ДИАГНОСТИКА НА ФИТОПЛАЗМЕНА БОЛЕСТ ПО ДЕКОРАТИВНОТО И МЕДИЦИНСКО РАСТЕНИЕ ВИНКА *CATHARANTHUS ROSEUS* (L.) G. DON. В БЪЛГАРИЯ
DIAGNOSTICS OF PHYTOPLASMA DISEASE ON THE ORNAMENTAL AND MEDICINAL PLANT PERIWINKLE *CATHARANTHUS ROSEUS* (L.) G. DON. IN BULGARIA

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Abstract

Periwinkle *Catharanthus roseus* (L.) G. Don*.,* family *Apocynaceae* is one of the most important medicinal and ornamental plant.

Periwinkle is grown commercially for its medicinal uses in different countries. It has medicinal value owing to the presence of alkaloids such as ajamalicine, serpentine and reserpine, which are well known for their hypotensive and antispasmodic properties.

The anti-cancer principles namely vincristine and vinblastine are also produced from periwinkle.

Periwinkle is also cultivated as an ornamental plant almost throughout the tropical and subtropical world and used in plant pathology as an experimental host for phytoplasmas.

In recent years many periwinkle plants can be seen in parks and gardens in many locations in Bulgaria. Some of them have symptoms of phytoplasma infection.

The diagnosis of the disease is based on symptoms of the plants and later on confirmed by electron microscopy method.

Key words: periwinkle *Catharanthus roseus* (L.) G. Don, phytoplasma, electron microscopy, Bulgaria.

INTRODUCTION

Periwinkle *Catharanthus roseus* (L.) G. Don is also called vinca or myrtle. *C. roseus* originates from the Indian Ocean island of Madagascar. This herb is now common in many tropical and subtropical regions a worldwide.

Madagascar periwinkle is a common decorative, easy growing and spreading perennial herb. As many other plants of the dogbane family, the Vincas are poisonous, although mildly. During the 1950s, *Catharanthus roseus* was discovered to contain a number of chemicals in the alkaloid class. There are also 2 drugs extracted from it; vinblastine (used in the treatment of Hodgkins' disease) and vincristine (used in the treatment of leukemia) (Duke, 1985).

Periwinkle *Catharanthus roseus (* L.) G. Don is an ornamental plant reported in different world regions as a natural phytoplasma host showing symptoms such as little leaf, witches'-broom, yellowing, virescence and phyllody.

The presence of phytoplasma disease poses a new threat to the periwinkle in Bulgaria.

Typical diseases include phyllody, little leaf, dense clusters of highly proliferating branches with shortened internodes.

Periwinkle plants showing severe chlorosis and little-leaf symptoms were observed in Bulgaria.

Catharanthus roseus (L.) G. Don, a high-valued medicinal plant, is severely affected by phytoplasmas present in the sieve tubes of the phloem. The infected plant shows malformation of leaves and flowers as well as virescence of flowers.

The presence of phytoplasma bodies in the ultrathin section of the midribs was confirmed by transmission electron microscopy.

MATERIALS AND METHODS

Some periwinkle plants naturally infected with phytoplasma were collected from public and private gardens in Plovdiv and in the suburbs.

The pathogens were maintained by successive grafting to healthy periwinkle plants produced by cuttings and grown in a growth chamber under controlled conditions. Healthy periwinkle plants were also kept in the growth chamber to be used as negative controls.

Symptoms and disease progress were periodically checked in the grafted plants.

Tests for transmission by grafting were conducted using infected periwinkle plants showing symptoms of phyllody, virescence, proliferation, little leaf and flower abortion.

Scions from naturally infected periwinkle were grafted into 7 healthy periwinkles by side grafting. The graft-inoculated area in which the diseased scion was inserted was then bound tightly with plastic tape and was covered with plastic bag for 1 week to minimize dehydration. The grafted plants were then kept in the growth chamber at about 25-30°C until symptoms appeared.

Observations were also recorded on season and the type of symptoms produced.

