中華農業研究 (Jour. Agric. Res. China) 37(3) : 313~319 (1988)

Mycoplasma-like Organism Associated with Eggplant Phyllody in Taiwan[1]

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Abstract : The phyllody of eggplant(*Solanum melongena*) was a newly recorded disease found in the Penhu Islands in Taiwan in 1986. The diseased plants showed the yellows symptoms comprising green flower and malformation of leaves. The disease agent was transmitted by grafting. Numerous pleomorphic bodies characteristic of a mycoplasma-like organism(MLO) were observed in veinal sieve elements of the diseased leaves under electron microscopy. They were spherical, ovoid, or ellipsoidal, and about 110 to 800nm in diameter. No such MLO bodies were found in the healthy plant. The stem and leaf sections were also examined under the light and fluorescent microscope with Dienes' staining, DAPI technique and direct fluorescent detection method, respectively. The results showed specific blue staining, DAPI fluorescent staining MLOs and direct yellow fluorescent reactions in the affected phloem cells, respectively, but no such reaction was found in those of the healthy ones. The staining which revealed the presence of MLOs in the sieve elements was in accord with results observed by electron microscopy. We suggest an MLO etiology for the eggplant phyllody.

Introduction

The phyllody disease of eggplant (*Solanum melongena* L.) was newly found in the Penghu Islands in Taiwan in 1986. The disease causes the production of enlarged calyxes of green flowers, malformation of young leaves with chlorosis, and abnormal conical shoots with singular large cup-like green flowers (Fig. 1). The symptoms were similar to those described for the little leaf disease of eggplant in India during 1968—1970 [1,2,6,9,14,15]. However, the relation between the eggplant phyllody in Taiwan and the eggplant little leaf in India was unknown. Because of the eggplant phyllody was first noticed in Taiwan, besides of the eggplant little leaf in India, it has not been recorded in any other country [6,14], this study was attempted to clarify the disease etiology by electron microscopy, Dienes' staining, DAPI staining, and direct fluorescent detection

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methods.

Materials and Methods

Graft inoculation

Nine healthy eggplant seedlings cultivated in pots were used as test plants for grafting. Scions taken from diseased eggplant were each grafted to the healthy eggplant by top-working for inoculation, and were maintained in greenhouse for 3 months for symptom development.

Electron microscopy

Small pieces of the diseased eggplant leaves containing veinal tissues were fixed in 5% glutaraldehyde in 0.1M phosphate buffer, pH 7.0, at 4°C for two hrs. They were rinsed and post-fixed in 1% osmium tetroxide at 4°C for two hrs. Following fixation, the tissue pieces were dehydrated in 2, 2-dimethoxypropane, treated with propylene oxide, and embedded in LX112 epoxy resin [3,5,17]. Ultrathin sections were cut with a glass knife fixed on a Reichert-Jung Ultracut E ultramicrotome. The thin sections were then double-stained with uranyl acetate and lead tatrate [3,7,17], and examined under JEM-200 CX electron microscope.

Light microscopy

Hand sections of stems and leaf veins were cut from both healthy and infected eggplant into distilled water. The sections were then transferred to Dienes' stain. After 10min, the stain was withdrawn and replaced with distilled water [4,16,17].

After further washing in distilled water, the sections were mounted in water and examined under an Olympus-Vanox type light microscope [16,17]. Both longitudinal and transverse sections were examined.

Fluorescent microscopy

For the DAPI (4'-6-Diamidino-2-phenylindole) technique [10,11,12], samples about 3×5 mm taken from the stems and leaf veins of both healthy and diseased eggplant were fixed in 5% glutaraldehyde in 0.1M phosphate buffer, pH7.0 at 4°C. Before sectioning, the pieces were washed in histological buffer for at least 3min, then longitudinal sections about 0.1mm thick were stained on slides for at least 1min with a 1 μg/ml solution of the DNA-specific compound DAPI (Sigma Chemical Co., St. Louis, MO) in histological buffer. Sections mounted in the DAPI solution and covered with coverslips were subsequently examined for DNA-specific fluorescence. Fluorescence was observed under a fluorescence microscope (Zeiss Standard XBO-75W).

For direct fluorescent detection method [8], small pieces of stems and leaves of both healthy and diseased plants were fixed in 2% glutaraldehyde in 0.1M phosphate buffer (pH7.0). Free hand sections of fixed or fresh stems and leaf veins were cut from both healthy and diseased plants, and observed under a reflecting fluorescence microscope (Ziess Standard XBO-75W).

Results and Discussion

By graft inoculation, 5 out of 9 grafted scions sprouted, and those graft-inoculated

eggplants developed phyllody symptoms with leaf malformation and chlorosis (Fig. 1) 2—3 months after grafting. This result confirmed that the eggplant phyllody was a graft transmissible yellows disease.

