INTRACELLULAR INCLUSION BODIES IN THE RICE PLANTS AFFECTED WITH TRANSITORY YELLOWING[1]

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Introduction

A strange disease on rice plant broke out in the paddy field in Pingtung during the summer of 1960. According to Chang (1962), the new disease was assumed to be attributed to suffocation due to soil conditions, since no pathogenic microorganisms were isolated from the diseased plants. The disease soon become very prevalent over the southern and central parts of this island mostly in the second crop season of rice, and caused great damage to rice production in Taiwan. More recently, Chiu *et al* (1964) confirmed that the disease, formerly called rice suffocating, was mainly caused by a new virus transmitted by green rice leafhopper, *Nephotettix apicalis apicalis*. They named the new virus disease "Transitory yellowing", because the diseased plants showing yellowing on the lower leaves usually recover from the suffering and do not exhihibit the symptom on their tillers.

So far, seven virus diseases of rice such as dwarf, stripe, yellow dwarf, black streak, hoja blanca, orange leaf, and tungro have already been reported (Fukushi, 1931, Kawai, 1939, Atkins, 1957, Shinkai, 1960, Rivera, 1964). Whether the present disease is quite different from these virus disease is still open to question. Rivera and Ou (1964) supposed that transitory yellowing was probably closely related to the tungro disease of rice occurred in the Philippines according to the similarities in symptomatology, vector relationship, and recovery from the symptom expression between the two diseases. However, Chiu *et al* (1965) considered that the two viruses might not be identical because they observed the difference in varietal reaction between this disease and tungro.

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X-bodies associated with virus diseases have already been observed in dwarf virus-infected rice by Fukushi (1931, 1934) and in stripe virus-infected rice by Kawai (1931). More recently, Hirai *et al* (1964) found large spherical inclusions in dwarf virus-infected cells which were stainable with Giemsa, but were distinct from X-bodies. In stripe virus-infected cells, there were varying shapes of large inclusions which also showed the same stain ability as the inclusions in dwarf virus-infected cells. Since the inclusion bodies have a diagnostic value for virus diseases, the present investigation on the pathocytology of the rice-plants affected with transitory yellowing was made in order to examine the occurrence of intracellular inclusions for obtaining a further evidence for the virus nature of this disease, and attempt to differentiate present disease from the other virus disease of rice already reported.

Materials and Methods

Transitory yellowing most widely occurs in *Japonica* variety of rice during the second crop season. The diseased rice-plants of *Japonica* variety showing the typical symptoms (Fig. 1) as described by Chiu *et al* (1965) were collected from Pingtung and Taichung districts in the summer of 1964. At the first time, the diseased and healthy plants of *Japonica* varieties, Taichung 65 and New 10, were collected from the same paddy fields in Pingtung one and one half months after transplantation. Thenceforth, the diseased and healthy plants of Taichung 65 were also collected from the Taichung District Agricuttural Improvement Station one month and 2.5 months after transplanting, respectively. Some plants were used for study soon after collection from the paddy fields. The other plants were planted in pots (10 cm in diameter). The potted plants were kept in the water tank containing overflowing water at a suitable level under green house conditions. Some healthy plants grown from seeds under the same conditions mentioned above were served as control.

The histological and cytological changes of the diseased plants in comparison with those of healthy ones were examined on fresh materials stained with Giemsa's stain and on embedded materials stain with Heidenhain's iron alum haematoxylin or safranin as a counterstain. In each trial, several diseased plants derived from about 5 hill were used.

The diseased and healthy leaf blade and leaf sheath were cut into small pieces and fixed in a alcohol-iodine-formalin solution. The inside epidermis was peeled from the leaf sheath and cross sections of both leaf blade and sheath were obtained by free hand sectioning. Tissues were then stained with Giemsa solution according to the Bald's method (1949) and the modified method of Rawlins (1957).

On the other hand, the small pieces of leaf blade, leaf sheath, stem and root of diseased and healthy plants were fixed in strong formalacetic alcohol

(FAA). The fixed materials were embedded in paraffin and sections were cut 7 or 10 microns in thickness and stained with Heidenhain's iron-alum haematoxylin or safranin as a counterstain.

