ON THE INTRACELLULAR BODIES ASSOCIATED WITH THE DWARF DISEASE OF RICE PLANT

By

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(With 5 Text Figures)

Since KUNKEL (8) demonstrated in 1921 the intracellular bodies associated with the corn mosaic and suggested that these bodies represent living organisms, the possible causative agents for the mosaic disease, a considerable number of publications have accumulated, dealing with the cytological aspects of the virus disease of plants. Consequently the occurrence of similar cell inclusions has been shown in mosaic diseases of tobacco (2, 3, 7, 11, 18, 21), tomato (5), pepper (5), potato (5, 25), *Petunia* (5, 23), *Datura* (23), and some other Solanaceous plants (5, 24); likewise in sugar cane mosaic (1, 11), wheat mosaic (14), Chinese cabbage mosaic (11), *Hippeastrum* mosaic (6, 11, 15), *Phytolacca* mosaic (23), *Dahlia* mosaic and dwarf (4), infectious chlorosis (in BAUR's sense) of *Euonymus japonicus* (23), Fiji-disease of sugar cane (10, 12, 13), and in spike disease of sandal (17).*

As to the nature of these intracellular bodies, there exists a remarkable difference in opinon but the general belief among plant pathologists seems to be in favor of the view that these bodies are the reaction products of the host cells rather than the causal agencies of the disease. Whatever their exact nature may be, it is evident that they are of diagnostic significance, because similar bodies have never been found up to the present time in assosiation with any plant diseases other than viroses.

In the course of studies on the dwarf disease of rice plant, the writer succeeded in revealing the presence of intracellular bodies in the cells of the diseased leaves. This will be the first record of the occurrence of intracellular bodies in association with the streak type of virus diseases of plants including

* In all probability neither SCHAFFNIT-WEBER's elytrosoma associated with the mosaic diseases of beet and broad bean (22), nor PLACKIDAS' bodies in the strawberry yellows and dwarf (19, 20), falls in the same category with the intracellular bodies above mentioned.

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the streak disease of corn and sugar cane (27, 28), the corn stripe disease in Cuba (26), the dwarf disease of rice plant, and others.

Description of the disease

No attempt will be made herein to describe in detail the dwarf disease of rice plant, as a more detailed account will be given in another publication presenting the results of the transmission experiments and discussions on the etiology of the disease.

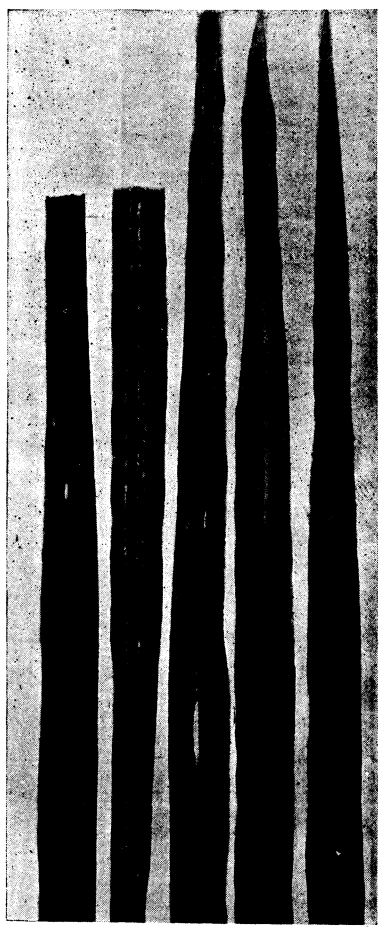

Fig. 1.
Diseased leaves, showing the typical streaks along the veins.

The dwarf disease of rice plant is characterized by small elongated chlorotic areas, or white specks when viewed by transmitted light, arranged along the veins of leaves, and by stunting of the whole plant followed by excessive tillering and a dark green coloration. The white specks which develop before the leaves unfold, elongate and spread out along the leaf parallel to the midrib, forming fine interrupted streaks. These range from mere dots to several millimeters in length and from 0.2 to 1 mm. in width. The plants infected in their very early stage of growth, become severely stunted and produce none or only a few small worthless panicles.