Electronic microscopic observation of tissues from diseased plants of the eggplant revealed the presence of numerous pleomorphic bodies resembling mycoplasmas in the phloem cells. These bodies were not found in the healthy leaves. The pleomorphic bodies had a distinct external membrane, and varied in size and concentration in the sieve elements (Fig. 2). The mature cells were filled with ribosomes and net-like strands in the cytoplasm and were spherical, ovoid or ellipsoidal. The spherical bodies had a diameter from 110 to 800nm. These different types of MLO cells were observed only in the phloem elements of the host plants.

Under the light microscope, the secions of stems and leaf veins with Dienes' stain showed a bright turquoise blue color in the xylem and a pale purplish blue color in the cortex. The phloem of healthy sections remained unstained. The phloem of infected sections contained regularly distributed areas of stained blue (Fig. 4—5).

By DAPI staining, in the sections of the diseased plants, numerous singular fluorescent particles of DAPI-stained MLOs were observed in phloem cells (Fig. 6), and DAPI-stained nuclei were seen singly in each cell under fluorescent microscopy. Xylem elements had bluish autofluorescence. No. abnormal fluorescence was observed in the phloem cells of the sections from healthy plants. Fluorescent particles of D API-stained MLOs were limited to sieve tube elements and were readily distinguished from other sources of fluorescence, including host nuclei, mitochondria, and secondary xylem thichenings [10,11,12,13].

Tissue sections under direct reflecting fluorescence microscopy specific bright yellow or greenish yellow fluorescence in the affected phloem cells, a yellow, yellowish green or green color in the vessel walls, a pale green or yellow color in the cortex and a red or reddish brown color in the chloroplasts under different filter combinations [8]. The specific fluorescence was observed in the affected cytoplasm of phloem cells only (Fig. 3). No such fluorescence was observed in the phloem tissues of healthy plants. The observed phloem reaction was specific to the infection of plants with phyllody disease.

Those singular reactions in the infected sieve cells indicated the presence or infections of the phleom limited MLOs in the infected plants [4,8,10,11,12,13]. It also appeared to be well correlated to the electron microscopic observations in that the MLO bodies were phloem-limited [3,6,11,12]. In this study, Dienes' stain, DAPI technique and direct fluorescent detection method showed a high specificity to plant MLO. Evidently, those detection method can conveniently be used to determine the distribution, and development of MLOs in infected plants [4,8,10,11,12,13,15,16]. In view of the fact that the morphological structures and the localization in plant tissues of the pleomorphic bodies observed in the diseased eggplant and their specific reactions with Dienes' stain, DAPI staining and autofluorescence were similar to those previously found in other plants infected with MLOs [3,4,6,8,9,10,11,12,13,14,15,16], our results suggest a mycoplasmal etiology for the

phyllody disease of the eggplant. In India, Anjaneyulu and Ramakrishman [1] first reported the eggplant little leaf disease in 1968, where the eggplant was widely cultivated and known as the name of brinjal, and the disease had been considered to be identical with tomato big bud disease, but no serological studies was available to support this assertion. It was also reported to be transmitted by the leafhopper vectors *Hishimonus phycitis* and *Empoasca devastans* in India [9], and was confirmed to be transmitted to *Vinca rosea* [2], and the MLOs were also noticed in the diseased plants [9,15]. The relation between the eggplant phyllody in Taiwan and the eggplant little leaf in India remains to be studied in the future by serological examinations.

Acknowledgements

The author is indebted to Drs. C. C. Tu, E. Shikata, S. Namba, H. J. Su, and W. Y Wang for their support and help in this study, and also wish to thank Mr. S. M. Wang for his assistance in this experiment.

