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Mycoplasma-like Organisms Associated With Witches' Broom of Wild Balsam Pear in Taiwan[1]

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Abstract : The witches' broom disease on wild balsam pear was newly found in an isolated areas near Shui-li, Nan-tou county in the central Taiwan in 1984. The diseased plants showed typical witches' broom symptoms comprising bushy little leaves, green flowers and abnormal conical shoots. Numerous pleomorphic bodies, characteristic of mycoplasma-like organisms (MLO), were observed in veinal sieve elements of the diseased leaves under electron microscopy. They were spherical, ovoid or ellipsoidal about 110 to 740 nm in diameter. No such MLO bodies were found in the healthy plant. The stem and leaf sections were observed directly under a reflecting fluorescence microscope and also examined under light microscope after treatment with Dienes' stain. The specific yellow fluorescence and blue staining reaction were noticed in affected phloem cells, but no such reactions were detected in those of healthy. The results revealed the presence of MLOs in the sieve elements were in accord with what observed under electron microscopy. We suggest an MLO etiology for the witches'broom of wild balsam pear.

Introduction

The wild balsam pear (*Momordica* spp.) is distributed in Nantou county in the central Taiwan. The witches' broom disease on wild balsam pear was found to be severe in some isolated areas near Shui-li by the first author, in 1984. The diseased plants produced numerous little leaves, phylloid blossoms and abnormal conical shoots (Fig. 1). The symptoms were similar to those described for other plant witches' broom diseases due to mycoplasma-like organisms (MLOs) [1,2,5,8,10,11]. Recently, a simple method of detecting MLOs in plant tissues based on the use of Dienes' stain and reflecting fluorescence has been described [3,7]. The witches' broom disease on wild balsam pear was observed only in an isolated area and has not been reported by any author in the world. We attempted to clarify the disease etiology by electron, light and reflecting-fluorescence microscopy.

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Materials and Methods

Electron Microscopy

Small pieces of both healthy and infected wild balsam pear 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 [4]. Ultrathin sections were cut with a glass knife fixed on a Leitz's Fernandez-Moran type ultramicrotome. The thin sections were then double-stained with uranyl acetate and lead tertrate [6] and examined under JEM-200CX electron microscope.

Light Microscopy

Hand sections of stems and leaf veins were prepared from both healthy and infected plants and placed in distilled water. The sections were then transferred to Dienes' stain. After 10 min, the stain was withdrawn and replaced with distilled water [3]. After further washing in distilled water, the sections were mounted in water and examined in an 01ympus-Vanox type light microscope [9]. Both longitudinal and transverse sections were examined.

Reflecting fluorescence microscopy

Small pieces of stems and leaves of both healthy and witches' broom diseased wild balsam pear 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 prepared from both healthy and diseased plants, and observed under a reflecting fluorescence microscope (Ziess Standard XBO-75W) as described by Namba *et. al.* [7]

Graft transmission

The true seeds of wild balsam pear were sown in pots and cultivated in green house. Twenty-eight healthy seedlings were used as stocks for grafting tests, and top-workings were made by grafting with diseased scions collected from field. The results of symptom developments of those graft-inoculated plants were observed about a half year in green house.

Results and Discussion

Electron microscopic observation of tissue from witches' broom diseased plants of wild balsam pear 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 bodies were filled with ribosomes and net-like strands in the cytoplasm and were spherical, ovoid or ellipsoidal [1]. The spherical bodies had a diameter from 110 to 740 nm. The large variation in the number and size of these MLO bodies in host cells have also been observed for other MLO-associated diseases [1,2,5,11].

Under light microscope, the sections of stems and leaf veins of diseased plants sh-

owed 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 stained blue (Fig. 3). The reaction appeared to be well correlated to the electron microscopic observations in that the MLO bodies were phloem-limited. In this study Dienes' stain showed a high specificity to plant MLO. Evidently, the staining method can conveniently be used to determine the distribution, and development of MLOs in infected plants [3].