For electron microscopy analyzes samples were fixed in 2,5 to 3 percent gluteraldehyde in 0,1 M phosphate buffer (pH = 7,2) for 24 h at 4°C and post fixed in 2 percent aqueous osmium tetra oxide in the same buffer for 2 h. Dehydrated in series of graded alcohols, infiltrated and embedded in araldite resin or spur resin (Spurr, 1969). Ultrathin (50 to 70 nm) sections were made with a glass knife on ultramicrotome LKB, mounted on copper grids and stained with saturated aqueous uranyl acetate and counter stained with Reynolds lead citrate (Bozzola and Lonnie, 1998). Viewed under TEM (Model: Hitachi, H-7500 from Japan) at required magnifications as per the standard procedures at Lab of Moscow Botanical Garden, Academy of Sciences, Russia.

RESULTS AND DISCUSSION

Results of the tests for transmission by grafting indicated that the disease is contagious. The periwinkles grafted with buds from diseased periwinkles, showed phyllody, virescence, proliferation, noticeably reduced leaf size and diminished plant development.

The periwinkle plants grafted with buds from healthy periwinkles had no symptoms.

In the field conditions, phytoplasma disease of periwinkle showed reduction in the height i.e., stunted growth with bushy appearance. Most typical symptom was phyllody, witches' broom and little leaf. Other symptoms observed on infected plants were chlorosis, sparse foliage, dull yellowing of the older and malformation of the younger leaves.

The percent disease incidence varied from location to location and even at some places it was zero. The variation in disease incidence in different areas might be because of the prevalence of different climatic condition favouring multiplication and migration of the vectors responsible for the spread of the disease.

In this study phytoplasmas were detected and characterized from naturally infected periwinkle plants grown in different locations in Plovdiv, Bulgaria.

The collected periwinkles exhibited typical symptoms of phytoplasma such as phyllody and virescence.

The pathogenicity of the phytoplasma was confirmed by graft transmission, as they were transmitted from diseased to healthy periwinkle plants by grafting and the symptoms of experimentally infected periwinkles appeared similar to naturally infected periwinkle.

The presence of phytoplasma bodies in the ultrathin section of the midribs was confirmed by transmission electron microscopy. The electron micrograph showed typical pleomorphic phytoplasma bodies inside the phloem cells of ultrathin section of the midribs. Because of the inability to obtain pure cultures of any phytoplasma, their detection and identification were never precise. The presence of characteristic symptoms in diseased plants and subsequent observation of phytoplasma bodies in ultrathin sections of diseased plants were the main criteria used to diagnose diseases of possible phytoplasmal origin (Lee et al., 2000).

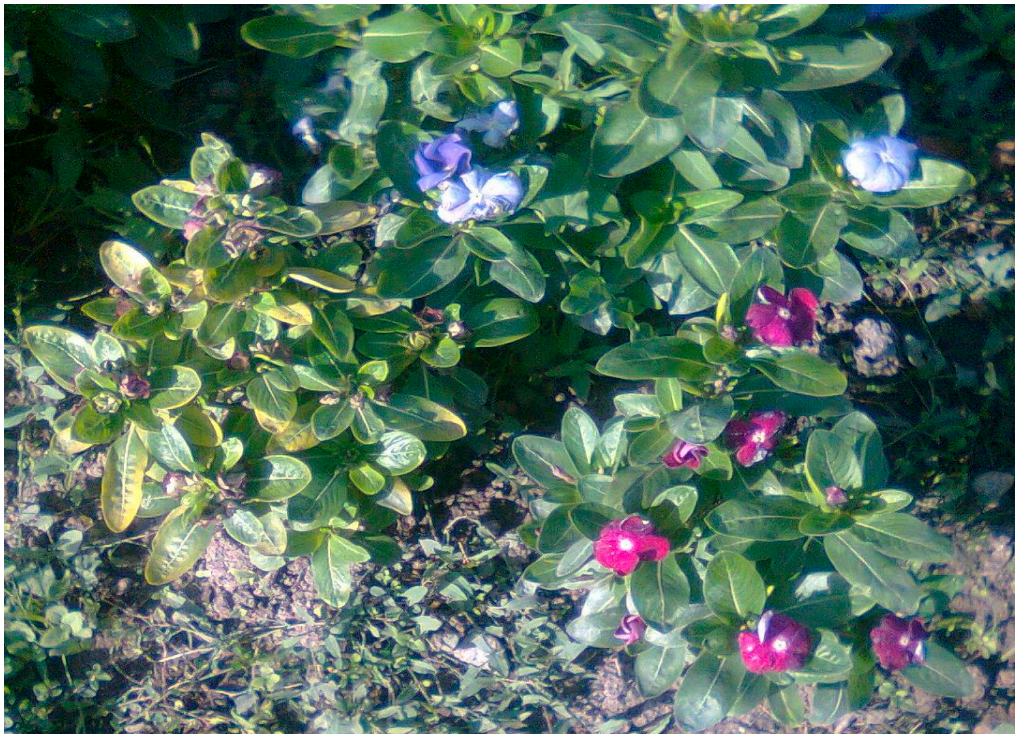

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Fig. 1. *Naturally infected with phytoplasma periwinkle Catharanthus roseus* (L.) *G. Don*

