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DETECTION OF PETUNIA PHYTOPLASMA IN ELMANSOURA – DAKAHLIA, EGYPT

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ABSTRACT

Phytoplasma like symptoms, foliar yellowing, witches' broom and phyllody were observed on diseased petunia hybrid vilm. In the gardens of El-Mansoura University, Egypt.

Grafting had been succeeded in transmitting the causal agent but not by infectious crud sap inoculation or by the aphid *Myzus persicae*. Light microscopy of dienes stained free hand section revealed staining of cambium with blue colore. Electron microscopy study confirmed the presence of phytoplasma in phloeim of diseased petunia flower petioles. Phytoplasm bodies were pleomorphic and they were not found in phloeim of healthy plants.

Results showed that spraying diseased petunia twice with oxytetracycline (200 ppm) caused disease recovery.

Keywords: Phytoplasma; petunia; symptoms; detection; dienes' staining; histology and Electron microscopy.

INTRODUCTION

Petunia (*Petunia hybrida* Vilm.) is one of the most winter ornamental flowering and gardening plants in Egypt and all over the world.

Such plants are susceptible to several pathogens including fungi, bacteria, virus, nematodes and recently prokariotical pathogens. (Maramorosch, *et al.* 1970), (Davis and Whitoomb, 1971), (Hiruki and Chen,1978); (Hussein and Freigoun,1978); (Thomas,1979) and (Agrios, 1988).

Phytoplasma is one of the most destructive pathogens which attacks certain plants including petunia and might reduce the flower yield. (Maramorosch, *et al.* 1970).

The present study was undertaken in El-Mansoura, Dakahlia government, Egypt, for the detection of phytoplasma pathogen of petunia plants, by using light and electron microscopy and certain biological properts under greenhouse conditions.

MATERIALS AND METHODS

Plant materials:

Sampels of phytoplasma like disease on naturally infected *Petunia hybrida* were collected from El-Mansoura University gardens and nurses. Then, they were transplanted into plastic pots with their soil. Transplanted petunia was saved for further study under insect proof greenhouse condition. The disease is characterized by the development of abnormal growth on

shoots (witches broom) or on petunia flowers (Phyllodey) and also on the petunia tissues (yellowing) Photo (1).

Different samples from both healthy and infected flowers, roots and leaves were collected for histological study of infected petunia tissues throughout light and electron - microscopy. Samples were fixed in 3% glutraldhyde solution for few hours before testing.

Phytoplasma causal pathogen had been detected based on the following means: Symptoms and symptomatology,modes of transmission (sab, insect and grafting), histopathological studies by light and electron microscope and oxytetracycline sensitivity. (Jones and Cockain, 1984).

Sap transmission:

Sap inoculum was prepared by grinding young infected petunia leaf tissues in 0.02 M phosphate buffer solution (pH 7.2) then rubbed on healthy carborandium dusted leaves of petunia seedlings. Inoculated plants were left for symptoms development.

Grafting transmission:

N-shaped grafting methods was performed by shaping a chip of small petunia twig from diseased petunia, which inserted into cleft made in the stem of healthy petunia seedlings and the wounds bound with parafilm sealing tape. The grafted plants were then kept in greenhouse. Grafted plants received normal practical processes of irrigation, foliar fertilization and nutrition. When the new growth was developed, it has been examined for phyotplasma transmission (Bos, 1967).

Histopathological studies:

This study was done on different organs of both healthy and diseased petunia plants (roots, leaves and flower petioles). The collected samples were prepared for examining by light microscope.

Free hand sections were prepared under distilled water using sharp blade. The sections were transferred to Diene's stain (Deeley *et al.*, 1979), which was prepared by dissolving 2.5 g of methylene blue, 1.25 g of azure II 10.0 g of maltose and 0.25 g of sodium carbonate in 100 ml distilled water, pH was adjusted to 7.2 according to Deeley *et.al.* (1979) and Hibben *et al.* (1986). The stained sections were washed and mounted in distilled water then examined by the light microscope.

Electron microscopy:

2.0 x 0.5 mm pieces of young flower petiole from phylloidy affected and healthy petunia were mounted in 3% glutaraldehyde/3 h in 0.05 M phosphate buffer pH 7.0. Then rinsed twice in the same buffer and left overnight in 1% aqueous Osmium tetra oxide (O_sO_4). two mm square blocks of the tested tissues were cut and fixed for 2h in 3% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2. Tissues were rewashed in the buffer and post fixed for 3h. at room temperature in 1% osmium tetraoxide, then were dehydrated by passing throughout a graded ethanol series. After two rinses in propylene oxide for 15 min. each. he tissues were embedded in Poly/Bed 812. Ultra thin sections, were cut with ultramicrotome, then stained with urinaile acetate and lead

citrate. These stained sections were examined with Joel 100s transmission electron microscope at 60 Kv. Healthy petunia ultrathin sections were used as control (Jones and Cockbain.1984).

Insect transmission:

Aphid (*Myzus persicae* Sulz.) insects were used in this test. Tested aphid were starved for 1 hour, allowed for 20-minutes for acquisition feeding period, and then transferred with fine hair brush to healthy test plants (4 aphids/plant) for 24 hours. Then sprayed with 0.15% malathion for aphid killing under greenhouse conditions. Tested petunia plants were left for 3 weeks for symptoms development. (Maramorosch *et.al.* (1970).

Tetracycline treatments:

The influence of oxytetracycline on the phytoplasma diseased petunia was conducted under greenhouse conditions. Two successive Foliar sprying of 200 ppm oxytetracycline were carred out. Control plants were sprayed with distilled water only. Plants were observed periodically for about 6 weeks and results were recorded (McCoy, 1982).

