite into leaves of sowthistle via the stomatal openings of the lower epidermis, where the surface is not punctured during oviposition (DeClerck and Steeves, 1988). *Cystiphora sonchi* prefers to oviposit into leaves that are in the final stages of growth, when mature stomata are available and tissues are still responsive to the gall-forming stimulus (DeClerck-Floate and Steeves, 1995).

Insect galls are the transformed abnormal growth tissues of the infected plants. The gall maker resides within. Although all plant organs are subject to insect galling, about 75% of insect galls occur on leaves (Dreger-Jauffret and Shorthouse, 1992; Meyer 1987; Williams, 1994). In spite of the appearance diversity in leaf, most of the galls must originate from leaf cells containing chloroplast.

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Herbivorous insects have been found to cause the total deficiency of some photosynthetic pigment-protein complexes, such as A1, AB1 and AB2, in an obovate and an oval-pointed cecidomyiid galls of *Machilus thunbergii* leaf though their grana morphology is still normal (Yang *et al*., 2003; Yang *et al*., 2007). The incomplete organization of PSI and PSII may affect the gall photosynthetic function of light-harvesting, energy transfer and photochemical energy conversion performed in pigment-protein complexes.

Differentiation of epidermis may or may not take place in the course of development of a gall on leaf. Sometimes there is no true epidermis on the surface. One of the striking features of the gall epidermis in general is that stomata are fewer than normal and in many cases totally absent. Even if some stomata are present, they are mostly functionless, due to the collapse of one or both the cells. The guard cells in the gall of *Diplolepis quercus-folii* often fused together because of swelling, causing stomata remain permanently open pores and functionless (Mani, 1964). On the other word, only limited literatures have answered these questions.

Therefore, many questions arise and remain to be answered, such as (1) How does the galler breathe despite confining itself inside the gall tissues derived from leaf cell? (2) Does gall have stomata as typical as leaf epidermis? (3) Does the galler acquire the oxygen through the plant metabolism? (4) Can the carbon dioxide produced by galler's respiration be reused by leaf cells? (5) Can the air inside the gall be directly exchanged to the environment by the incomplete enclosure of gall or other structure? And so on.

Therefore, this short paper aims at demonstrating a new possible stomata morphology of a pink cup-like gall of *Litsea acuminata* (Bl.) Kurata leaf.

Materials and Methods

Plant and gall

The cup-like gall, residing on the lower epidermis of *Litsea acuminata* (Bl.) Kurata leaf (Lauraceae), was collected from Mt. Chung-Cheng of the Yangminshan National Park near Taipei City at northern Taiwan (Fig. 1). The gall takes about four months to develop and grow from oviposition to maturity. The gall is pink and its center is

Figure 1. The pink cup-like galls on the lower epidermis of *Litsea acuminata* leaf. These galls were at the mature stage. White bar indicates 1 cm.

concave, looking like a crystallite cup or bowl.

Scanning electron microscopy

The detached young and mature galls and mature leaf were fixed for 3 h in 2.5% glutaraldehyde and then washed three times with 0.1 M phosphate buffer. Each wash took 15 min. A series of ethanol mixtures, 30, 50, 75, 90 and 100%, were used to dehydrate the fixed samples and finally 100% acetone was used to further dehydrate them. A thin gold film was set on the sample's surface (Falk, 1980). The morphology of gall's epidermis was examined with a Zeiss DSM 950 scanning electron microscope.

Results and Discussion

Leaf stomata

The lower epidermis of *Litsea acuminata* (Bl.) Kurata leaf showed a morphology of surface and stomata similar to the epidermis of other leaves (Fig. 2A). The uninfected area of healthy leaf contains approximately 450 stomata and 2 trichomes per mm^2. The narrow aperture of each stoma in a close condition is approximately 16.5 μm in length and 12.5 μm in width. The size of neighboring cells surrounding the stoma is close to that of the stoma. The packing of epidermal cells and guard cells forms many continuously connected troughs.