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Fig. 2. *Naturally infected with phytoplasma periwinkle Catharanthus roseus* (L.) *G. Don*

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Fig. 3. *Symptoms of phytoplasma disease on periwinkle Catharanthus roseus* (L.) *G. Don., experimentally infected by grafting*

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Fig. 4. *Symptoms of phytoplasma disease on periwinkle Catharanthus roseus* (L.) *G. Don. Virescence of flowers*

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Fig. 5. *Symptoms of phytoplasma disease on periwinkle Catharanthus roseus* (L.) *G. Don. Phyllody, virescence, proliferation, little leaves*

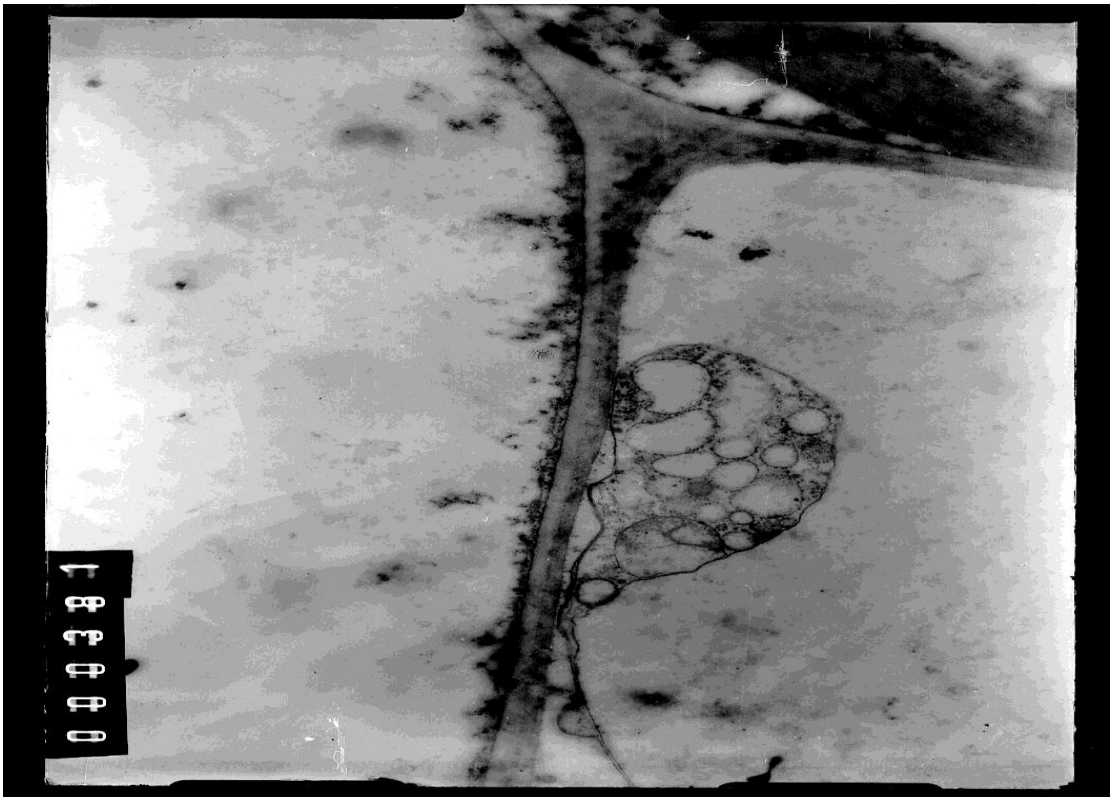

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Fig. 6. *Phytoplasma bodies in the ultrathin section of the midribs of periwinkle Catharanthus roseus* (L.) *G. Don*

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