Leaf Surface Fine-structures in Rice Plants Cultured Under Shaded, and Non-shaded Conditions*

Yoji Takeoka, Kiyotaka Kondo, and Peter B. Kaufman**

(Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464
**Department of Cellular and Molecular Biology, Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109, U.S.A.)

Received in April 30, 1983

According to Takeoka and Shimizu[1], epidermal tissues of the lemma of the rice plant were transformed in a manner similar to that of the foliage leaf at a time prior to their gross morphological change under the influence of spikelet proliferation. They showed in that paper that structural changes in the epidermal tissue are of significance for the development and adaptation of the rice crop to the surrounding environment. We therefore considered it necessary to study rice epidermal tissues from the following points of view: (1) structural changes in the epidermal surface and (2) tissue silicification. These points of view are important because the surface structure of plants is influenced directly by the external environment.

Waxy cuticle and epicuticular wax (EW) that cover the entire shoot portion of plant body[2] contribute not only to drought resistance[3,4,11,15,17] and frost hardiness[5] by water loss protection from the leaf surface, but also, to the "wettability"[20] of the leaf surface. So they play an important role in determining spraying efficiency of growth substances, and in protecting against damage by air pollution[2] and acid rain[7].

There are few investigations that have been carried out on hygrophytes (semi-aquatic species) like the paddy rice plant especially concerning the influence of environmental factors on EW structures, though numerous studies have been reported on mesophytes (upland species) and xerophytes (arid-or semi-arid species)[3,5,9,10].

The primary objective of this study is to make clear the difference in leaf surface fine-structures, especially the EW on shoot surfaces of rice plants cultured under shaded as compared with non-shaded conditions.

Materials and Methods

Three germinated seeds of paddy rice (Oryza sativa L. cv. 'Akihara'), seeded previously in a dark growth cabinet at 30 °C, were planted in each plastic pot (12 cm diameter) filled with washed sand, and cultured with the use of Kasugai's culture solution. Plants were grown in the greenhouse with natural light under two kinds of light conditions: restricted 80% by shading with cheesecloth and non-shading. Twenty-eight days after the beginning of culture, the fifth leaf blade were harvested from shaded and non-shaded plants at the stage of sixth leaf emergence. Five mm length sections of the leaf blade were cut at one-third position from the tip with a razor blade, and then fixed by modified Karnovsky's solution[11] for 12 hours. The fixed materials were dehydrated in ethyl alcohol and acetone, and dried with a critical point drier. The fixation and dehydration processes were undertaken at 4°C. Then, the dried pieces were mounted on polished brass stubs with bilateral adhesive tape, coated with gold, and stored in a desiculator until examination in the microscope.

Form and qualitative frequency of EW formed on epidermal cells and idioblasts in costal and intercostal zones of both sides of leaf epidermis were compared between

* An outline of this paper was presented at the 173rd meeting of the Crop Science Society of Japan, April, 1982.
shaded plants and non-shaded ones using a scanning electron microscope (JEOL, model JSM-F7) operated at 15 KeV.

Results

Upper epidermis: Among idioblasts arranged in the costal zone of the upper epidermis in non-shaded plants, cork-silica cell pairs (Fig. 1), and both types of cells (guard and subsidiary cells) composing the stomatal apparatus (Fig. 3) were observed to be covered by dense irregular sized fibrillar or platelet-like EW. Especially small papillae (Figs. 1, 2, 3) projected from the outer tangential walls of these cells were most densely covered by the EW. The long epidermal cells were also covered by the EW densely, but trichomes (Figs. 5, 14) and microhairs (Fig. 18), in contrast, were scarcely covered by EW except only at base of the trichomes. Even at the apertural ledge and inside the apertural separating the guard cells (Fig. 7), fragments of EW has been observed to form. In the costal zone, bulliform cells (Fig. 4) were also covered by the EW; however, the EW covering was relatively thinner than on outer surfaces of other idioblasts in the same locus of the leaf. Papillae on the outer surfaces of the guard cells were covered by the EW most densely (Fig. 3). In shaded plants, it was the same as non-shaded ones that papillae are most densely coated by EW. On the other hand, trichome and microhairs were scarcely covered by the EW. The most striking effects of the shading treatment on EW deposition appeared on cork-silica cell pairs, the stomatal apparatuses, and the long epidermal cells. Fig. 7 showed a thinner coating of EW on guard cells and subsidiary cells; as a result, the stomatal apparatus surface had larger areas of low electron density. Fig. 6 indicated that most of the surface area of the cork-silica cell pairs was devoid of the EW. Furthermore, the EW formed on surface of the shaded plant has changed its form more likely to platelet-like or rodlet-like (Fig. 9) comparing with non-shaded plant (Fig. 8).