Experimental Results

1. *Free-hand seotions embedded in water*

The leaves showing the mottling symptom and healthy leaves were transversely sectioned by free hands in usual way. The sections mounted in water were observed under microscope and the cytological changes of living cells of the leaf tissue were examined. The mesophyll cells of chlorotic tissues were lighter in color and the chloroplasts in these cells seemed to be disintegrated. In the parenchyma cells or parenchymalike cell surrounding the vascular bundle, certain foreign bodies were found and some cells were fully filled with them. Such foreign bodies were ususally round in transversal view and varied in size. No such foreign body was observed in the corresponding cells of healthy leaves. The intracellular inclusions quite strange in structure and appearrance.

2. *Materials stained with Giemsa's stain*

In the sections stained with Giemsa, Fukushi (1931) found X-bodies in the leaf cells of rice plant infected with dwarf virus, and the large spherical inclusions, which were stainable with Giemsa but distinct from X-bodies, were observed in diseased cells stained with Giemsa and safranin by Hirai *et al* (1964). The present experiment was made in order to clarify the affinity to these stains and the structure and distribution of the inclusion bodies mentioned above. The free-hand section of diseased and healthy leaves and inside-epidermal strips of leaf sheath were used for Giemsa staining. No such a inclusion as those found by Hirai *et al* (1964) in inside-epidermal cells of dwarf virus-infected leaf sheath was found in the corresponding cells derived from the present diseased and healthy plants. The cross sections of healthy and diseased leaves showing chlorotic modification were fixed and stained in the same manner mentioned above. Some alternations similar to those found in the fresh sections embedded in water were clearly observed in vascular and mesophyll tissues of diseased leaves in comparison with those of healthy ones. The large round inclusions, stained violet with Giemsa, were found in parenchyma cells surrounding vascular bundle and also in the phloem parenchyma cells (Fig. 2). No intracellular body and chloroplastic change were noticed in the free-hand section of healthy leaves stained with the same stain.

3. *Materials stained with Heidenhains iron-alum haematoxylin*

More comprehensive studies on the intracellular bodies under consideration were made in the microtomed sections properly stained with haematoxylin.

The resultant data of pathocytological studies on different portions of diseased and healthy rice-plants will be given below.

a. Microtomed sections of leaf blades

In cross sections of a leaf blade with the transitory yellowing symptom of early stage, the large inclusions as those found in the leaf sections stained with Giemsa solution were also seen very clearly in the parenchyma cells of vascular bundles. Haematoxylin brought out the intracellular bodies very sharply, since they took the stain as intensively as the host nuclei. The inclusions were stained dark in color. They were mostly formed in the parenchyma cells around vascular bundle and were also present in the smaller parenchyma cells surrounding xylem vessels and sieve tubes (Figs. 3 & 3a). It was probable that the companion cells in phloem contained the inclusion. The present inclusion seemed to be similar to those in the corresponding cells of stripe virus-infected leaf observed by Hirai *et al* (1964). These inclusions were found in the materials derived from the diseased plants of Taichung 65 and New 10 varieties (Figs. 3 & 4). They were round and varied considerably in shape and size, which were confined to the parenchyma cells. The size of the inclusion in parenchyma cells surrounding vascular bundle ranged from 2.4 to 8 microns in diameter and those in the phloem parenchyma cells ranged from 1.4 to 2.4 μ in diameter. Inclusions were mostly spherical and oval, but sometimes were ring-shaped, hemispherical, or elliptical in transversal view. The content of the intracellular bodies appeared to be homologous without vacuoles and seemed not to be surrounded by a membrane. Chloroplasts in the mesophyll cells of chlorotic leaf-tissue looked to be disintegrated and showed less affinity for the stain. No intracellula bodies mentioned above were noticed in the cross section of a healthy leaf and the mesophyll cells contained full-shaped normal chloroplasts stained deeply with the dye (Fig. 5).

Longitudinal sections were preferable to demonstrate the intracellular bodies in comparison with cross sections. Accordingly, the inclusions were examined further in the longitudinal section stained with haematoxylin. The inclusion bodies looked round in a cross section of leaf blade mentioned previously, whereas they were found cylindric in the longitudinal section of leaf, from the lateral view (Figs. 6, 6a & b). These cylindric inclusions varied in length and some of them occupied most space of a cell. In consequence, the round large inclusions found in parenchyma cells of vascular tissue were actually cylindric in shape. These cylindric inclusions were distinct from the X-bodies observed in dwarf virus-infected rice by Fukushi (1931), and in stripe virus-infected rice by Kawai (1939). The typical X-bodies described by the both workers generally occurred in chlorotic mesophyll cells, occasionally in moter cells, and were usually round in shape, surrounded by a membrane, and possessed

