Bulletin of Insectology ***60*** *(2): 377-378, 2007*
ISSN 1721-8861

Occurrence, distribution and detection of potato purple top phytoplasma disease in the Punjab (Pakistan)

Mian Muhammad NASIR, Sardar Muhammad MUGHAL, Sultan Mahmood KHAN
Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Abstract

The incidence in Punjab of potato purple top phytoplasma disease, ranged between 4.1 to 80.1% in seven locations, with a mean incidence of 20.7% which was mainly attributed to the use of infected tubers. The disease could be easily identified on the basis of proliferation of axillary buds and mass production of aerial and under ground tubers which are purplish in colour, malformed in shape and unmarketable. Evidence of phytoplasma presence was achieved by positive reaction with Diene's and gallocyanine-chromalum stains in the free hand cut thin sections under light microscope. Antibiotics of tetracycline group almost completely restored the dormancy of infected tubers.

Key words: Phytoplasma, proliferation of axillary buds, weed hosts, tetracycline, emerging disease.

Introduction

Phytoplasmal diseases described in potato in the world include: potato purple top (PPT), witches' broom, stolbur, marginal flavescence, virescence, hairy sprouts (Salazar and Jayasinghe, 1997). PPT was considered as a major and widely distributed disease, responsible for colossal loss to potato production. The disease syndrome was first recognized in Canada in 1933, and by 1953, 20-70% of the seed failed to produce normal crop. In Mexico and Guatemala, PPT has been catalogued as the second most important disease of potato after late blight (Cadena-Hinojosa, 1996). The disease is widely distributed in India and causes 15-80% loss in yield (Giri and Nagaich, 1971). In Pakistan, PPT was first observed in 1986 and then described by Shafiq *et al.* (1995), Khalid *et al*. (2000) and Fouqia *et al*. (2001).

The identification of phytoplasmal pathogens requires expensive equipment like electron microscopy, fluorescent microscopy, ELISA, PCR and nucleic acid hybridization. In view of the emerging nature of the disease, we have achieved evidence of PPT presence through staining of infected tissues using light microscope. Some aspects of disease i.e. its distribution, weed hosts, symptomatology, transmission and reaction to tetracycline antibiotics were also studied.

Materials and methods

Potato fields were surveyed at 7 locations with 10 sites each. In each field, PPT symptomatic and asymptomatic plants were counted in a 5x5 m area and % disease incidence was calculated. Symptomatic tissue and tubers were collected in polythene bags and stored at 4°C until processed. Weeds showing typical symptoms of phytoplasma were collected within PPT-infected fields.

Symptomatic tissue was top and wedge grafted on healthy plants of potato cv. Diamont, Cardinal and Desirée, according to the method of Bos (1978). All plants were maintained in an insect-free environment for 8 weeks and symptom expression was recorded.

About 2 mm long pieces of various parts of symptomatic and asymptomatic potato tissues were cut with a fine razor blade, and fixed in Karnowsky fixing solution for 24 - 48 hours (Salazar and Jayasinghe, 1997). Diene's stain (Deeley *et al*., 1979) and gallocyanine-chromalum (Petzold and Marwitz, 1980) were then used. Free hand cross and longitudinal thin sections were cut with a fine sterilized razor blade, floated in distilled water and stained for 5 minutes. Excess stain was washed off with distilled water and then by a drop of xylol. Sieve tubes in sections were examined under Nikon light microscope model EFB-3 at 850 X.

Twelve antibiotics (tetracycline, oxytetracycline, vibramycin, terramycin, achromycin, aureomycin, erythromycin, amplicin, amoxicillin, lincomycin, streptomycin and penicillin) were selected and tested against PPT-infected tubers that had lost dormancy at low temperature. Cut pieces of diseased and healthy tubers (25/treatment, 4 replicas) were dipped in each antibiotic at 250 and 125 ppm for 24 hours then stored at 4 °C for several weeks. Effectiveness of antibiotics was evaluate on the dormancy restoration of infected tubers.

Results

The incidence and distribution of PPT indicate that the disease was prevalent in the autumn and spring crops: incidence ranged between 4.1 to 80.1% with a mean of 20.7%; maximum incidence (68.6-89.1%) was encountered at Chiniot followed by 25-72.4% at Faisalabad. At other places, disease incidence was significantly lower (4.1-7.5%), and the diseased plants were generally scattered in the field.

Due to hormonal disturbance in the diseased plants, height was increased by 30%, haulms 37.6%, formation of numerous aerial tubers (212) with poor diameter (2 cm) and weight (8 gm), and underground tubers (57) which were all malformed. These differences were statistically significant but of no practical value, because

tubers harvested from the infected plants were not marketable except 1 or 2, as against 8-9 of healthy plants.

Six weed species pig weed (*Amaranthus viridis*), pimpernel (*Anagallis arvensis*), black medic (*Medicago arvensis*), bind weed (*Convolvulus arvensis*), spurge (*Euphorbia granulata*), and cape weed (*Phyla nodiflor*a) showed bushy growth, proliferated buds, sterile flowers and chlorotic appearance. *C. arvensis*, with typical phytoplasmal symptoms was predominantly present in the infected potato fields.

The disease was graft transmitted from potato to potato as well as reproduced from infected tubers. Leafhoppers could not be found in the potato crops.

The sieve tubes of the symptomatic potato samples were stained dark blue which indicate the presence of phytoplasmas. Stains were stronger in the thin sections of mid rib, petiole, axillary buds and crown than stem and tuber sprouts. No stain appeared in the phloem sieve tubes of asymptomatic plants. The strong colouration was coincided with high concentration of phytoplasma, and longitudinal sections gave better distribution than cross sections. Diene's and gallocyanine-chromalum gave similar results, but Diene's stain was better because of convenience in preparation, and its 0.25% solution was better than 0.5 or 0.125%.