Direct fluorescence microscopy of tissue sections from diseased plants showed a distinctly bright yellow of greenish yellow color in the phloem cells, a yellow, yellowish green or green color in the vessel well, a pale green or yellow color in the cortex and a red or reddish brown color in the chloroplasts under different filter combinations. The specific bright yellow fluorescence was observed in the cytoplasm of affected phloem cells only (Fig. 4). No such fluorescence was observed in the phloem tissues of healthy plants. Accordingly, the observed phloem reaction was specific to the MLO infection of diseased plants. This result indicates that a phloem-limited MLO etiology may be involved [7].

In grafting tests, diseased wild balsam pear used as inoculum-scions were graft-inoculated to 28 healthy wild balsam pear seedlings, Six out of 28 grafted scions sprouted, and the 6 inoculated plants successfully developed green flower and typical witches' broom symptoms. The results confirmed that the wild balsam pear witches' broom disease was graft-transmissible and was similar to other plants infected with MLOs. The etiological relations among witches' broom diseases of wild balsam pear, balsam pear and loofah are not known [2,10]. Those etiological relations deserve further research attention.

The morphological structures and the localization of the pleomorphic bodies in diseased wild balsam pear plants and their specific reaction with Dienes' stain and fluorescence and the results of graft-inoculation and symptom expression, were similar to those previously found in other plants infected with MLOs [1,2,3,5,7,9,10,11]. Our results suggest a mycoplasma etiology for the wild balsam pear witches' broom disease. Recently, we are making the protoplast culture of MLO-diseased phloem cells, and attempt to clarify the morphology of MLOs in single cell-wall free phloem cell and to confirm the specificity and etiological relations of Dienes' stain and reflecting fluorescence to these MLO agents.

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Explanation of plates

Fig. 1. The witches' broom-diseased plants of wild balsam pear showing bushy little leaves (A), and the healthy plant with normal leaves, flowers and fruits (B).

Fig. 2. Cross-sections of leaf veins of witches' broom diseased wild balsam pear showing numerous pleomorphic mycoplasma-like bodies in a sieve cells. Bar in figure indicates 0.3μm.
Cw : cell wall. M : mycoplasma-like bodies. SP : sieve pore.

Fig. 3. Light micrographs of transverse sections of witches' broom-diseased wild balsam pear treated with Dienes' stain. A section containing MLOs in the phloem cells (P) stained distinctly blue. P : phloem cells. V : vessel wall.

Fig. 4. Fluorescence microphotographs of vascular bundles of witches' broom-diseased wild balsam pear. Specific fluorescence reaction was observed in the phloem cells of diseased plants. S : specific fluorescence, P : phloem cells, V : vessel cells, C : chloroplasts.

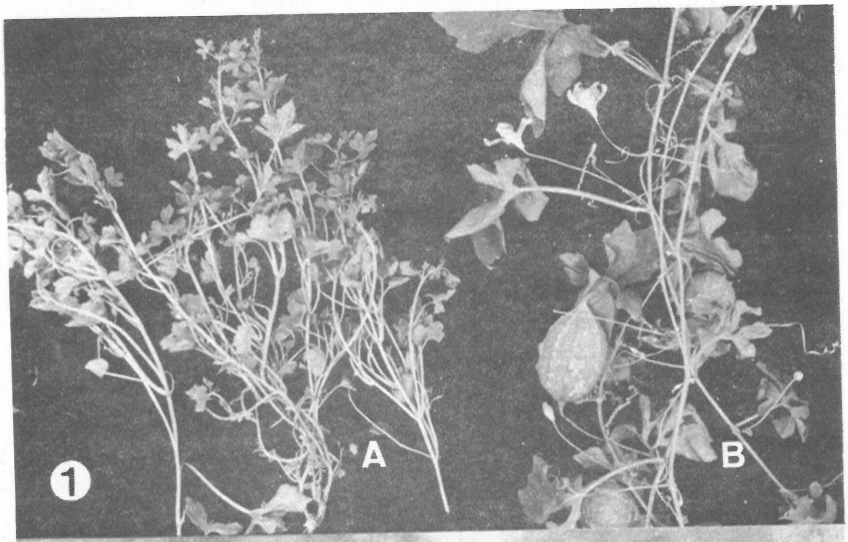
臺灣野苦瓜簇葉病之擬菌質體病原[1]

