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Fluorescence Microscopic Diagnosis of Mycoplasma Infections in Jujube, Mulberry and Periwinckle Plants

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螢光顯微鏡的 技法에 依한 대추나무, 뽕나무 및 일일초의 마이코플라스마 感染診斷

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ABSTRACT

Attempts were made to evaluate the efficacy of three fluorochromes, i.e., DAPI (4'-6-diamidino-2-phenylindole·2HCl), aniline blue and quinacrine (quinacrine mustard dihydrochloride) for the detection of mycoplasma infections in jujube (*Zizyphus jujuba*), mulberry (*Morus alba*) trees and periwinckle (*Catharanthus roseus*) plant by fluorescence microscopy. Stem sections from these plants infected with mycoplasma-like organisms (MLO) produced distinct fluorescence in the phloem when stained with DAPI, aniline blue or quinacrine, while fluorescence was absent in the healthy plants. The use of these fluorochromes provided simple and efficient techniques for the diagnosis of MLO infections. Of the three fluorochromes tested, DAPI was found to be most efficient.

Key words: diagnosis, fluorescence microscopy, DAPI, mycoplasma.

要 約

마이코플라스마에 感染된 대추나무, 뽕나무 및 일일초의 組織切片을 蛍光染色素인 DAPI(4'-6-diamidino-2-phenylindole·2HCl), aniline blue 그리고 quinacrine(quinacrine mustard dihydrochloride)으로 染色하여 蛍光顯微鏡下에서 觀察함으로서, 이들 蛍光染色素의 마이코플라스마 感染診斷에의 效用價値를 比較調査하였다.

罹病植物의 줄기切片의 師部에서 特異蛍光反應이 나타났으나 健全植物의 組織切片에서는 特異蛍光反應이 觀察되지 않음으로써, DAPI, aniline blue 및 quinacrine 等의 蛍光染色素에 의한 組織染色法은 대추나무, 뽕나무 및 일일초의 마이코플라스마 感染을 신속 正確하게 診斷하는 데 매우 有用한 方法임을 보여 주었다. 한편, 이들 蛍光染色素中에서는 DAPI가 가장 效果的이었다.

INTRODUCTION

Electron microscopy is one of the most important diagonistic tools in diagnosis of plant diseases caused by mycoplasmalike organisms (MLO). Its disadvantages, however, are the use of small amount of plant tissue from ultrathin sections and the laborious techniques required for preparation of test material. Due to these disadvantages of electron microscopy, simple histochemical techniques using fluorescence microscope for the detection of MLO infections in plants have been attempted by several researchers. Hiruki et al. (2) reported the usefulness of aniline blue stain for the diagnosis of MLO infections in sandal tree. Recently, Seemüller (6) demonstrated that DAPI (4'-6-diamidino-2-phenylindole·2HCl) was very effective for demonstrating MLO presence in pear and apple trees, thereby the efficiency of DAPI stimulating the study in this field subsequently (1,3,5). The objective of our researches was to evaluate the efficacy of three fluorochromes, i.e., DAPI, aniline blue and quinacrine (quinacrine mustard dihydrochloride) for the detection of MLO infections in jujube (*Zizyphus jujuba*), mulberry (*Morus alba*) and periwinckle (*Catharanthus roseus*) plants.

MATERIALS AND METHODS

Plants: Jujube tree samples diseased with witches' broom were collected in Boeun, Korea. Dwarf diseased-mulberry tree samples were provided from the Sericultural Experimental Station in Suweon, Korea. Periwinckle plants artificially inoculated with mulberry dwarf using insect vector, *Hishimonus sellatus*, were also used in this study. Healthy seedlings of jujube, mulberry and periwinckle plants were used as controls.

Staining procedures: DAPI staining procedure used in this experiment was performed by the method of Seemüller (6). Young stem samples were fixed in 5% glutaraldehyde in 0.1M phosphate buffer(pH7.0) or in 4% formalin at 4°C for 2 hours. After washing in the phosphate buffer, longitudinal or transverse sections of ca. 30 μm thick were made by free-hand and then stained in the phosphate buffer containing DAPI at a concentration of 1 μg/ml for 20 minutes. After rinsing with the phosphate buffer, they were mounted in the buffer and examined with a fluorescence microscope.