Lower epidermis: The most striking feature in non-shaded plants was that large inflated papillae of long epidermal cells in the intercostal zone had a lack of EW at their heads, as shown in Figs. 10, 13 and 17. As in the upper epidermis, the small papillae (Fig. 11) were most densely coated by EW among the epidermal cells and idioblasts though cork-silica cell pairs (Fig. 11), stomatal apparatus (Fig. 12), and long epidermal cells also were covered densely by the EW. This nature of EW coating for the non-shaded plants was thus not different from that in the shaded plants. Therefore, it was seen that primary influence of shading on EW deposition was manifest in the upper epidermis as compared with non-shading. Figs. 15 and 16 showed a clear contrast with the upper epidermis in the dense covering of EW on cork-silica cell pairs and stomatal apparatus.

Discussion

This scanning electron microscopy analysis of rice leaf epidermis derived from plants grown under shading and non-shading conditions has revealed following features of surface fine structure, especially with reference to epicuticular wax formation: (1) a remarkable contrast was apparent in the EW formation that depended on the type of epidermal cell and idioblast; namely, plenty of EW was on cork-silica cell pairs, stomatal apparatuses, and long epidermal cells (especially on their papillae), and a lack of the EW was on trichomes and microhairs; (2) it was evident on both sides of leaves in shaded and non-shaded plants that intercostal zone cells (bulliform cells and long epidermal cells) were covered thinner EW or without EW at their heads, respectively, compared with costal zone cells; (3) in non-shaded plants no striking difference was seen in EW form and frequency between the same types of cells formed in the upper epidermis and in the lower one; (4) the influence of shading treatment on the EW formation was expressed more clearly on the upper epidermis than on the lower one, as indicated by a lack of EW or a thinner coating of the EW.

Many investigators have emphasized the
role of cuticular waxes in retarding water loss$^{6,14}$, and as a being an important feature of drought-resistant plants$^{14,15,17}$. For example, O'Toole et al.$^{17}$ concluded from their findings with two cultivars of rice, *Oryza sativa* L. cv. '63-83' (an upland adapted rice from West Africa), and cv. 'IR-20' (bred and selected in submerged paddy culture in Philippines) that high leaf cuticular resistance, resistance to water loss through the cuticle, indicates an important component related to the adaptation of plants to drought-prone regions. Removal of EW from rice leaves by a chloroform dip significantly reduces the cuticular resistance. Further, stressed plants have increased the cuticular resistance, and upland rice having drought resistance shows high cuticular resistance and a more complex wax structure and chemical components than paddy rice. Demna$^{6}$, in his cuticular transpiration experiments with seven glaucous and non-glaucous sibling lines of *Brassica oleracea*, reported that the presence of a waxy bloom was associated with a reduction in the rate of water loss by the intact plants. Daly$^{1}$ with population of *Poa coloni* has shown that those plants growing in regions of high precipitation are uniformly green while those growing in semiarid or summer-dry habitats are uniformly glaucous. He concluded that there were strong negative correlations between leaf surface wax of *P. coloni* and precipitation, and that there was a slight positive correlation between amount of wax present and mean temperature. Furthermore, Leyton and Juniper$^{15}$ revealed with Monterey pine (*Pinus radiata*) that pine needles show a marked resistance to desiccation by drought, possibly due to inhibition of cuticular transpiration by the thick wax covering over the cuticles of the needles. Judging from those investigations mentioned above, it is clear that differences in EW covering over cell surfaces due to the type of the epidermal cells and idioblasts in rice leaves may be attributed to differences in the transpiration among them. If so, then trichomes and microhairs would have the greatest amount transpiration from their surfaces, while papillae would leave the least. The fact$^{1}$ that trichomes have an important role in facilitating the uptake of externally supplied nutrients in solution also support this consideration. And then the result that the intercostal zone cells contrasting with the costal zone ones are covered by either thinner or no EW suggests that the intercostal zone of rice leaf has greater loss of water by transpiration than does the costal zone. This structural difference is associated with the fact that rice leaf rolling is caused by shrinkage of bulliform cells under conductive to wilting (drought).