homologous matrix containing vacuoles. However, the present inclusions were mostly found in the parenchyma cells of vascular tissue, and cylindric in shape, and consisted of homologous protoplasm without vacuoles. The nucleus generally disaappeared in the diseased cells containing a large compact inclusion, but the former could be seen in the cells with an inclusion loose in texture. Therefore, it seems likely that the nucleus may be buried in the compact inclusion. Most of the X-bodies were usually formed near or in close contact with the host nuclei, while the present intracellular bodies seemed to imply the nucleus within them. The chloroplasts in chlorotic mesophyll cells were disintegrated, and light colored after staining. Some deeply stained foreign bodies could also be distinguished in the chlorotic mesophyll cells containing degenerated chloroplasts. They were spherical or irregular in shape (Fig. 6c), although they were not more sharply differentiated from the cytoplasm than the inclusion bodies formed in vascular parenchyma cells. In the longitudinal sections of healthy leaf, any kinds of foreign bodies were not found (Fig. 7).

b. Microtomed section of root

Some intracellular bodies were also found in the pericycle and parenchyma cells within vascular cylinder, and occasionally in endodermal cells (Fig. 8). These inclusions were identical in shape and structure with these found in the sections of leaf blades. They were seen to be round in cross sections (Fig. 8a) and cylindric in longitudinal sections (Fig. 9). In both cross and longitudinal sections of healthy roots, no cellular modification was noticed (Fig. 10).

c. Microtomed section of stem and sheath

The same attempt was also made in order to confirm the occurrence of the inclusion bodies under consideration in the stem and leaf sheath of diseased plants. So far, no cytological abnormality was detected in the cross section of diseased stems (Fig. 11), while the parenchyma cells surrounding sieve tubes of leaf sheath were filled with the intracellular bodies (Fig. 12).

d. Microtomed section of leaf blades of the rice plants recovered from the disease

The present disease is characterized by its recovery from yellowing. The same staining process was also applied to both cross and longitudinal sections of leaf blades and roots derived from the recovered rice plants (Taichung 65). In these trials, the mesophyll cells and parenchyma cells of vascular tissue were seen normal, as deeply stained full-shaped chloroplasts were abundant in mesophyll cells and no intracellular bodies or the other viroplasms were found in the cells of vascular tissue. Accordingly, the infected plants were able to recover not only from the external symptom but also from the internal symptom.

Discussion

So far, seven virus diseases of rice have already been reported. Whether the present disease is distinct from those virus diseases is still open to question. Rivera and Ou (1964) supposed that transitory yellowing was probably closely related to the "Tungro" disease of rice informed from the Phylippines according to their similarities in symptomatology, vector relationship, and recovery from symptom expression. However, Chiu *et al* (1965) considered that the two viruses might not be identical because they observed very mild yellowing in the seedlings of the variety Taichung (native) 1 inoculated with the present virus, while the response of the same rice-variety to tungro virus showed conspicuous color change. The intracellular inclusions including crystalline and amorphous bodies have been designated "viroplasts". They are usually proteinaceous, and some are nearly pure virus nucleoprotein. The inclusion bodies have diagnostic value, since the inclusion bodies appear to be specific for virus diseases. The different viruses differ greatly in frequency and distribution of inclusion production, and in the type of inclusion structure. Some simple staining techniques to demonstrate the presence of inclusion were devised and proposed as a diagnostic aid for virus diseases (Lindner, 1961). Under these assumptions, the present pathocytological study was made. X-bodies associated with rice virus diseases have already been observed in dwarf virus-infected rice by Fukushi (1931), and in stripe virus-infected rice by Kawai (1939). Those X-bodies were found distinctly in the chlorotic mesophyll cells of microtomed sections stained with Giemsa stain or iron-alum haematoxylin. More recently Hirai *et al* (1964) found large spherical inclusions stainable with Giemsa in dwarf or stripe virus-infected cells, and the large inclusion of varying shapes in stripe virus-infected cells which also showed the same stain ability. Those large inclusion were distinct from X-bodies.