Aniline blue staining procedure was performed by using the modified method of Hiruki and Dijkstra (2). Free-hand sections were made soon after excising the stem or after fixing it in 5% glutaraldehyde or 4% formalin. The sections were fixed immediately by boiling in tap water for 3 minutes and then stained in 0.01% aniline blue in 1/15M K_2HPO_4, pH8.0 for 20 minutes. The preparations were examined under a fluorescence microscope.

After fixing stem samples in 5% glutaraldehyde or in 4% formalin for 2 hours, sections of ca. 30 μm thick were made and stained in quinacrine solution at the concentration of 0.0001% in distilled water for 20 minutes. Then, they were examined under a fluorescence microscope.

Fluorescence microscopy: The stained sections were examined using Olympus VANOX fluorescence microscope equipped with a super pressure mercury burner USH-200MB200W and set up with exciter filter UG1 or UG5 and barrier filter L420, L435 or L450.

RESULTS AND DISCUSSIONS

Efficacy of DAPI: When examined under the fluorescence microscope, DAPI-stained stem sections of diseased jujube and mulberry trees showed strong bluish fluorescence distributed only to the phloem (Fig. 1, 2). The similar strong fluorescence reaction was observed in the inner and outer phloem of periwinckle plant infected with mulberry dwarf mycoplasmas (Fig. 3-left). However, such fluorescence was absent in the phloem of stem sections from healthy jujube, mulberry and periwinckle plants (Fig. 1, 2).

DAPI has been known to possess specific binding properties with DNA of mycoplasmas and viruses

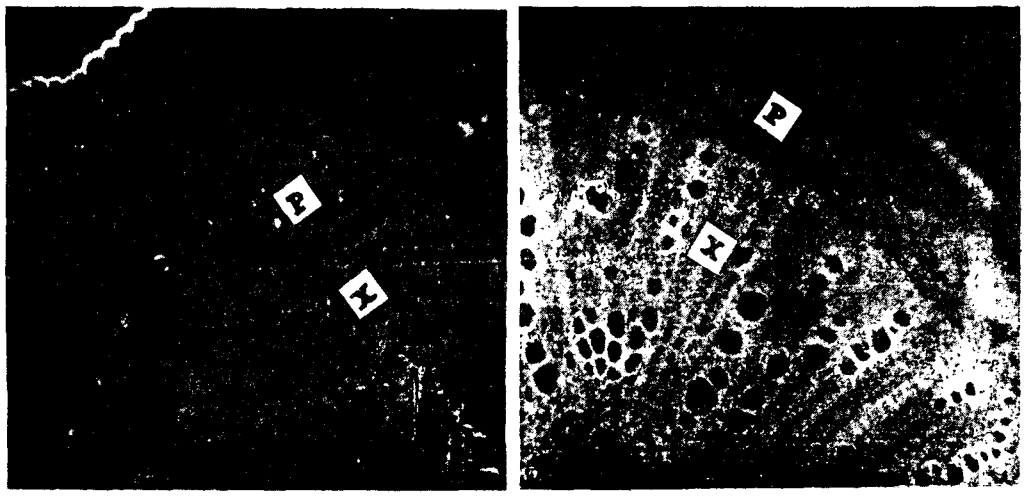

Fig. 1. Transverse sections of stems of *Zizyphus jujuba* stained with DAPI. (Left: diseased, Right: healthy). The fluorescent spots are visible in the phloem of diseased plant. P=phloem, X=xylem.