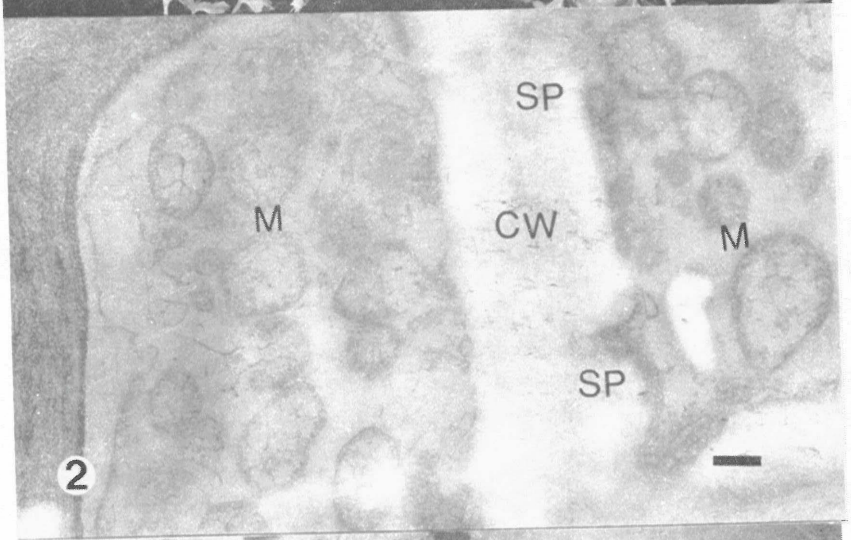
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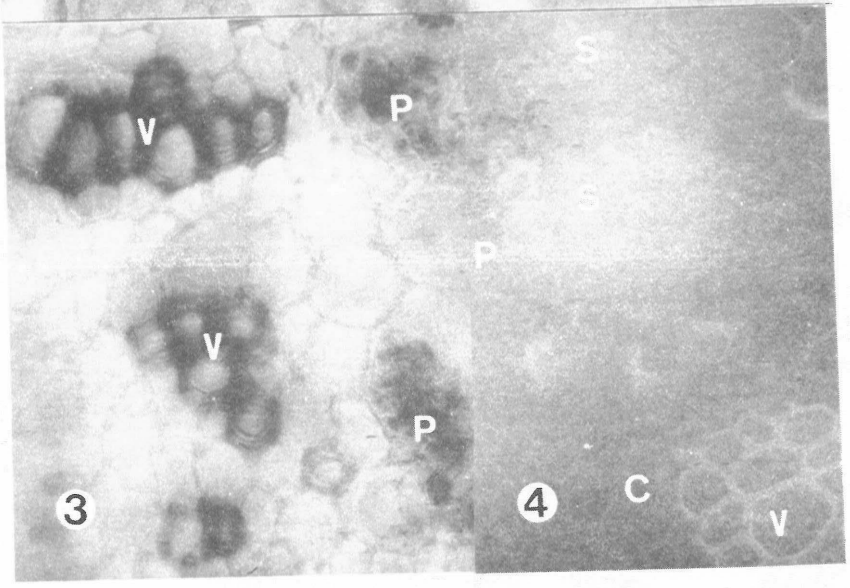
摘 要

臺灣之野苦瓜簇葉病（Wild balsam pear witches' broom），係第一作者於1984年，在南投水里地區發現之新病害。病株叢生小葉、異常分蘖、綠化花，顯現典型簇葉病徵。田間採集之病株葉脈組織超薄切片之電子顯微鏡觀察結果，篩管細胞內有多數多形性擬菌質體（mycoplasma-like organisms）存在，呈球形、楕圓形及圓形等大小不同之各種形態，直徑約 110-740nm。健全株則沒有擬菌質體。病株莖葉徒手切片，經 Dienes' stain 染色，並以光學顯微鏡觀察，結果在罹病篩管細胞內，顯現專一性藍色之 Dienes' stain 染色反應，健株者則無此反應。另將病組織徒手切片，直接以反射型螢光顯微鏡觀察結果，在篩管細胞，呈現顯著特殊螢光反應，這些特殊螢光反應局限於篩管組織，健全株之篩管組織則無此反應。上述專一性反應表示其擬菌質體之存在，其存在位置及數量與電子顯微鏡觀察結果相似。以上結果顯示擬菌質體似爲野苦瓜簇葉病之病原。

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