PATHOLOGICAL STUDIES ON RICE BLAST CAUSED BY PIRICULARIA ORYZAE

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PART II. ON THE MODE OF INFECTION OF THE PATHOGENE

I. Structure of Epidermal Tissue of the Rice Plant

Observation of External Form

The materials used in the experiment included early and late growing Jinriki, Asahi, Togo, No 132 Rikuu, and Senho rice plants. These leaf blades, sheaths, and stems were observed fresh or after affixation. The epithelium was sometimes observed as is or by obtaining an ash image of a section of it. On the upper surface of the leaf blades, there were long cells in the form of long rectangles arranged vertically outside of the assimilatory tissues. The edge was undulante with many papilla-like silica processes on the surface. The existence of stinging hairs and rather pliant slender hairs were observed between the long cells. The stomata were in vertical rows which are commonly found in several rows each on both sides of the vascular bundles. The epidermal cells in front and back are short rectangles. This structure is the same as the long rectangular cells (Fig 1). The long and short epidermal cells were generally rectangular when observed in cross section and came in direct contact with the assimilatory tissue underneath. On the outer edge of the vascular bundle, there was a row of highly silicified dumbbell-shaped cells
Fig 2A). This row of cells is located directly above on the outer edge of the small vascular bundle, but on both sides of the large vascular bundle and the part directly above, the vascular bundles consist of rows of large sting hair cells instead.

The special structure on the surface of the leaf blade is the row of motor cells which have developed on the surface of the center part of the assimilatory tissues (Figs 3 and 4).

Fig 1. Reverse surface of leaf blade (Early Jinriki)
  a. stoma; b. papilla-like silicate; 
  d. small sting hair; e. long cells; 
  f. slender
Fig. 2 A: Dumbbell-shaped cells (right side)

Fig. 2 B: Papilla-like silicate processes and cuticle layer.

Fig. 3: Surface of motor cells. Black spots correspond to p in Fig. 4.

Fig. 4: Cross section of motor cells. s. silica layer, p. pectin layer, l. lignin layer.
The upper and lower surface of the leaf when seen in a vertical cross section, indicate a condition in which several long rectangular cells are gathered together but a horizontal cross section shows the gathering of several pocket shaped cells, the ones in the center being larger and those away from the center gradually becoming smaller as both edges are approached, the latter usually being connected to the rectangular epidermal cells. Among the motor cells, the largest ones in the center displayed marked silicification over the entire walls. This should be detected in various spots in young leaves at tillering time (plate 1: A, B). The vessel terminal at the tip of the leaf blade and is a water spore. Usually, one should detect many non-pathogenic microorganisms in this area. The leaf tongue and ear [literally] grow at the base of the leaf blade and are wrapped around the succeeding cormus. The leaf ear is at the tip of the sheath with brachiat-like extensions to the left and right, and surrounds the new cormus which later emerges. The epidermis is formed from long cells which are slow in thickening. It is smooth and lacks both processes and stomata. However, coarse, long needle-like sting hairs have grown on the top part of the perimeter which has become free. The tongue is located intermediate of the base of the two ears and is a triangular organ which usually comes in contact with new cormus. Although the inner and outer tissue is quite similar to that of the sheath, it lacks assimilatory tissue and stomata and the epidermis is a smooth, thin membrane. That part which moves toward the sheath of the leaf blade, i.e. the petiolule, is deficient in chloroplasts and is a banded part. There are no processes on the outer surface of the epidermis and almost all inner and outer organs consist of young tissue. It is apparent that the petiolules, "ear" and "tongue" have a structure that is easily attacked by *pirlicularia oryzae*.

Although the epidermis on the outer surface of the sheath (Fig. 5), is in general similar to the reverse surface of the leaf blade, the cell walls are very thick, lack silicate processes, have a reduced amount of dumbbell shaped cells and sting hairs, and the stomata are completely closed off (Fig. 7, 5, 6, 7).
Fig. 5: Outer surface of sheath (Early Jinriki).

a. stomata, b. long cells, c. silicified short cells, d. small hairs.

There were many silicified short cells in the connecting part of the long epidermal cells or, in some instances, slender hairs grew there. Near the node section, the long cells gradually became smaller and there was a gradual increase in the rate of occurrence of silicified short cells in a specific area. Frequently the silicified short cell region is seen, or the so-called cork cells which adjoin it. While these are cells which have likewise become highly silicified, they show a marked relic of cutin on account of the depressions on the upper surface, and they are not suberised cells. The epidermis on the inner surface of the sheath is much simpler than the structure of the outer surface. It lacks hairy processes, is covered by long, smooth, thin, membrane epidermal cells and, while having stomata in places, it lacks motor cells. Many of the stomata occupying the central part of the inside of the sheath are closed but there are many open ones in the section close to the perimeter. (Fig. 6, 6). Neither of them has an opening and closing function.