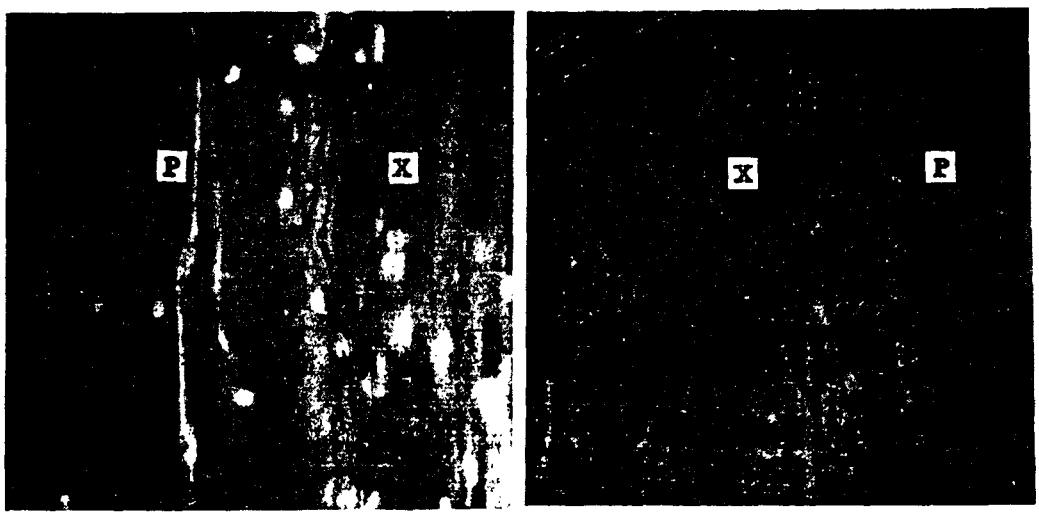

Fig. 2. Longitudinal sections of stems of *Zizyphus jujuba* stained with DAPI (Left: diseased, Right: healthy) The fluorescences are seen in the phloem of the diseased plant. P=phloem, X=xylem.

(4). In applying DAPI to jujube, mulberry and periwinckle plants affected with MLO diseases, the use of DAPI proved to be a considerably efficient method for detecting MLO in the diseased plants (Table 1). The fluorescence observed in the phloem of stem sections diseased was consistent with that described by other researchers (1,3,5,6). Because of the absolute absence of fluorescence in the phloem of healthy jujube. Mulberry and periwinckle plants, the specific fluorescence visible in the phloem of diseased plants is regarded as an evidence of MLO infections.

Efficacy of aniline blue: Specific fluorescent spots were also observed in the phloem of aniline blue-stained stem samples from diseased jujube, mulberry and periwinckle plants (Fig. 3-right), whereas such fluorescence was not detected in the phloem of healthy plant samples.

As aniline blue has been known to demonstrate not only MLO infections but also callose in the

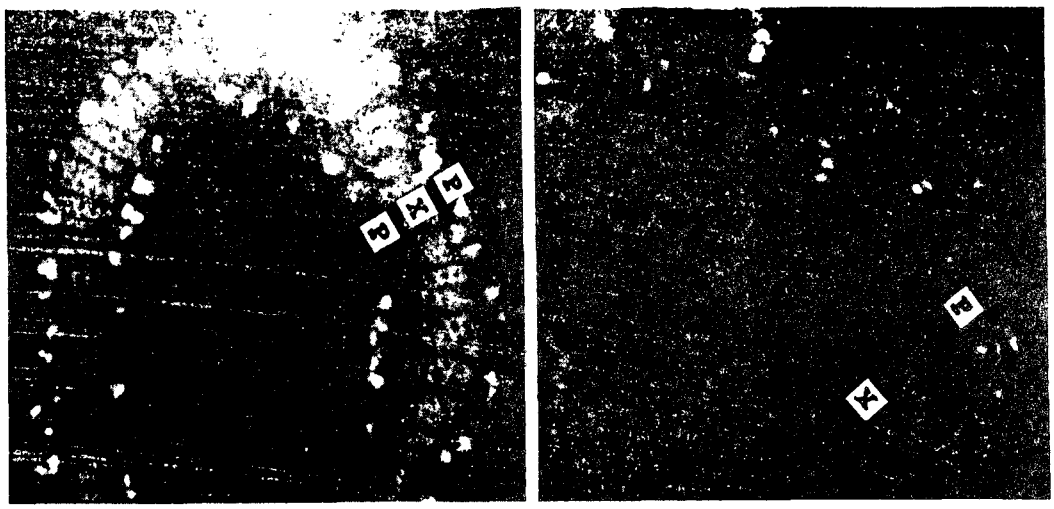

Fig. 3. Left: inner and outer phloems of diseased stem of *Catharanthus roseus* show the fluorescent spots after staining with DAPI. Right: aniline blue-stained section of diseased stem of *Zizyphus jujuba* demonstrates the fluorescent spots in the phloem. P=phloem, X=xylem.