Although much has been written of the nature of the EW, rarely has any information been reported on the variation in the frequency of the EW due to the cell type formed in the epidermis. Further we can

**Explanation of figures**

Fig. 1~Fig. 5 are scanning electron micrographs of upper leaf epidermis in *non-shaded* rice plants; Fig. 1. Dense network of wax covering cork-silica cell pairs and their neighboring long epidermal cells. x6, 500. Fig. 2. Dense network of plate-like wax covering small papilla on surface of cork cell of a cork-silica cell pair. x15, 500. Fig. 3. Dense network of wax covering a stomatal apparatus especially papilla on surface of a guard cell. x7, 300. Fig. 4. Plate-like structure of wax covering a bulliform cell. x27, 200. Fig. 5. Very sparse wax covering the surface of a trichome. x4, 100.

Fig. 6~Fig. 9 are scanning electron micrographs of upper leaf epidermis in *shaded* rice plants except Fig. 8 (non-shaded); Fig. 6. Very sparse development of wax network on the surface of cork-silica cell pairs and their neighboring long epidermal cells. x6, 100. Fig. 7. Sparse development of wax over surface of a stomatal apparatus. x36, 600. Fig. 8. Dense network of fibrillar and plate-like wax covering long epidermal cells in *non-shaded* plants. x27, 200. Fig. 9. Thinner covering of wax on the surface of long epidermal cells in *shaded* plants, as compared with that shown in Fig. 8. Note plate-like form of the wax contrasting with fibrillar one in Fig. 8. x27, 200.
not find any studies on rice plants in which comparison is made on the extent of transpiration between upper epidermis and lower one grown under restricted light conditions. However, several studies revealed that thickness of cuticle and EW development was positively correlated with light intensity; Tribe et al., worked with oat and barley, found that the total amount of cuticle was proportional to light intensity, although the specific pattern of cuticular lipid constituents was constant under different environmental conditions. In Brassica napus, a 60% restriction of sunlight gave the impression of a severe reduction in wax deposition, while wax produced in the 40% light treatment was very irregular as well as of smaller size and lesser in quantity.

Scanning electron microscopic analysis of rice leaf epidermis have been reported by Kaufman et al., and Maeda and Miyake. According to Kaufman et al., 1) the leaf adaxial epidermis is covered with waxy fibrillar material; 2) the waxy fibrillar coating is absent on the cork cells in between the paired silica cells but is present on the silica cells; 3) many small and large knobs covered the epidermal surface; 4) 2 to 4 small knobs were found on either side of the stomatal pore of the guard cells, and the knobs were absent on the bulliform cells. Maeda and Miyake noted from their investigation of the second leaves of paddy rice cv. 'Norin No. 8' and its chlorophyllless mutant that no essential difference was observed between green and albino plants in the surface structure of the leaf blade. The small and large knobs observed by Kaufman et al., are the same as small papillae and large inflated papillae, respectively, in this study. The knobs are also found to be present on the bulliform cells and cork cells of cork-silica cell pairs in this study. They are not different from the other types of epidermal idioblasts in being densely covered by the EW. The reason why our results differ from those of the above workers with respect to cork cells and bulliform cells is unknown, though fixing procedure and rice cultivar they used vary from ours; they froze the mature leaves of cv. 'Colusa' in liquid nitrogen immediately after cutting them, and then dried them at 0°C in an automatic freeze-drier.