The disease is transmitted by the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL. but not through the seed or the soil. All attempts to transmit the disease by mechanical inoculations with unfiltered juice of the diseased plants or by means of leaf mutilation have failed.

The intracellular bodies

Certain intracellular bodies were revealed both in fresh and embedded

material of leaves of the rice plant affected with the dwarf disease. For fixation of the material several fixatives were used including medium chromo-acetic solution, Flemming's fluid, Merkel's fluid, Allen's modification of Bouin's fluid, Gilson's fluid, Zenker's fluid, acetic alcohl, formol-acetic alcohl and Schaudinn's fluid. Chromo-acetic solution and formol-acetic alcohol were considered most suitable for general purposes. The fixed material was embdded in paraffin and sectioned by means of a microtome in the usual way. Transverse and longitudinal sections were cut 5 microns thick and stained with Heidenhain's iron-alum haematoxylin or safranin and Gentian violet. From the protozoological viewpoint, some of the material was fixed in Schaudinn's fluid and stained with Giemsa's stains.

Microscopic studies of free hand sections of the diseased leaf show chlorotic modifications in the mesophyll cells adjacent to certain of the vascular bundles. In sections mounted in water, the chlorotic tissues are lighter in color or nearly colorless, the chloroplasts in these cells being light colored and smaller in size and number, due to their disintegration. In the cell where most of the chloroplasts have been disintegrated, a certain foreign body is found in close proximity to the nucleus. Such foreign bodies are usually round in shape, more or less larger than the host nucleus and contain many vacuoles of various sizes. These bodies stain yellow in dilute iodine potassium iodide solution, brick red in aceto-carmin, Grenadine pink (after RIDGWAY) in the alcoholic solution of Sudan III, and pinkish cinnamon in Millon's reagent.

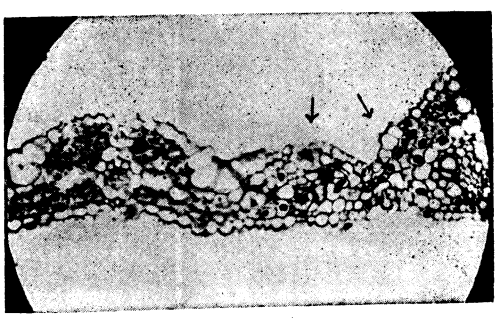

Fig. 2.
A portion of a cross section of diseased leaf showing the chlorotic tissue. (Fixed in chromo-acetic solution and stained with safranin.)

However, more comprehensive studies of the intracellular bodies can be done in the microtomed sections properly stained. In the preparations stained with Heidenhain's haematoxylin, the chlorotic areas are easily recognized since the chloroplasts in these tissue show less affinity for the stain, and they are, according to the degree of chlorosis, smaller and less regular in shape and

fewer in number than those in the apparently healthy tissues of the same leaf. Haematoxylin brings out the intracellular bodies very sharply, since they take

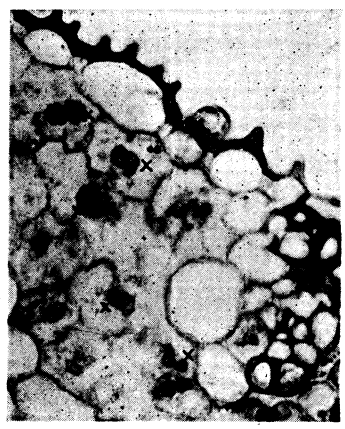

Fig. 3.

Fig. 4.

Intracellular bodies marked × in the mesophyll cells of a diseased leaf. (Fixed in chromo-acetic solution and stained with safranin and Gentian violet.)

the stain as intensely as the host nuclei. When preparations are stained with safranin and Gentian violet, the bodies show a special affinity for safranin while Gentian violet stains the host nuclei violet in color. The foreign bodies under consideration occur singly in the host cells but occasionally two of them are found in the same cell. They are more abundant in the mesophyll cells but it is not unusual to find them in the epidermal cells. The bodies are located near or in close contact with the host nucleus which sometimes becomes more or less enlarged. They vary considerably in shape and size. Round to oval are the most usual forms, but it is not uncommon to find bodies rather irregular in shape. In size they range from 3 to 10 microns in length and 2.5 to 8.5 microns in width, frequently much larger than the

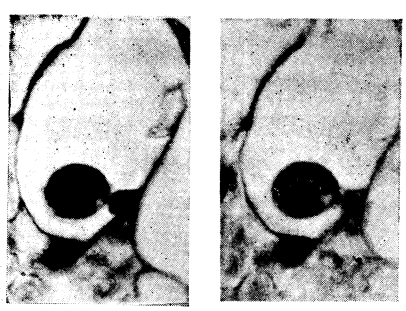

Fig. 5.