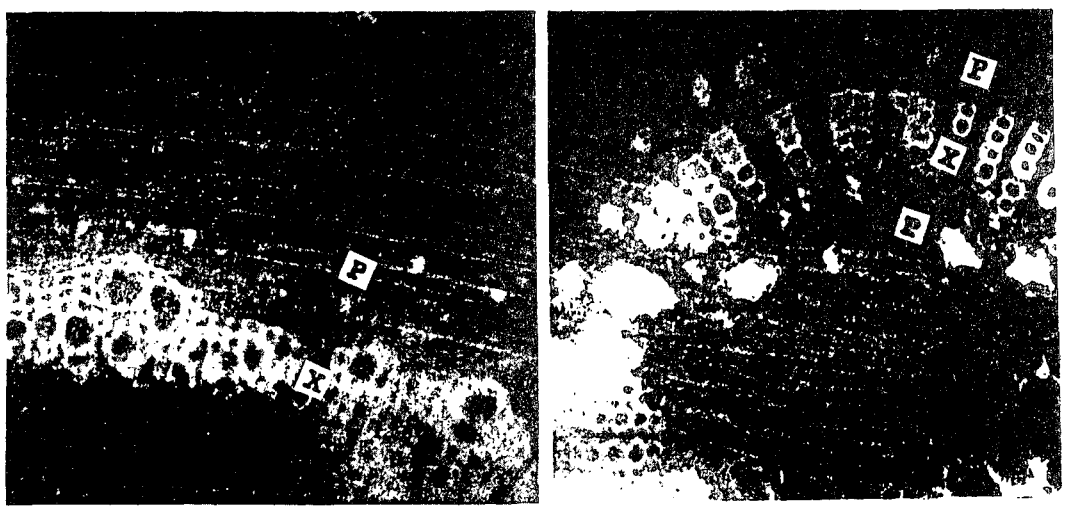

Fig. 4. The fluorescent spots are present in the phloem of diseased stems stained with quinacrine. Left: *Morus alba*, Right : *Catharanthus roseus*. P=phloem, X=xylem.

phloem (1,2), the possible presence of callose in healthy plant sections must be carefully interpreted when using aniline blue staining method for the diagnosis of MLO infection.

Efficacy of quinacrine: With quinacrine staining, specific fluorescence was observed in the phloem of the stem sections from diseased jujube, mulberry and periwinckle plants (Fig. 4), whereas it was not detected in that of the healthy plant stems. However, the fluorescence was less distinct compared with staining methods of DAPI or aniline blue. Quinacrine is generally used for detecting bacterial infections in the animal tissues. When applied to plant tissues, quinacrine was useful for demonstration of MLO infections in the diseased plants (Table 1).

Table 1. Efficacy of three fluorochromes for the fluorescence microscopic detection of MLO in plant stems

Fluorochrome	Jujube		Mulberry		Periwinckle	
	D[a]	H	D	H	D	H
DAPI	+~++[b]	–	+~++	–	+~++	–
Aniline blue	+	–	+	–	+	–
Quinacrine	+	–	+	–	+	–

[a] D: diseased, H: healthy.

[b] –: no fluorescence, +: distinct fluorescence, ++: strong fluorescence.

In conclusion, all the fluorochromes used in this study proved to be useful for detecting mycoplasma infections in jujube, mulberry and periwinckle plants. Among the three fluorochromes examined, DAPI was most effective. Thus, the use of DAPI in fluorescence microscopy may provide an efficient histochemical technique for rapid and accurate detection of MLO infections in these plants.

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REFERENCES

1. HIRUKI, C. (1981). Fluorescence microscopy in diagnosis of tree diseases associated with mycoplasmalike organisms (MLO). *Proceedings-Referate-Exposes (XVII IUFRO) Div.* 2:317-322.
2. HIRUKI, C. & DIJKSTRA, J. (1973). Light and electron microscopy of Vinca plants infected with mycoplasmalike bodies of the sandal spike disease. *Neth. J. Pl. Path.* 79:207-217.
3. PETZOLD, H. & MARWITZ, R. (1980). Ein verbesserter fluoreszenzmikroskopischer Nachweis für mykoplasmaähnliche Organismen in Pflanzengewebe. *Phytopath. Z.* 97:327-331.
4. RUSSEL, W. C., NEWMAN, C. & WILLIAMSON, D. H. (1975). A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. *Nature* 253:461-462.
5. SAMYN, G. & WELVAERT, W. (1979). The use of 4'-6-diamidino-2-phenylindol, 2HCl (DAPI) compared with other stains for a quick diagnosis of mycoplasma infections in ornamental plants. *Med. Fac. Landbouww. Rijksuniv. Gent* 44:623-628.
6. SEEMÜLLER, E. (1976). Fluoreszenzoptischer Direktnachweis von mykoplasmaahnlichen Organismen in Phloem pear-decline- und triebsuchtkranker Baume? *Phytopath. Z.* 85:368-372.