Acknowledgement

The present work was partially supported by a research Grant-in-aid from the Ministry of Education, Japan (No. 36480029). The authors wish to express their thanks to Dr. T. Wada, Nagoya University, for his discussion and advice on this subject.

Fig. 10~Fig. 14 are scanning electron micrographs of lower leaf epidermis in non-shaded rice plants; Fig. 10. The intercostal zone showing the long epidermal cells with large inflated papillae on the surface. Note that heads of the papillae lack EW wax. x1, 100. Fig. 11. Dense network of wax covering cork-silica cell pairs. x4, 100. Fig. 12. Dense EW network on outer surface of a stomatal apparatus, even adjacent to the stomatal aperture (closed). Two papillae show even more dense EW. x4, 100. Fig. 13. Lack of wax on the rounded head of a large inflated papilla of the long epidermal cell in Fig. 10. x4, 100. Fig. 14. Lack of wax on the surface of trichome (prickle hair). x1, 200.

Fig. 15~Fig. 18 are scanning electron micrographs of lower leaf epidermis in shaded rice plants; Fig. 15. Dense network of wax covering cork-silica cell pairs and their neighboring long epidermal cells. x4,000. Fig. 16. Dense network of wax covering a stomatal apparatus and its neighboring long epidermal cells. x4,000. Fig. 17. Lack of wax on the oblong-shaped head of large inflated papilla on the long epidermal cell in the intercostal zone. x2, 400. Fig. 18. Lack of wax on the surface of microhair. This contrasts with a dense covering of wax on its neighboring small papilla. x8, 500.

Abbreviations

References


* In Japanese with English summary.
【和 文 摘 要】

遮光および無遮光条件下におけるイネ葉身の表面微細構造

武 岡 洋 治・近 藤 清 貴・PETER B. KAUFMAN*
（名古屋大学農学部，*米国ミシガン大学生物科学部）

作物の恒常的な発育を促す上で外気に関与する表皮系組織の果たす役割は大きい。表面構造の変化と結実化の面からこの組織の構造と機能を明らかにする必要があると考え本研究を行った。水稻葉身の表面構造をとくにエピカチファックス（EW）の形態と構造ならびに遮光による影響を走査電子顕微鏡で観察した。水稻品種 “秋晴”を春日井氏栽培液で砂耕し、播種後28日間寒冷処理による80%遮光処理を行った。別に無遮光区を設けて対照区とした。自然日長ガラス室内で栽培し、第6葉展開期に第5葉葉身の先端から1/3の部分を5mmずつ切り取って改良カルバソースキーボーで12時間 Dancingした。アルコール・アセトン脱水、臨界点乾燥ののもつコーティングを施し、JSM-F7型走査電子顕微鏡で加速電圧15KeVで葉の上面（向軸側）と下面（背軸側）を観察した。

上面表皮：無遮光区葉身の顔上部では、コルナー細胞細胞，孔辺細胞，下部細胞のいずれも，表面が不規則な大きさの繊維状ないし小片状のEWが密に覆われていた（第1，3図）。これらの細胞中乳頭状突起の部分はEWがとりわけ密であった（第1，2，3図）。他方毛と無毛では果実基部以外はEWが認められなかった（第5，14，18図）。隔壁部の細胞でもEWの形成が密であったが，機動細胞では他の細胞に比べてやや薄であった（第4図）。この部位でも乳頭状突起のEWは最も密であった。遮光区葉身では全体にEWの形成が悪く，電子密度の低い部分が多く見られた（第6，7，9図）。この変化はコルナー細胞細胞（第6図），孔辺，下細胞（第7図），表皮長細胞に著しく現われ，EWも小片状のものが多くなった（第7，9図）。

下面表皮：無遮光区の顔上部でEWは上面表皮と同様な形成が認められ，乳頭状突起に最も密にEWが見られた（第11，12図）。隔壁部では長細胞の大きさ突起部はEWを欠いていた（第10，13図）。遮光区葉身も無遮光区と同様にEW形成が良好で，下面表皮では遮光の影響はほとんど見られなかった。

水稻葉身表面でのEW形成と遮光の影響が場所により細胞により異なることを明らかにし，これに若干の考察を加えた。