The epidermal structure of the stem in general is similar to that of the outer surface of the leaf sheath (Fig. 9), and the membrane wall thickening is generally quite stronger. It lacks motor cells, large sting hairs, and dumbbell shaped cells. There
is considerable wall thickening of the stomata in both the closed cells and the subsidiary cells and none of them was observed to be open.

In the section, the surrounding vascular bundle directly beneath the epidermis recedes towards the inside together with part of the thick membrane epidermal tissue and, as in other parts of the stem, it was difficult to observe the straight lines of the vascular bundle from the outer section as well as the residual hypodermal thick membrane. Tissue gradually decreases in thickness and is very smooth when seen from the outside.

The spikelets on the nodes of the spikes were usually branched. The base of the ear, moreover, includes scaly bracts. Also, many hairs presenting an antler-like configuration grew at the branching points of all spikelets. It is thus believed that this structure facilitates the attraction of pathogenic spores in the node section of the spikes.

In the varieties of rice used in the experiment, although the tissues on the leaf stomata in some cases were observed to be slightly opened during the early growth period, most of them were closed. Those which had been observed to have opened, the closing function was observed when inspected at 10 A.M. (Figure 6, 1, 2). To summarize the examinations made at 10 A.M. and 9 P.M. of the stomata on the blade of the rice leaf which had already grown (branching off period), the stomata in general were almost or completely closed and it was observed that they completely lacked the opening and closing function (Fig. 6. 3, 4, 5; Fig. 7. 1, 2, 3, 4). They were completely closed as far as the stomata in the stem and outer surface of the leaf sheath. (Fig. 7. 5, 6, 7; Fig. 9a). However, the stomata in the perimeter section of the inside of the leaf sheath and the neighborhood of the growth line of the cormus were finally beginning to open (Fig. 6, 7, 8).
Fig. 6: Stomata of each part of rice plant (I)
1, 2: stomata at tillering time for young plant.
3, 4, 5: stomata at tillering time for matured plant.
6: stomata on perimeter of inside of sheath.
7, 8: stomata in vicinity of young tissue of un-tillered leaf base of matured rice plant.
3, 4 are the same stomata; 3 is outer and 4 inner surface.
7, 8: likewise the same; 7 is the outer and 8 is the inner surface.
Fig. 7: Stomata of each part of rice plant (II).
1-4: stomata in adult tillering leaf.
5-7: stomata on surface of sheath.

In other words, while opening and closing of stomata fissures in young rice plants is observed, after maturation the stomata in the leaf sheathes and others generally close and lose their opening-closing function. The distinction between young and mature stages should be clear from the appearance of the brachial-like walls of the assimilatory tissue cells.
While the plants in the young stage usually appear as multilateral on the cross section of a leaf blade, those after maturation are brachial. It can be said that the appearance of brachial cells indicates the transverse growth accompanying the longitudinal growth of each leaf cell. This brachial shape can be explained by loosening as a result of the difficulty of transverse elongation of width due to the various motor tissues. Thus, this transverse loosening should be found not only in assimilatory tissue but in soft cells in general and can be explained as the result of the growth of undulant edges on the short and long epidermal cells on the leaf surface. This is explained by the fact that the stomata are thus compressed from the edge as they grow thicker and horizontally looser, and finally the fissures are closed or their opening-closing function is so highly restricted that they reach the point where it is impossible to see them open or close.

Microscopic Observations

Although the existence of cutin can be confirmed in the elongation and growth in the epidermis of a rice leaf, it is difficult to detect this in the plant after maturation, or it is broken in places. Especially in those which have produced papillary
silicate processes, it was only observed slightly in the smooth part of the base (Fig. 2B). While the lignin coloration reaction is generally clear at first in the epidermal tissue, it is especially marked in the section which forms the thick membrane. Thus there is an increase in the accumulation of silica in the outer epidermal wall several days after emergence of the leaf stem. Plate 1; A, B, C, is an ash image of the abverse and reverse of a young leaf at tillering time and of a grown leaf. In the upper layer of the accessory cells of the stomata section and the upper layer of the closed cells, the silica accumulation is slightly retarded, or the silica layer is thinner than in other sections (based on ash image of cross section). The lignin coloration reaction is also generally marked.