The writers succeeded in revealing the presence of intracellular bodies in the parenchyma-cells in fresh and embedded materials of diseased rice-plants showing the symptoms of transitory yellowing. It may be assumed that the existence of the inclusion bodies is a further evidence for the virus causality of the present disease. The large inclusion were generally cylindric from the lateral view and looked round in transversal view. They were mostly formed in the parenchyma cells around vascular bundle and were also present in the parenchyma cells surrounding xylem vessels and sieve tubes. These large cylindric inclusion varied in length and thickness, and some of them occupied the most space of a cell. The present inclusions were quite different in size, shape, distribution and structure from X-bodies found in dwarf virus-infected rice by Fukushi (1931) and in stripe virus-infected rice by Kawai (1939). The

typical X-bodies discribed by the both workers usually occurred in chlorotic mesophyll cells, occasionally in motor cell, and phloem parenchyma-cells of root; and were generally round in shape, vacuolate, and homologous in matrix surrounded by a membrane; and located in close contact with the host nucleus. However, the present inclusions were formed in the parenchyma cells within a vascular bundle mostly in leaves and roots, occasionally in leaf sheathes; and were usually cylindric in shape, consisted of homologous protoplasm without vacuoles; and seemed to imply the nucleus within them. Accordingly, the present virus is not identical with dwarf and stripe viruses in view of the type and distribution of inclusion bodies.

Some deeply stained foreign bodies which were spherical or irregular in shape could be distinguished in the chlorotic mesophyll cells containing disintegrated chloroplasts. They were also distinct from X-bodies, since they were not so sharply differentiated from the cytoplasm as X-body.

No such large spherical and variously shaped large inclusions as those found by Hirai *et al* (1964) in inside-epidermal cells, and parenchyma cells of dwarf or stripe virus-infected leaf sheath, was found in the Geimsa-stained corresponding cells derived from the present diseased and healthy plants. Anyhow, the present cylindric inclusion seemed to be similar to the round inclusion present in parenchyma cells near vascular bundles of stripe or dwarf virus-fnfected leaf and leaf sheath observed by Hirai *et al* from transversal view.

The all bodies in question were not artifacts since they could be found in fresh and embedded materials of diseased plants, while could not be noticed in the sections derived from healthy plants. It is uncertain at present whether the large cyclindric inclusions are the viroplasm or the by-product of the virus invasion.

In the microtomed sections of leaf blades of the rice plants recovered from the disease, no inclusions mentioned above was observed. Accordingly, the infected plants were able to recover not only from the external symptoms but also from the internal symptom. It seemed that the symptom expression of transitory yellowing was correlated with the occurrence of the large inclusions.

According to Rivera and Ou (1964), transitory yellowing is probably closely related to the "Tungro" disease of rice. It is possible to identify the two viruses by the fact of inclusion formation. There is still no report concerning the pathocytological study on tungro disease, therefore the writers could not differentiate the two viruses on this basis.

Summary

Some alternations were revealed in vascular and mesophyll tissues of rice plants affected with transitory yellowing. In the fresh cross-sections of diseased

leaves, certain large foreign bodies were observed in the parenchyma cells of vascular bundles. The large round inclusion-bodies, stained violet with Giemsa, were mostly found in the parenchyma cells around vascular bundles and were also present in the smaller parenchyma cells surrounding xylem vessels and sieve tubes. Haematoxylin brought out the inclusion bodies very sharply, and stained the inclusions dark in color. Actually the intracellular inclusions were cylindric in longitudinal sections of the diseased leaves from the lateral viow, although the inclusions looked round in cross sections of a leaf blade. These cylindric inclusions varied in length and thickness, and some of them occupied the most space of a cell. The present inclusion consisted of homologus protoplasm without vacuoles, and seemed to imply the nucleus within them. They were distinct from the X-bodies observed in dwarf or stripe virus-infected rice plants.

The similar cylindric inclusion-bodies were also formed in the pericycle and parenchyma cells within vascular cylinder, and occasionally in endodermal cells of diseased roots. No cytological abnormality was detected in the cross section of diseased stems, while the parenchyma cells surrounding sieve tubes in a leaf sheath were filled with the intracellular bodies.

The chloroplasts in chlorotic mesophyll cells were disintegrated and showed less affinity for the stains used. Some deeply stained foreign bodies could be distinguished in the chlorotic mesophyll cells. They were spherical or irregular, although they were not so sharply differentiated from the cytoplasm.

In the microtomed sections of leaf blades of the rice plant recovered from the disease, the mesophyll cells and parenchyma cells of vascular tissue were seen as normal as those of healthy plants.