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Explanation of plates

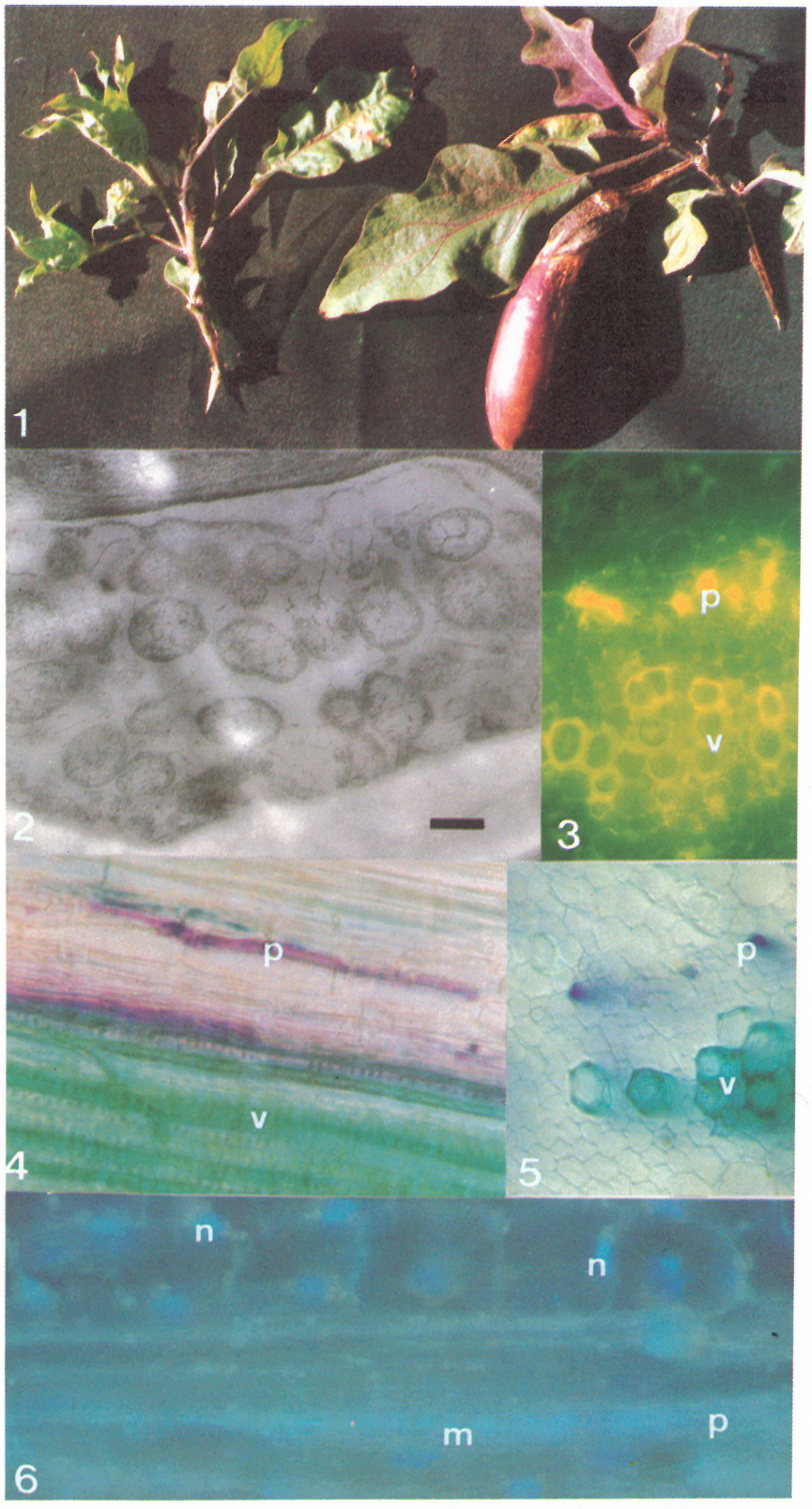
Fig. 1. The symptoms of phyllody diseased eggplant showing green flowers and leaf malformation (left), and the healthy plant with normal leaves and fruit (right).

Fig. 2. Cross-section of leaf vein of diseased eggplant showing numerous pleomorphic mycoplasma-like bodies in a sieve cell. (Bar in figure indicates 0.5μm).

Fig. 3. Fluoresce micrograph of vascular bundles of phyllody diseased eggplant. Specific yellow fluorescent reaction (p) was observed in the phloem cells of diseased plants. p: phloem cells. v: vessel cells.

Fig. 4-5. Light micrographs of longitudinal(4) and transverse (5) sections of diseased eggplant treated with Dienes' stain. In which the phloem cells (p) stained distinctly blue. p: phloem cells. v: vessel cells.

Fig. 9. Fluorescence micrograph of section of diseased eggplant stem stained with DAPI, in which the phloem cells (p) show numerous singular DAPI-stained MLO fluorescent particles (m). p: phloem cells. m: MLOs. n: nucleus.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臺灣茄子葉化花病之擬菌質體病原[1]

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摘 要

茄子葉化花（egplant phyllody）係作者於1986年在澎湖地區發現之新菌質病害。病株呈現葉片畸型狀及多數杯狀葉化花之花器葉化病徵。本病可經嫁接傳染。病株切片之電子顯微鏡觀察結果，篩管細胞有多數擬菌質體（mycoplasma-like oganisms）存在，呈球形及橢圓形等大小形態，直徑約110—800nm。健株則沒有。病株切片之 Dienes' stain 染色，以光學顯微鏡觀察結果，罹病細胞有特殊藍色反應。健株則沒有。病組織切片之 DAPI 螢光染劑處理後，以螢光顯微鏡觀察結果，罹病篩管細胞內，呈現多數擬菌質體羣落狀螢光顆粒，惟與細胞核之螢光有明顯之差異。健株篩管則無此反應。病組織切片，直接以反射型螢光顯微鏡觀察結果，罹病篩管呈顯特殊黃色螢光。健株即沒有。上述罹病篩管細胞之專一性反應表示其擬菌質體之存在，其存在情況與電子顯微鏡觀察結果相似。以上結果顯示擬菌質體爲本病之病原。

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