Streaks and little cracks of young gall

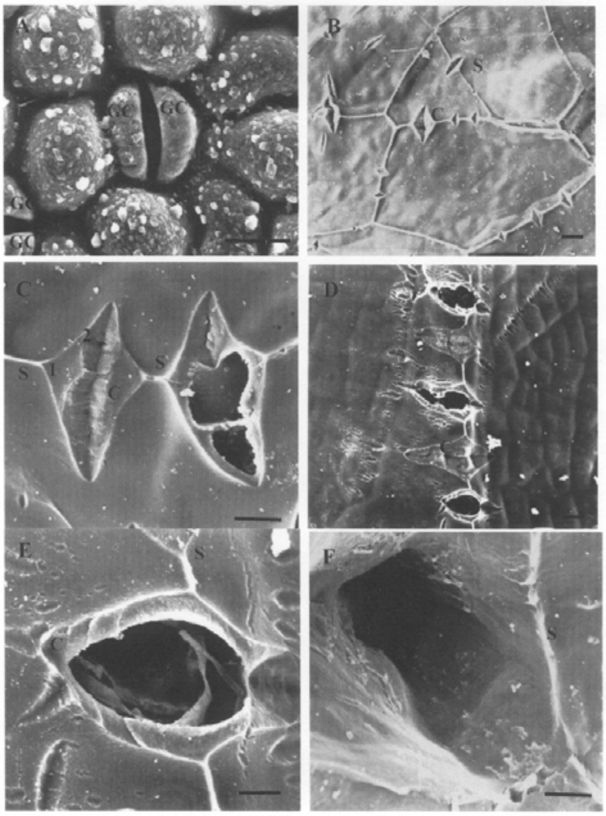

Figure 2. The epidermal morphology of cup-like gall at young and mature development stages on the lower epidermis of of *Litsea acuminata* leaf. A, lower epidermis of mature leaf; B, epidermis of young gall; C, D, E and F, epidermis of mature gall. Notes: C, crack; GC, guard cells; S, streak; T, trough. 1 and 2 indicate the first and second rip, respectively. Black bars indicate 10 μm.

Four points relative to the epidermis morphology of the cup-like galls, in spite ofdevelopment stages, are extremely different from that of regular leaf (Fig. 2). During the young development stage, no stomata, trough, or trichomes were found in the epidermis of galls at any growth stages, and the epidermis of the gall is much smoother than that of its host leaf. Line-like streaks form irregular networks with various geometric patterns (Fig. 2B). A series of little cracks are formed along the streaks and the axes of cleavage are perpendicular to its residing streaks. At the young stage, all cracks keep close and the space underneath the crack remains invisible. The sizes of little cracks of gall epidermis fluctuate between 16.5×6.7 μm^2 and 23.3×20.0 μm^2, bigger than the size of normal stoma in a closed condition.

Wide cracks and tunnel-like network of mature gall

As the cup-like galls grow older, usually 2-3 months after the young stage, the original little cracks on the streaks widen and deepen (Fig. 2C-F). Many new little fissures appear on the surface of gall epidermis, but it is still smoother than leaf. Hence, many wider and deeper holes or caves underneath the gall epidermis are exposed. The hollow hole can reach a size of 20.2x21.0 μm^2-24.9x17.1 μm^2. It seems that the rip process includes at least two steps; the first rip takes place at the young stage causing a little crack, and a second widening and deepening stage comes later. Therefore, two distinct fissure types can be identified (Fig. 2C).

The data indicated that there possibly exist certain very complicated tunnel-like networks underneath the epidermal surface of the pink gall. As the insect larvae grow older and bigger, they may require more exchange of oxygen and carbon dioxide between the growth chamber inside the gall and host tissue or surrounding gall tissue. If the air exchange is not sufficient, an extra mechanism may be needed to solve the problem.

It is unknown in the present observation whether or not this tunnel-like network is related to the air exchange function of the leaf stomata. Another possibility is that these structures have no role in air exchange or physiology, but just dead cells torn by mechanical or physical surface tension force. However, both require further exploration.

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一種 *Litsea acuminata* （Lauraceae）葉片杯狀蟲癭表皮氣孔之觀察

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摘　　要

本文以掃描式電子顯微鏡觀察 *Litsea acuminata* 葉上一種杯狀蟲癭的表皮之形態與結構。此蟲癭在任何生長階段，其表皮都無正常形態之氣孔，且其表皮都比葉表皮平滑。在幼年階段，其表皮出現網線，沿著網線分佈連串小裂縫，其大小介於 16.5×6.7 μm^2-23.3×20.0 μm^2 之間，此時看不到裂縫下之洞隙。隨著蟲癭增長，裂縫變更大更深，其大小介於 20.2×21.0 μm^2-24.9×17.1 μm^2 之間，此時其裂縫下之隧道狀網路洞隙完全曝漏。迄今尚不知道這些隧道狀網路洞隙是否與蟲癭光合作用之空氣交換或與癭內蟲呼吸有關。

關鍵詞：氣孔、表皮、杯狀蟲癭、*Litsea acuminata*、裂縫、隧道狀網路洞隙

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