The outer surface of the epidermis of the motor cell section has a special structure (Fig. 4). While the outermost layer becomes a silica layer abundant in papillary silica processes, as is the case with the other sections, there are cases where there is an especially thin wall on both sides of the upper epidermis of the center pocket-shaped cells. Also, while the lignin reaction is pronounced inside the upper epidermis of other tissue, the pectin reaction is marked on the inside of the upper epidermis of the center purse shaped cells. Moreover, the innermost layer is sometimes silicified and sometimes plainly attached by ligno-cellulose to the inner wall. This type of structure should be observable in leaf blades of rice even after propagation and death.

In other words, generally, the upper epidermis on the outer part of the assimilatory tissue in the leaf blade has a thinner silica layer than the upper epidermis of the outer perimeter of the vascular bundle, especially in the upper epidermis of the cells constituting the stomata, and the upper epidermis of the motor cells is generally less silicified and its rate of silica accumulation is seen to drop. This is especially true with young leaves at tillering time. The upper epidermal wall of the leaf sheath and stem is generally thicker than the leaf blade and the lignin reaction is extreme (Fig. 9). Among them, however, only the slender hairs are not markedly thickened. The upper epidermis of the emerged part of the stem has a gradual accumulation of silica which is much greater than on the leaf blade. The epidermis of the node is only slightly silicified, while lignification on the other hand, is very pronounced.
II. Inoculation with *Piricularia oryzae*.

**Method of Experiment**

This experiment was repeated several times from 1931 to 1935. It is an inoculation test on leaf blades and the base of the ear-Hokubi. The materials used were early growing Jimriki and No. 132 Rikumi. Now to summarize the method of testing the above. Principally young leaves were selected during the sprouting stage, just after tillering, (sometimes old leaves were used after emergence of spikes). The leaves were taken with the trichomes still on them, placed in contact (obverse or reverse surface) with *Piricularia oryzae* prepared beforehand and removed after three days. Then they were
fixed in an alcohol solution of formalin acetate, kept for several days in an aquatic solution of hydrogen fluoride after washing in water, an attempt thus being made to remove the silica. Later a paraffin cross section was made and dyed with various dyes such as Methyl green / Acid fuchsin, Methyl green / Ruthenium red, Gentian violet / Safranin, and examined under the microscope. Sometimes a suspension of fungus spores was painted on and used for the experiment. The stems of Rikuu No. 132 and early Jiryoku were used together.

The stem was divided into the coarse part which had already emerged and the part which was about to emerge. These were then cut to a suitable length and immediately placed in contact with a suspension of previously prepared *Piricularia oryzae* and kept in this condition for four days. They were fixed in the same manner as the leaves above and then 20 days were spent in treating them to remove the silica. Then 6-12 μ thick paraffin cross sections were made and dyed in the same manner as the leaves.

**Observation**

When the *Piricularia oryzae* comes in contact with the epidermis an appressorium should grow there. The size of the appressorium is not fixed. Its free surface forms a generally globular shape and forms a thick brown membrane. It generally invades from the undersurface of the appressorium. It apparently can invade, but with relative difficulty, from all parts in a young leaf excepting the outer perimeter of the vascular bundle. According to results of observations made 3 days after inoculation, it apparently invades the upper epidermis of the motor cells most easily. (Fig. 10, 11)
Fig. 10: Invasion by piricularia oryzae I (Early Jinriki)
1, 2 invasion of stomata auxiliary cells; 3, 4 invasion of short cells; a. appressorium, b. invasion hypha, c. swelling of inner wall, d. small vesicle, e. hypha inside.

It generally adheres to these regions first, sends out very slender invasion hyphae almost perpendicular to the surface of adhesion, from about the center of the appressorium under the surface. The invasion hypha passes through the upper epidermids, finally reaches the inside of the epidermal cell and is observed to grow a small vesicle here. The inner walls of the invaded cells usually swell in the vicinity of the wall of the section invaded by the hyphae. Within the scope of this experiment, however, no case was observed where swelling prevented the epidermal cells from being invaded. That is, in those parts where the inner wall was swollen, tips of invasion hyphae had already reached the inner center of the swollen parts and small vesicles had formed. Internal hyphae then grew from the vesicles, extending into the invaded cells. They then apparently extend into neighboring epidermal cells or invade the internal assimilatory tissue (Fig. 12).
Fig. 11: Invasion by *piricularia oryzae* II (Early Jinriki).
Invasion of motor cells.

