

マイコプラズマ様微生物による一新病害-Brugmansia candidaの萎縮病

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Little-Leaf Disease of *Brugmansia candida*, A New Disease Associated with Mycoplasma-Like Organisms*

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Abstract

Little-leaf, a new disease of *Brugmansia candida* was found in Brisbane, Queensland, Australia. Petiole and stem tissues of both affected and healthy plants as well as tomato plants, graft-inoculated and uninoculated, were subjected to histochemical examination and electron microscopy for possible association with mycoplasma-like organisms (MLO). Pleomorphic bodies of MLO were detected in the phloem elements of the diseased specimens but not in healthy plants. In fluorescence microscopy with a DNA specific stain, DAPI (4', 6-diamidino-2-phenylindole·2HCl), these MLO fluoresced specifically. Disease specific accumulation of callose in affected phloem cells was detected after staining hand sections with aniline blue.

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Key words : Mollicutes, ultrastructure, histochemistry.

Introduction

The genus *Brugmansia*¹⁷⁾, often treated as a section of *Datura*, is distributed in central and South America, Asia and North Africa. It is represented in Australia only by a few species in cultivation¹⁰⁾. *B. candida* Pers. is a woody shrub or small tree growing to a height of about 3 to 5 m. Because of its showy flowers, pendulous with peduncles 3 to 6 cm long, it is frequently planted as ornamental plant in gardens. The genus *Datura* and its relatives contain tropane alkaloids, especially scopolamine,²²⁾ and cases of poisoning in humans, especially children, are common in the cultivated *Brugmansia* species, which have also received attention recently through their use as hallucinogenic drugs¹⁰⁾.

In February, 1985, *B. candida* cv. Angel's Trumpet cultivated in the Brisbane Botanic Gardens, Mt. Coot-tha Road, Toowong, Brisbane, Queensland, was found to display little-leaf symptoms, characteristic of a mycoplasma disease. In an attempt to determine if mycoplasma-like organisms (MLO) were associated with the disease, healthy plant material and diseased specimens were subjected to both electron and light microscopy.

This paper reports the results of the observations as well as evidence of graft transmission of the disease agent.

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Materials and Methods

***B. candida* little-leaf agent.** Samples of *B. candida* showing little-leaf symptoms as well as samples of healthy plants were collected from the Brisbane Botanic Gardens. The pathogen was graft-transmitted to healthy tomato (*Lycopersicon esculentum* L. cv. Grosse Lisse). Infected plants which displayed big-bud symptoms were tested for the presence of viruses by electron microscopy and by mechanical inoculation to indicator plants *Nicotiana glutinosa* L., *N. clevelandii* Gray, *Gomphrena globosa* L., *Chenopodium quinoa* Willd., *C. amaranticolor* Coste and Reyn., *Vigna unguiculata* (L.) Walp. cv. Blackeye and *Phaseolus vulgaris* L. cv. Bountiful.

Graft transmission. Healthy tomato plants, about 15 cm high, grown from seed were excised at about 5 cm from the soil level and were wedge-grafted with a scion from diseased *B. candida*. The grafted plants were covered with a plastic bag to maintain high relative humidity for about a month. A union was considered successful if the scion survived for four weeks in the greenhouse.

Histochemistry. Two histochemical techniques were used for locating MLO or MLO-infected cells in hand sections, about 25 μ m thick, of stem and petiole tissue samples. For DNA staining, sections were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.0 for 2 h. After rinsing, they were stained in a solution (1 μ g/ml) of 4', 6-diamidino-2-phenylindole $\cdot$ 2HCl (DAPI, Sigma Chemical Co., St. Louis, MO, USA.). Sections mounted in the DAPI solution were examined for DNA-specific fluorescence. For aniline blue staining, hand sections were fixed either in boiling water for 2-3 min or in a 5% glutaraldehyde solution for 2 hr, rinsed, and stained in 0.01% aniline blue in 1/15 M K_2HPO_4 pH 8.0 for 20 min. The sections were mounted in a drop of aniline blue and examined under an Olympus Reflected Light fluorescence microscope, BHS-RF-A with an HBO 100W/2 mercury lamp, an excitation filter UG1, a dichroic mirror U(DM-400+L-410) and a barrier filter L-420.