RESULTS

Different symptoms yellowing, phylloidy and witches broom were expressed on the naturally infected petunia plants growing in El-Mansoura University gardens and nurses.

Symptoms of the causal pathogen were usually associated with weakening petunia plants, abnormal green structures (Phelloidy), folial yellowing of shoot system and development of abnormal growth of basal and axillary shoots (wiches broom growth). Similar symptoms had been described on petunia by Jones and Cockain (1984) in sudan on the phylloidy diseased broad bean.

Sap transmission:

No symptoms had been expressed in the mechanically inoculated petunia plant with phytoplasma infectious sap.

Insect transmission:

The causal agent of phytoplasma diseased plants could not be transmitted by aphids (*Myzus persicae* Sulz.) to healthy petunia seedlings since no symptoms were observed on the tested plants.

Grafting transmission:

Phytoplasma causal pathogen was transmitted by grafting technique. The new developed growth on the grafted shoot, showed yellows symptoms similar to those of naturally infected plants.

Histopathological studies:

Light microscopy:

Light microscopy using Dienes stained free hand section of stem phyllody diseased petunia showed a blue colour in the infected phloem. No blue colour was appeared in cambium, of healthy plants which were used as control in this study (Photo 2).

Electron microscopy:

Ultra thin section of phyllody diseased plants revealed the presence of phytoplasma in flower petioles, pleomorphic. Phytoplasma bodies were observed in the phloem elements. No phytoplasma bodies were observed in control healthy tissues (Photo 3).

Tetracycline treatment:

Results of two successive foliar spraying with 200 ppm oxytetracycline caused recovery of the symptoms on the new leaflets of the infected petunia.

Photo1. Natural phyllodey disease of petunia
a) healthy
b) infected

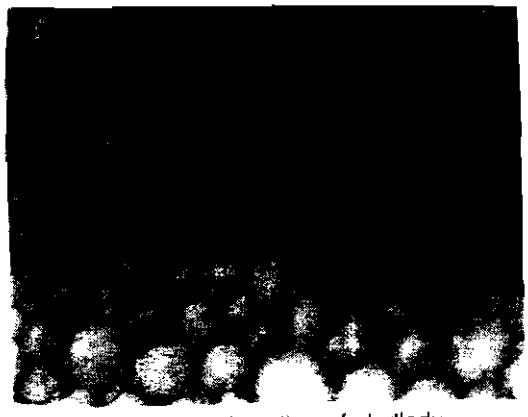

Photo 2. Free hand section of phyllody infected petunia stem showing that the phloem tissue stained blue after staining with Dienes stain

Fig 1 A: Transverse section in diseased petunia plants phloem cells showing phytoplasma organisms (x27000)

B) Transverse section in phloem containig phytoplasma of phyllody infected petunia (x20000)

C) Transverse section in healthy petunia plants showing ploem cells without phytoplasma organisms (x27000)

DISCUSSION

On the basis of symptoms, sap, insect and grafting transmission. light and electron microscope as will as effect of oxytetracycline, the causal pathogen of disesed petunia under study was identified as phytoplasma.

The symptoms of diseased petunia expressed as yellowing, witches broom and phyllody (green flowers) are similar to those indused by phytoplasma as recrded by Nour, (1962); Hussein and Freigoun, (1978); Nienhaus and Sikora,(1979); Jones and Cockbain,(1984) and Hibben et.al. (1986).

Present work show that, phytoplasma was not transmitted mechanically or by aphid. Such results are in agreement with others Maramorosch, (1970).

Our study succeeded to transmit the causal pathogen by grafting. This finding was previously reported by other researchers Bos,(1967) and Deeley *et.al.*,(1979). Electron microscopy revealed that the phytoplasma organisms were associated directly to phloem tissues of infected petunia. In addition staining of cambium with blue colore in free hand section preparation supported the evidence that the causal pathogen from diseased petunia was phytopolasma organisms Deeley *et.al.*,(1979) and Tones and Cockbin, (1984).

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تشخيص الميكوبلازما على نباتات البتونيا المنزرعة فى المنصورة – دقهليـــة – مصر.

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تم مشاهدة أعراض تشبه أعراض الميكوبلازما على المجموع الخضرى وهى الأصفرار – تشوهات التفريع – الزهرة الغير ملونة الخضراء وذلك على نباتات البتونيا المريضة فـــى حدائـــق جامعة المنصورة –جمهورية مصر العربية.

نجح التطعيم فى نقل المسبب المرضى للمرض ولكن لم ينجح النقـــل بواســـطة العـــدوى بالعصير المعدى وكذلك عن طريق حشرة من الخوخ.

الفحص بالميكروسكون الضوئى للقطاعات اليدوية المصبوغة لأعناق الأزهار أظهر لـــون أزرق فى منطقة الكامبيوم مما يدل على وجود الميكوبلازما فى الأنسجة.

أكدت دراسات الميكرسكوب الإليكترونى وجود الميكوبلازما فى نسيج اللحـــاء لقطاعـــات أعناق الأزهار وكانت أجسام الميكوبلازما متعددة الأشكال ولم تكن موجودة فـــى لحـــاء النباتـــات السليمة.

أوضحت النتائج أن رش نبات البتونيا المصابـــة مرتيـــن متتـــاليين بمركبـــات الأوكســـى تتراسيكلين بتركيز ٢٠٠ جزء فى المليون أدى إلى شفاء الأوراق الجديدة لنباتات البتونيا المصابة.