An intracellular body in the motor cell photographed at different optical levels to show the host cell-nucleus and the vacuoles in the body. (Fixed in formol acetic alcohol and stained with safranin and Gentian violet.)

host nuclei which are 2.5 to 3.5 microns in diameter. The bodies must be much smaller in their earliest stage of development but in such a case it is rather difficult to distinguish them exactly from the cellular elements of the host cells. The contents of the bodies appear to consist of a somewhat homogeneous structure, surrounded by an indistinct membrane, and contain many vacuoles of various sizes. All attempts to demonstrate a nucleus or chromatin granules in these bodies were unsuccessful. Although these bodies occur invariably in the chlorotic areas of the leaf, as yet they have not been found in the intact tissue of the diseased leaf or in plants free from the disease. The bodies in question are not artifacts, since they can be found in the cells of material studied in the living condition, as well as in material fixed by various method and stained with various stains. It is also evident that these intracellular bodies are neither deformed or degenerating nuclei, nor hypertrophied chloroplasts of the host cells, although their exact nature remains still unknown. Beyond doubt they are similar to the intracellular bodies which have been found in association with certain virus diseases of plants and animals.

Summary

Certain intracellular bodies are invariably present in the chlorotic tissue of the leaf of the rice plant affected with the dwarf disease. These bodies occur near or in close contact with the host cell nucleus. They are usually round to oval but occasionally irregular in shape and much larger than the host nucleus. They are of rather homogeneous structure containing many vacuoles of various sizes and resemble the intracellular bodies in association with certain virus diseases of plants and animals.

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摘　　要

萎縮稻組織內に發見せらるゝ細胞包含体に就て

福　士　貞　吉

萎縮病に冒されたる稻は新葉の表面に、葉脈に沿ひて黃白色の微細なる斑点を生ず、此斑点は葉の未だ展開せず葉鞘中に包まるる時より存在し、反射光線にて見る時は黃綠—黃色を呈するも、病葉を日光にかざして見る時は黃白色—白色の斑點となりて現はる。斑點の大さ0.2-0.5×0.3-3mm. 單獨に散在するものあるも多くは斷續して條線をなす。かゝる葉の切片を檢鏡するに病斑に相應する部分は維管束に隣接する組織にして色彩淡きか無色なるを以て容易に識別し得。且つ病斑部の細胞內には徑5ミクロン前後の球形の小体存在するを認め得らる。此小体は薄き沃度沃度加里液にては黃色に、醋酸カーミンに依りて煉瓦色に、Sudan III に依りて淡紅色に、ミロン氏指藥に依りて肉桂色に着色す。固定せられたる病葉のミクロトーム切片を檢するに、病細胞に於ては被害の程度に準じ、葉綠体崩壞して少數となり形も小さく、色素に染み難し。かかる細胞內には核に相接して一個稀に二個の小体存在す。その形多くは球形なるも時には不規則なる形をさることあり。大さ3-10×2.5-8 ミクロンにして細胞核より遙かに大なるもの多し。核と同じくヘトマキシリンによく染む。されどサフラニン及ゲンチアン紫にて染色すれば核はゲンチアン紫に染まるに反し小体はサフラニンにて赤く着色す、又細胞核內には仁及びクロマチン粒を認むるも小体內にはかくの如き物体存在せず單に多くの空胞を認め得るのみ。此小体は病斑の細胞內にのみ存在し他の組織及び健葉の組織中には發見せられず。その形狀性質より見るに諸種の動植物のウイルス病に冒されたる組織內に見出さるゝ intracellular bodies に著しく類似せり。