The infected potato tubers sprouted quickly when stored at 4 °C, therefore this stage was selected to study the antibiotic effect. Results indicated that tetracycline antibiotics at 250 ppm, but not at 125 ppm, were significantly effective against PPT phytoplasma. Tetracycline, oxytetracycline, vibramycin, terramycin and aureomycin significantly restored the dormancy by 80-100% as against 40-60% by other tetracycline antibiotics. Streptomycin and Penicillin were not effective at all.

Discussion

Some of the potato fields (farms in Chiniot and Faisalabad) were heavily infected (40-80%) by potato purple top (PPT). Surveys of potato growing areas at Jhang, Gojra, Okara, Sahiwal and Taxilla confirmed the prevalence of PPT in the autumn and spring crops, but to a lesser extent (4-8%). The disease incidence may be attributed to the use of infected seed tubers, but the possibilities of leafhopper vectors can not be excluded.

It appears that the inoculum of PPT has been adequately built up in different weed hosts; similar situation was reported by Ahmad *et al*. (1995) and Cadena-Hinjosa (1996). Host range of PPT is wide and distributed in distinct, botanical family (Nagaich *et al*., 1982; Valenta *et al*., 1961; Welliver, 1999). This work was limited to potato however the six weed hosts, with the prevalence of bindweed, with typical phytoplasmal symptoms might be alternate/reservoir hosts of PPT and hibernating host for leafhoppers.

Simple staining of sieve tubes of infected phloem can provided a satisfactory evidence of phytoplasma under a light microscope. These results are in close conformity with those of Deeley *et al*., (1979) and Petzold and Marwitz, 1980.

Infected potato tubers had no dormancy and tetracycline, oxytetracycline, terramycin and aureomycin were highly effective in restoring the dormancy by 80-100%, whereas penicillin and streptomycin had no effect as described for eggplants phytoplasma infected (Anjaneyulu and Ramakrishnan, 1969; 1972).

References

AHMAD I., SOOMRO M. H., IFTIKHAR S., BURNEY K., 1995.- Recent distribution of potato disease in Pakistan, pp. 20-21. In: *Proceedings of National Seminar*, NARC Islamabad, 23-25 April.

ANJANEYULU A., RAMAKRISHNAN K., 1969.- Therapy of egg plant little leaf disease with tetracylines.- *Current Science*, 18: 271-272.

ANJANEYULU A., RAMAKRISHNAN K., 1972.- Chemotherapy of egg plant little leaf disease.- *Mysore Journal of Agricultural Science*, 6: 145-146.

BOS L., 1978.- *Symptoms of virus diseases in plants*.- PUDOC, Wageningen, The Netherlands.

CADENA-HINOJOSA M. A., 1996.- La punta morada de la papa en México: Efecto de cubiertas flotantes, genotipos y productos químicos.- *Revue Mexican Fitopatologia*, 14: 20-24.

DEELEY J., STEVENS W. A., FOX R. T. V., 1979.- Use of Diene's stain to detect plant diseases induced by mycoplasma-like organisms.- *Phytopathology*, 69 (11): 1169-1171.

FOUQIA F., AZIZ-UD-DIN S. M., IFTIKHAR A., 2001.- Incidence of phytoplasma in potato growing areas of Punjab confirmed through fluorescent microscopy, pp. 120-123. In: *Proceeding 3rd National Conference of Plant Pathology*, NARC, Islamabad. 1-3 October.

GIRI B. K., NAGAICH B. B., 1971.- Purple top roll- A menace to newly evolved potato varieties, pp. 110. In: *2nd International Symposium Plant Pathology,* New Delhi.

KHALID S., IFTIKHAR S., MUNIR A., AHMAD I., 2000.- *Potato diseases in Pakistan*.- PSPDP/PARC, Islamabad, Pakistan.

NAGAICH B. B., GIRI B. K., 1973.- Purple top roll disease of potato.- *American Potato Journal*, 50: 79-85.

NAGAICH B. B., KHURANA S. M. P., SINGH S., SUNAINA V., PARIHAR S. B. S., 1982.- Studies on the transmission and control of potato yellow virus and their effect on crop production, pp. 294-303. In: *Potato in Developing Countries*. *Indian Potato Association conference*, Simla.

PETZOLD H., MARWITZ R., 1980.- The gallocyanine-chromalum staining for the detection of mycoplasma like organisms.- *Phytopathology Zeitschrift*, 87: 46-50.

SALAZAR L. F., JAYASINGHE U. 1997.- *Techniques in Plant Virology Training Manual*.- CIP, Lima, Peru, Section 3.1.2.

SHAFIQ M., AHMAD M., CHAUDHRY M. S., CHAUDHRY M. H., 1995.- Incidence of mycoplasma infection of potato in Punjab, pp. 317-323. In: *National Seminar on Research & Development of Potato Production in Pakistan*, PSPDP/PARC, Islamabad, 23-25 April.

VALENTA V., MUSIL M., MISIGA S., 1961.- Investigation on European yellows type viruses. I. The stolbur virus.- *Phytopathologische Zeitschrift*, 42: 1-38.

WELLIVER R., 1999.- Diseases caused by phytoplasmas.- *Regulatory Horticulture, Pennsylvania Department of Agriculture, Bureau of Plant Industry*, 25 (1) Plant Pathology, Circular No.82: 17-22.

Corresponding author: Mian Muhammad NASIR (e-mail: miannsr@gmail.com), Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.