It apparently becomes very difficult for this fungus to invade a grown leaf several days after tillering (this refers to a complete leaf). No invasion was detected on any part of an adult leaf (after the spikes had emerged) 3 days after inoculating its surface.

Fig. 12: Invasion by *piricularia oryzae* III (Early Jinriki).
Extension of hyphae after invasion.

It appears that the most easily invaded portions of the stem are the scaly bracts and the stem epidermis near the region where the spikes branch out at the node. Figure 13.4 shows the upper epidermis of the bract in the process of being invaded. While it cannot be judged that invasion of this section will cause inflection of the "neck" of the rice-plant by extending as far as the base of
the ear if this section is diseased at the time of emergence. As for the stem itself, it is apparently invaded with much more difficulty than a young leaf and invasion frequently stops by the mere formation of an appressorium on the thick upper epidermal membrane. Nevertheless there were a few cases of invasion through the upper epidermis (Figs. 13, 14, 15).

Very slender invasion hyphae are formed at the time of invasion which penetrate the upper epidermis and reach the interior of the cells. Small vesicles are formed as in the case of the leaves. Fig. 15.3 shows the behavior of this fungus growing on the fissures of the stomata near the section where the first small spikes branch out from the base of the ear. While appressoria became attached to the top of the closed cells, invasion hypha did not grow. The stomata clearly indicated a deterioration of the cell walls between a and b. Seen from above, it would appear that invasion from the stomata fissures should be observed, but in reality this does not occur. Invasion is apparently even more difficult in stems which have already emerged, as no invasion whatsoever was observed even four days after inoculation.

Fig. 13: Invasion of *Piricularia oryzae* IV (early Jinriki).
1-3, invasion of young weak spikelets during swelling period; 4, invasion of scaly bracts in base of ear-Hokubi during period of emergence of the ear.
Fig. 14: Invasion of *piricularia oryzae* V (Rikuu No. 132)
Invasion of epidermal wall above base during period of emergence of ear.

Generally the protoplasm in invaded cells expands first, its existence is clearly shown, and its chromaticity apparently increases. At the same time, even the cell walls deteriorate. The pathogenic hyphae branch out and the branches which reach the walls on the other side penetrate the latter and extend into the neighboring cells.

Fig. 15: Invasion by *piricularia oryzae* VI (Rikuu No. 132).
1, 2: the vicinity of the point where the spikelets branch out at period of emergence of ear.
3: growth of appressorium on upper part of stoma of spikelet as above. Although hyphae did not grow, the cell wall between a and b deteriorated.
III. Discussion.

The first person to present empirical evidence of the means of invasion of a host by *piricularia oryzae* was Matsuura Yoshi (1). At about the same time Sueda (9) obtained the same evidence and then Ito and Kuribayashi (3) corroborated Sueda's hypothesis. Moreover, Ikata, Matsuura, and Taguchi (2) further corroborated the results obtained by Matsuura. Now to sum up the results obtained empirically. While Matsuura and Ikata merely observed the penetration and invasion of the epidermis, Sueda, Ito, and Kuribayashi found that while the epidermis is generally penetrated and invaded, rarely can the stomata be infected from the hyphae themselves.

Moreover, Nagai and Suzuki (8) and Imamuma (7) found a close interrelationship between the resistance to *piricularia oryzae* in the nodes of the rice leaf and the number of stomata on them, and hypothesized (7) that those with a large number of stomata had a greater chance of being invaded through the latter. According to them, moreover, there is a greater density of stomata in dry-land rice than in water-grown rice. Suzuki (10, 11) in opposition to this, states that there is no greater difference in the number of stomata in grown rice leaves whether irrigated or on dry land. However, a high rate of attack by *piricularia oryzae* was observed in dry areas in the results of the inoculation experiment. Suzuki thus assumed that variation in the number of stomata has no great influence on the outbreak of *piricularia oryzae*. He makes no reference, however, to the possibility of invasion of stomata.