Electron microscopy. Small pieces, about 2 mm $\times$ 2 mm, of main veins from healthy and little-leaf diseased plants were cut and fixed in 3% glutaraldehyde in 0.025 M Sørensen's buffer containing 0.24 M sucrose overnight at room temperature, then washed four times in 0.025 M Sørensen's buffer containing 0.25 M sucrose and twice in 0.025 M buffer alone at 4 C. The material was postfixed in 1% osmium tetroxide in 0.25 M Sørensen's buffer containing 0.25 M sucrose for 4 h at 4 C, dehydrated at room temperature through a graded acetone series, and placed in two changes of propylene oxide. After removal from propylene oxide, the tissue was embedded in Spurr's resin²⁶⁾. Sections were cut on an LKB Ultratome III equipped with a glass knife, and stained with uranyl acetate and lead citrate. Micrographs were taken with a Hitachi H-180 electron microscope at 120 kV.

Results

Symptomatology

B. candida plants with little-leaf symptoms showed severe stunting, extremely short

internodes and general yellowing of leaves, which were smaller in size and more numerous than normal. The development of the little-leaf symptoms was irregular and severely affected branches were often suppressed under apparently normal looking branches (Figs. 1 and 3). Affected plants rarely produced flowers and even when flowers were formed, their development was morphologically abnormal and their colour was greenish, whereas healthy plants produced vigorous green leaves and numerous, attractive flowers (Fig. 2).

Symptoms of tomato plants artificially inoculated by grafting were cessation of apical growth, reduction in internode growth, and general yellowing of leaves. Floral buds were never formed after inoculation. Secondary shoots formed after graft-inoculation developed big-bud symptoms (Fig. 4).

Graft transmission of the *B. candida* little-leaf agent to tomato plants

Of 5 grafts from little-leaf affected *B. candida* plants to tomato plants, 3 were successful and developed disease symptoms in 4 weeks. Five tomato plants were grafted with healthy *B. candida* scions, and all five remained healthy.

Histochemistry

After staining with DAPI, stem-internode sections from healthy *B. candida* plants showed two kinds of UV fluorescence: small fluorescent spots scattered outside of xylem elements and autofluorescence of xylem walls (Fig. 5a). No abnormal fluorescence was observed in the phloem elements. In comparable sections from infected plants, pale to bright yellow fluorescence was observed in both the external and internal phloem elements. DAPI staining of the nuclei was seen as small, faint fluorescent spots. Xylem elements had bluish autofluorescence (Fig. 5b). After staining with aniline blue, sections of petioles from healthy and diseased plants showed characteristic differences in the accumulation of callose in the phloem elements (Figs. 5c, 5d).

Hand-sections from graft-inoculated tomato plants which displayed typical big-bud symptoms suggested substantial concentration of MLO in both external and internal phloem elements in DAPI staining. DAPI fluorescence was intensive and widely distributed in the phloem regions (Fig. 6).

Electron microscopy

Many of the sieve elements observed in both naturally infected *B. candida* plants and experimentally infected tomato plants contained numerous, typically pleomorphic MLO (Fig. 7). No helical forms were observed. Neither viruslike particles nor other plant pathogens were found. MLO were not found in sections from healthy *B. candida* and tomato plants.

Other tests

No evidence of virus infection was obtained by bioassays and electron microscopy. Further light microscopic examinations of diseased leaf, stem and root tissues did not yield evidence of infection by other pathogens such as bacteria, fungi and nematodes.

Discussion

In this investigation, the association of MLO with the little-leaf disease of *B. candida*

was established by graft-transmission and by electron microscopy as well as by histochemistry with DAPI and aniline blue as fluorochromes. This is the first report of an MLO-associated disease affecting *B. candida*.