No intracellular body and chloroplastic changes were noticed in fresh and embedded materials of healthy leaves, roots and stems.

水稻黃葉病之封入體試驗

蘇鴻基 黃潔華

民國四十九年夏天以來，本省二期作水稻發生一種新病害爲害，至爲猖獗。當時一般認爲此病係爲窒息病。據邱人璋等氏最近鑑別結果，認爲是一種新的毒素病，並命名爲黃葉病。水稻的毒素病有記載者共有七種，而本省目前發生的黃葉病是否與此等已發表的毒素病完全不相同，至今仍未定論。對毒素病所具有特異性的封入體，一向被認爲在毒素病的鑑別與診斷上有利用價值。本試驗即欲究明該病的病理細胞學上的變異，則或對該病病因的判別上有所參考。

罹病水稻之維管束及葉肉組織中，均發現有細胞學上之變異；在病株的生細胞及經染色的細胞內均可看到奇異的封入體（Inclusion body）存在。於病葉的切片中此大形之封入體通常存於環繞着維管束的薄壁細胞中，有時在導管及篩管周圍的薄壁細胞中也可見到。此封入體可用 Giemsa 染料染成紫色，或用 Haematoxylin 染成暗黑色以利觀察。此封入體自橫切面上看似圓形者爲多，若自縱切面看，則呈長形故實際上應爲圓柱形，其似由均勻之原生質構成，細胞核亦似包被於其中，不具空泡。若病葉中的葉綠體崩潰，則對所用染料的親合力變低，在此等葉肉細胞中，可看到未完全由細胞質分化之圓形或不整形封入體。

此圓柱形的封入體亦存於根部維管束的薄壁細胞，內鞘（pericycle）細胞及皮層細胞中。在葉鞘，環繞篩管的薄壁細胞中也有此種封入體存在。但是在病癒的稻株任何部位之細胞內皆無封入體，其葉綠體亦正常。因此黃葉病病株復原後，不僅其外部病徵消失，即其內部病徵亦消失。此細胞封入體之形成似與葉黃病徵之表現有關。在健康的稻株之任何組織細胞中皆無上述之任何細胞學上的異常現象發生。因而此特異封入體之存在可做爲本病病因仍係毒素病之另一證據。

此封入體與水稻萎縮病（dwarf）及縞葉枯病（stripe）等病株中所發現的 X—封入體（X-body），在性狀及分佈上都不相同。即此種病毒與後二種病毒相異。此病與水稻 Tungro 病在病徵及媒介昆蟲之關係上皆相同。但在病理細胞上由於 Tungro 病尚無此方面的研究報告發表，故不能互相比較。待有關 Tungro 病的病理細胞學研究報告出來以後即可判定二者之異同。

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Explanation of plate figures

Fig. 1. Three diseased leaves on the right hand showing the yellowing and mottling symptoms. A healthy leaf on the left (Taichung 65).

Fig. 2. A portion of a cross section of a diseased leaf, showing the intracellular bodies in parenchyma cells of vascular tissue (stained with Giemsa solution).

Fig. 3. A portion of a cross section of a diseased leaf (Taichung 65) showing the inclusion bodies in parenchyma cells of vascular tissue (microtomed section stained with iron-alum haematoxylin).

Fig. 3a. A portion of the section shown in Fig. 5, highly magnified.

Fig. 4. A portion of a cross section of diseased leaf (New 10) showing the inclusion bodies in parenchyma cells of vascular tissue (stained with iron-alum haematoxylin).

Fig. 5. A portion of a cross section of a healthy leaf (Taichung 65, stained with iron-alum haematoxylin).

Fig. 6. A portion of a longitudinal section of a diseased leaf (Taichung 65) showing the cylindric inclusions (stained with iron-alum haematoxylin).

Figures 6a and b. A portion of the section shown in Fig. 6, highly magnified, showing the large cylindric inclusions in parenchyma cells of vascular tissue.

Fig. 6c. A portion of the section shown in Fig. 6, highly magnified, showing intracellular bodies in mesophyll cells of palisade tissue.

Fig. 7. A portion of a longitudinal section of a healthy leaf (Taichung 65, stained with iron-alum haematoxylin).

Fig. 8. A cross section of a root of a diseased rice plant, showing inclusion bodies in parenchyma cells of a vascular cylinder (Taichung 65, stained with iron-alum haematoxylin).