As explained previously, the stomata in a leaf during the tillering period of grown rice are almost or completely closed and the opening-closing function is seen to be lacking. It is also observed that the outer surface of the fissures, judging from their structure, is very unsuitable for the adherence of the undersurface of an appressorium. Therefore, even in cases where an appressorium focuses on the upper part of a stoma, the invasion hypha grow directly over the fissure and this is explained by the fact that it is next to impossible for a hypha to invade the inner part from the fissure section which is almost completely closed. This is why it was impossible to discover any invasion when a slide section was observed. This is explained by the fact that the stomata on the epidermis of the leaf are closed even more tightly and invasion from this section becomes increasingly difficult. We must therefore, conclude that invasion of the host by *piricularia oryzae*, at least as far as water-grown rice is concerned, can occur only through penetration of the epidermis.
The author discovered that in the invasion of a leaf by penetration of the epidermis, there were many cases where the upper epidermis of the motor cells had been invaded and he concluded that the latter is one of the areas most easily invaded (12, 13). This fact is also reported by Hayashi (1) Ito and Hayashi (4) and Ito and Shimada (5). As already explained above, the upper epidermis of the motor cells has a special structure and while there is a gradual deposition of silica on the upper surface of the upper epidermis, it is generally slow, and the major accumulation is on the upper surface of the pocket-shaped cells and ligum not infrequently exists for a long time on the upper surface of both sides. Moreover, the inside part of the upper epidermis of the center pocket-like cells had a marked pectin reaction and was not observed to lignify. Therefore, as soon as the appressoria of the *pyricularia oryzae* attach themselves to these parts, it is observed that they can easily invade the inside part. Thus, as long as all the walls of the motor cells of the invaded section do not become highly silicified, it is seen that invasion hyphae can easily extend themselves fully into other tissue. Although the author stated (13) that invasion should be possible between cells from the sides of the slender tri- chomes in the base section of the ear-Hokubi, this was not observed in the experiments and this statement should thus be rescinded here.

While it would be premature to discuss the problem of where the *pyricularia oryzae* can most easily invade from the inside of the epidermal tissue, except as regards the motor cells which were invaded with especial severity, in view of the fact that no measurements were made in these experiments, and as the results are based on study of cross sections only, the considerations given below concern the total results of observation. In the leaf, the motor cells and the upper epidermis of the accessory cells and stomata should occupy the first position. As for the other tissue, it is apparent that at tillering time, invasion is possible from any part of the leaf blade where the appressorium has adhered, except for the dumb-bell shaped cells which have already hardened considerably at tillering time, and the sting hairs and silica processes. It also seems that in the vicinity of the base of the ear-Hokubi the long cells of the bract or stem near the point on the node where the spikelets branch out in the area which is most easily invaded.

As for the shape of the infection hyphae, Ikata et al (2) and Ito et al (5) state that they have a wedge shape, but when observed in cross section they present a fine thread shape. A small vesicle is first formed at the point inside the cell that is reached
by the invasion hypha. The latter then branches out or extends without branching, ultimately forming an extremely long phypha.

IV. Summary.

This report is on a study of the inoculation of rice tissue with *piricularia oryzae*. For the purpose of supplementing the results of this experiment, an examination was first made of the form of the epidermal tissue of the leaf blade, stem, sheath, and of the microscopic characteristics. According to the results of inoculating the surface or reverse of a young leaf at tillering time with *piricularia oryzae*, the fungus forms at first an appressorium on the host. The host is invaded when brought in contact with the disease, except when the latter touches the very hard dumb-bell shaped cells on the upper epidermis, the various hairs or the silica processes. Also, it is apparent that the motor cells and the accessory cells of the somata are the most easily invaded. According to results of inoculating the base of the ear-Hokubi just at earing time, the fungus entered, just as in the case of the blade, but with difficulty outside of the highly hardened parts.

The long cells on the outer surface of the assimilating parenchyma of the stem near the branching point of the spikelets, and the long cells of the bracts projected near the nodes of the ears are apparently infected rather easily. Infection is apparently impossible through the leaf stem from the fissures of the stomata.

Penetration begins with the emergence of slender penetration hyphae on the surface where the appressorium has become attached to the host. This pierces the upper epidermal tissue and enters the epidermal cells where swelling takes place and small vesicles are formed. After this, branches may be formed from the vesicle and hyphae formed inside, or the hyphae may extend without branching and extend into the neighboring epidermal cells or to the inner assimilatory tissue cells.
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EXPLANATION OF PLATES

A and B. Image of young leaf of adult rice plant at tillering time.

A is an ash image of the obverse surface of the leaf blade. The silica layer on the outer most surface of the upper epidermis is very thin and broken in spots. In the stomata section especially, no silica membranes remain and the black spots which are seen in the center of the plate are the center pocket shaped cells of the remaining motor tissue.

B is an image of the reverse surface of the leaf blade. An ash image was made after stripping away as much of the obverse surface as possible. In the stomata section especially, the dropping off of silica membranes can be observed.

C is an image of the reverse surface of a blade of fully grown adult rice.

Complete silicified portions remain in the center pocket-like cells of the motor tissue. The stomata are also silicified, although the membrane is very thin.