DAPI was first synthesized as a trypanocide³⁾. The value of DAPI as a fluorescent DNA probe was quickly recognized^{16,19,23,27)} and DAPI has been widely used for practical diagnosis of diseases of trees and shrubs^{4,5,12,21)} as well as herbaceous species^{13,18,20,24)} thought to be associated with MLO. DAPI is particularly useful for those MLO diseases from which causal agents have not been isolated. While there is no problem with DAPI concerning background fluorescence due to nonspecific reactions, there are certain reactions of host components with DAPI that are possibly influenced by host species and perhaps plant age or physiological condition of the host plant. In this investigation, small fluorescent spots were found scattered in the sections from healthy plants (Fig. 5a). A similar observation was previously reported on *Hydrangea* sp.²⁰⁾. Since DAPI is highly specific to DNA, host organelles such as nuclei,²⁾ mitochondria,²⁷⁾ plastids²⁾ and DNA viruses¹⁹⁾ are stained by DAPI and detected as fluorescent foci. Generally speaking, however, in histochemical studies, sections are examined at low magnifications and small faint fluorescent spots do not interfere with assessment of the results.

When aniline blue was used as a fluorochrome, differentiation between healthy (Fig. 5c) and diseased plants (Fig. 5d) was done quickly and easily. Similar results were obtained with a variety of hosts^{6-8,25)}. Careful fixation was an important factor in avoiding callose accumulation in healthy plant samples that is unrelated to MLO infection^{12,14,15)}.

On the basis of the characteristic growth habit, particularly abundant production of abnormally small leaves, the name "little-leaf" is proposed for the abnormality of *B. candida* reported in this paper. Since *B. candida* is not native to Australia, its cultivation in Australia appears to be limited to display gardens and experimental plots. No information is available on the geographical distribution of the disease other than that it is present in Queensland, Australia. However, *B. candida* is no doubt highly susceptible to MLO infection.

The interrelationships of MLO observed in *B. candida* and those previously reported from other plant species in Queensland such as tomato and legumes,¹⁾ eggplant¹¹⁾ and native rosella hibiscus (C. Hiruki unpublished data) have not yet been established. It will be of interest to investigate leafhopper-vector relationships⁹⁾ as well as a possible relationship between this disease and both eggplant little-leaf and potato purple top, since the disease agents of these three diseases cause similar big-bud symptoms of tomato.