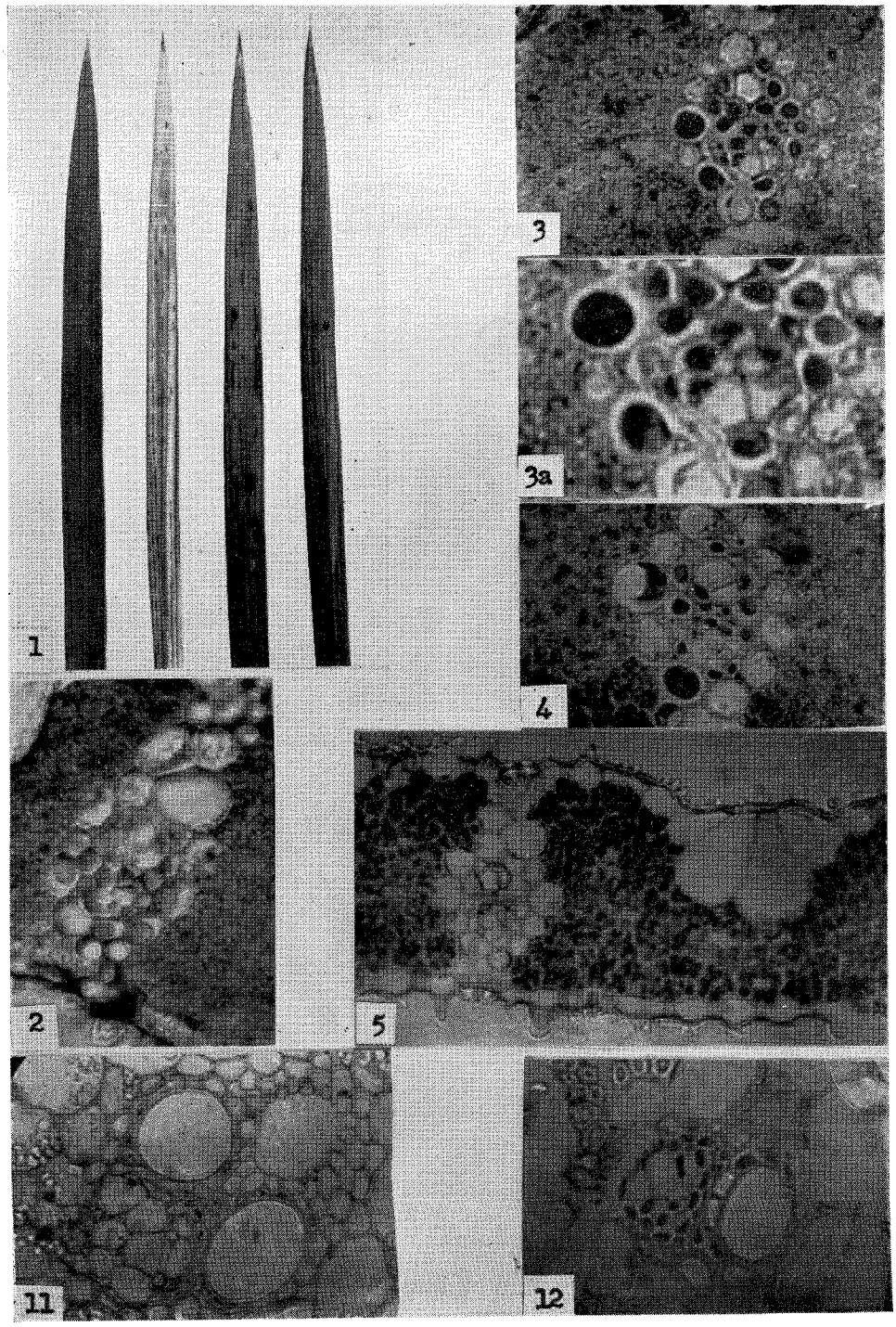
Fig. 8a. A portion of the section shown in Fig. 8, highly magnified.

Fig. 9. A portion of a longitudinal section of a diseased root, showing cylindric inclusions in parenchyma cells of a vascular cylinder (Taichung 65, stained with haematoxylin).

Fig. 10. A portion of a longitudinal section of a healthy root (Taichung 65, stained with iron-alum haematoxylin).