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Literature cited

1. Bowyer, J. W. (1974). J. Agr. Res. 25 : 449-457.
2. Coleman, A. W., Maguire, M. J. and Coleman, J. R. (1981). J. Histochem. Cytochem. 29 : 959-968.
3. Dann, O., Bergen, G., Demant, E. and Volz, G. (1971). Liebigs Ann. Chem. 749 : 68-89.
4. Deutsch, E. and Nienhaus, F. (1983). Z. PflKrankh. PflSchutz 90 : 278-284.
5. Douglas, S. M. (1984). Phytopathology 74 : 798-799.
6. Ghosh, S. K., Varma, A., Raychaudhuri, S. P. and Sang, A. (1974). Ind. J. Exptl. Biol. 12 : 586-587.
7. Ghosh, S. K., Ali, M. I. M. and Balasundaran, M. (1984). Phytopathol. Z. 110 : 207-212.
8. Gocio, M. J. and Dale, J. L. (1982). Plant Disease 66 : 244-245.
9. Grylls, N. E. (1979). In 'Leafhopper Vectors and Plant Disease Agents'. (Maramorosch, K. and Harris, K. F., eds.). Academic Press, New York. pp. 179-214.
10. Haegi, L. (1976). Aust. J. Bot. 24 : 415-435.
11. Harding, R. M. and Teakle, D. S. (1985). Aust. J. Agric. Res. 36 : 443-449.
12. Hiruki, C. (1981). Proc. XVII IUFR0 World Congress, Div. 2, pp. 317-322.
13. Hiruki, C. and da Rocha, A. (1984). Can. J. Plant Pathol. 6 : 262.
14. Hiruki, C. and Shukla, P. (1973). Phytopathology 63 : 88-92.
15. Hiruki, C., Giannotti, J. and Dijkstra, J. (1974). Neth. J. Pl. Path. 80 : 145-153.
16. Kania, J. and Fanning, T. G. (1976). Eur. J. Biochem. 67 : 367-371.
17. Lockwood, T. E. (1973). Bot. Mus. Leaflet. Harv. Univ. 23 : 273-283.
18. Petzold, H. and Marwitz, R. (1980). Phytopathol. Z. 97 : 327-331.
19. Russell, W. C., Newman, C. and Williamson, D. H. (1975). Nature 253 : 461-462.
20. Samyn, G. and Welvaert, W. (1979). Med. Fac. Landbouww. Rijksuniv. Gent 44 : 623-630.
21. Schaper, U. and Converse, R. H. (1985). Plant Disease 69 : 193-196.
22. Schultes, R. E. and Hofmann, A. (1973). In The Botany and Chemistry of Hallucinogens. Thomas, C. C. (ed.), Springfield, Ill., U.S.A. pp. 166-181.
23. Schweizer, D. (1976). Exp. Cell Res. 102 : 408-413.
24. Seemuller, E. (1976). Phytopathol. Z. 85 : 368-372.
25. Silvere, A. -P. A. (1983). Sel'sk. Biol. 9 : 108-112.
26. Spurr, A. R. (1969). J. Ultrastruct. Res. 26 : 31-43.
27. Williamson, D. H. and Fennell, D. J. (1975). In Methods in Cell Biology (Prescott, D., ed.), Vol. 12. Academic Press, New York pp. 335-351.

和 文 摘 要

比留木忠治：マイコプラズマ様微生物による一新病害—*Brugmansia candida* の萎縮病

マイコプラズマ様微生物 (MLO) による一新病害 *Brugmansia candida* の萎縮病がオーストラリア国クイーンズランド州ブリスベン市において発見された。罹病 *B. candida* およびその健全植物ならびに接木接種したトマトと非接種のトマトから採取した葉柄と茎について、組織化学的検査と電子顕微鏡観察をおこない、本病と MLO との関連について検討した。罹病組織の篩管部には MLO が見出されたが、健全植物には同様のものは認められなかった。DNA 染色剤 DAPI (4',6-diamidino-2-phenylindole·2HCl) を用いた蛍光顕微鏡観察では、これらの MLO に DNA 特有の蛍光発色がみられた。罹病植物の篩管部に aniline blue 染色により callose の異常集積による蛍光が見出された。

Explanation of plates**Plate I**

- Fig. 1. Little-leaf symptoms of *Brugmansia candida*.
Fig. 2. Healthy *Brugmansia candida* with numerous flowers.
Fig. 3. A close-up photograph showing little-leaf symptoms of a severely affected shoot of *Brugmansia candida*.
Fig. 4. Big-bud symptoms of tomato (large arrow) after graft-inoculation with a scion (small arrow) from a little-leaf diseased *Brugmansia candida*. An arrow head shows the graft-union.

Plate II

- Fig. 5. Fluorescence micrographs of *Brugmansia candida*.
(a) A healthy stem section stained with DAPI.
(b) A stem section from a little-leaf diseased plant stained with DAPI.
(c) A healthy petiole section stained with aniline blue.
(d) A petiole section from a little-leaf diseased plant stained with aniline blue.
Bars represent 100 μm .
Fig. 6. Fluorescence micrograph of a transverse section, stained with DAPI, of tomato graft-inoculated with the little-leaf agent from *Brugmansia candida*. Bar represents 100 μm .
Fig. 7. Electron micrograph showing mycoplasma-like organisms in a transverse section of the sieve tube of young stem of little-leaf diseased *Brugmansia candida*. Bar represents 1 μm .

Plate I



Plate II