Fig. 11. A portion of a cross section of a diseased stem (Taichung 65, stained with iron-alum haematoxylin).

Fig. 12. A portion of a cross section of a diseased sheath, showing deeply stained bodies in parenchyma cells within phloem tissue (Taichung 65, stained with iron-alum haematoxylin).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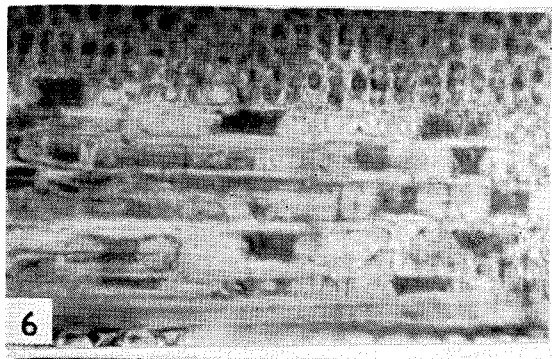
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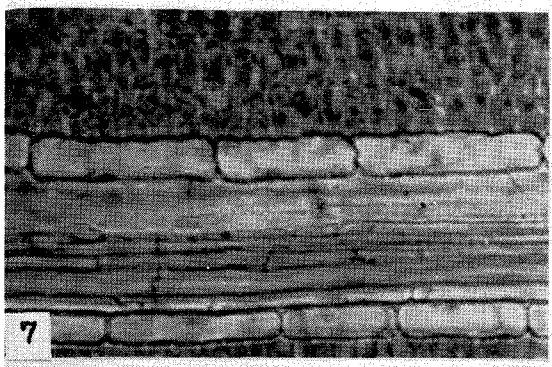
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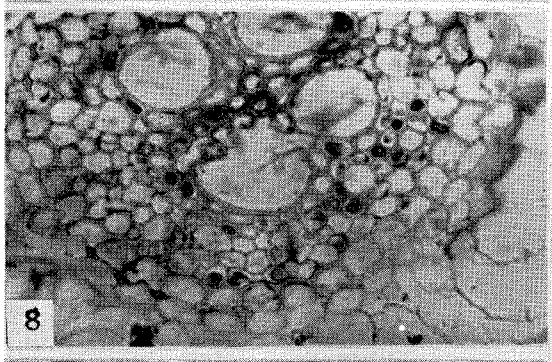
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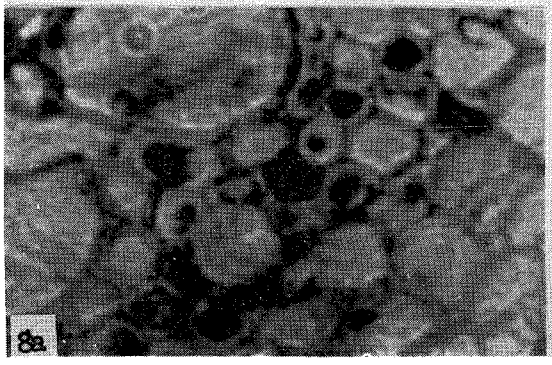
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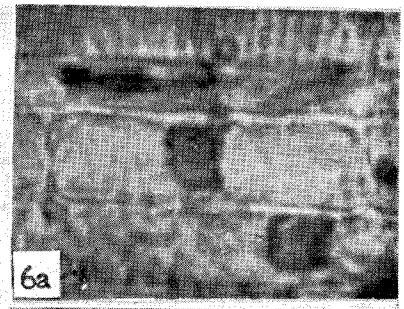
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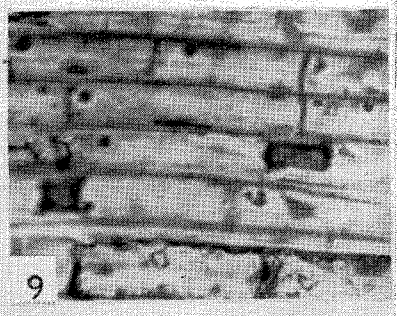
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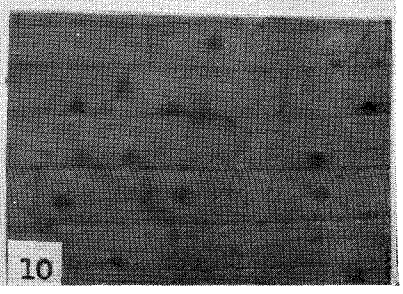
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