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BIOLOGICAL DIVERSITY OF THE WITCHES' BROOM DISEASES

BY

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More than 69 witches' broom like diseases have been reported from different countries including India. In West Bengal, 7 witches' broom and little leaf diseases are commonly found. Apparent biological diversities between some of the pathogens of these diseases could be found following dodder transmission, tetracycline sensitivity and tetrazolium sensitivity tests. Sophisticated methods including serodiagnostic, electron microscopy and culturing techniques are necessary for established the precise biological diversity of the pathogens associated with such unique diseases.

Key words : Biological diversity, Witches' Broom Diseases

INTRODUCTION

Witches' broom is a common English word which denotes a typical syndrome of growth abnormalities expressed in the form of a typical broom. This syndrome in a plant was first observed by Rutgers in 1913. He recorded the transformation of groundnut plants into witches' brooms. Similar syndrome was recorded by Tubeuf in 1919, who found the development of such syndrome in conifers due to the parasitization by dwarf mistletoes. Stewart (1924) described a witches' broom disease of Shagback hickory plant caused by a fungus *Mirostroma juglandis* (Berens). Sacc. Hengerford and Dana (1924) recorded the same syndrome in potato. Subsequently witches' broom syndromes in plants belonging to widely different families have been recorded from time to time by different workers in different countries without having much informations on the nature of the cusal agents of these syndromes (Martyn, 1963; Ganguly, 1986). After the discovery of the association of mycoplasma-like organisms with the witches' broom like diseases by Doi *et al.* (1967), Ishiie *et al* (1967), and Nasu *et al.* (1967), a large number of similar diseases were reinvestigated and the association of mycoplasma-like organisms in these diseases were confirmed (Ploaie, 1981). Studies made so far on witches' broom diseases of plants indicate that most of them are associated with mycoplasma like organisms. Thus similar symptoms are produced by a group of organisms in different plants

belonging to widely different families. The biological diversity of these organism which infect widely different plants needs to be established. In this paper, a comparative assessment of the witches' broom like diseases found particularly in West Bengal has been made to facilitate the understanding of the biological diversity of mycoplasma-like diseases.

WITCHES' BROOM-LIKE DISEASES

More than 69 witches' broom like diseases have been reported from India and abroad by 1983 (Appendix I). Amongst these, more than 22 diseases have been reported from India (Table 1). All produce either broom-like or little-leaf symptoms

Table 1. *Common witches' broom type diseases found in West Bengal.*

Name of the disease	Host plant	Remarks
Brinjal little leaf	*Solanum melongena*	Common in brinjal field
Datura little leaf	*Datura* sp.	Common
Jatropa little leaf	*Jatropa* sp.	Common
Witches' broom of tuna	*Cedrela tuna*	Occassional
Witches' broom of Jujuba	*Zizyphus* sp.	Occassional
Witches' broom of potato	*Solanum tuberosum*	Rare
Little Leaf of Solanum	*Solanum indicum*	Common

Ganguly (1986) made a survey on the incidence of witches' broom like diseases in West Bengal (Table 2). During the visits, two witches' broom like diseases were found in the field crops, potato and brinjal egg plant, *Solanum melongene*). Brinjal little leaf disease has been very common all over the state, whereas potato witches' broom has been found only occassionally in potato fields. Three very common disease were found in the perennial weeds viz., *Datura*, *Jatropa* and *Solanum*. In addition, one disease was observed in a timber plant, *Cedrela*, and another has been recorded on wild Ber plant (*Zizyphus* sp).

DIVERSITIES OF WITCHES' BROOM LIKE DISEASES

The symptoms of witches' broom-like diseases which are found in West Bengal can be categorized into two groups, typical witches' broom and little-leaf. In the witches' broom disease apical dominance is completely lost and excessive aggregation of extremely reduced branchlets occur. The leaves in these cases become very rudimentary and complete virescens takes place. In case of little-leaf disease the suppression of apical dominance occurs to a lesser extent with aggregation of branchlets changes, the leaves become very much reduced in size and partial virescence takes place (Table 2). Further diversities became apparent from transmission and detection studies.

Table 2. *Symptom of common witches' broom like diseases in West Bengal.*

Name of the disease	Common symptoms			
	General changes	Foliar changes	Floral changes	Remarks
Brinjal little leaf	General stunting of plant. Height of the plant ranges 1 to 5′ (3′). Excessive branching. Angle of the branch axis ranges 60°—70° (45°-50°).	Reduction in leaf size, normally 2.2 sq. cm. (38-58 sq. cm.) Angle of the leaf axis 60°-70° (40°-50°) yellowing of the leaf.	Phyllody of flowers, shoots developed from the virescence flower	Multiplication is severely affected
Jatropa little leaf	Plant reduced in size 1′-2′ (2′-4′). Excessive branching, angle of the branch axis 70°-75° (40°-75°).	Drastic reductions of leaf size, It was about 5.25 (48.75) sq. cm. Angle of the leaf axis was 60°-70° (30°-40°). Leaf showed mosaic pattern of yellowing.	Seldom flower, flowers are with short stalk and smaller in size	Seed production hampered
Witches' broom of Jujube	Standard size of the plant, plants with bush appearance due to excessive hairy branching. Height of the plants 2-3′ (4′-6′) Branching axises with 70°-80° (46°-50°).	Leaves are very small & minute. Leaf size average 0.21 sq. cm. (2.66). Angle of leaf axis 60°-70° (30°-40°). General yellowing of leaf.	No flowering noticed	Found in waste lands. Complete suppression of multiplication
Datura little leaf	Plants are bushy at the top of the branches due to excessive branching. Branch axis angle ranges 75°-80° (35°-50°). A great reduction of height 2′-2½ (3′)	A great reduction in leaf size shape 16.75 sq. cm. (51). Angle of the leaf axis 60°-80°. Leaf colour remain green. No resemblence in leaf shape.	Phyllody of flower	Found more or less all over West Bengal

(1) indicates healthy plant.

Transmission

Witches' broom-like diseases are usually graft transmissible. Several of them may also be transmitted by vectors and dodders. An attempt was made to transfer the pathogens of brinjal little leaf, *Jatropa* little-leaf and Datura little-leaf to different host plants using dodders. The host plants grown in pots were arranged in a circular way around the source plants under insect proof conditions. Dodders, fixed on the source plants were then simultaneously rolled around the test plants Seedlings of brinjal, *Jatropa, Datura,* potato, chilli and tomato were used as test plants for brinjal little-leaf ; brinjal, *Datura* and *Jatropa* were used as test plants for *Jatropa* little-leaf and *Datura* little-leaf The plants were kept under observation for 60 days. It was found that the brinjal little-leaf pathogen could be transferred to brinjal and chilli, whereas *Jatropa* and *Datura* little-leaf could be transferred only to *Jatropa* and *Datura* respectively within 30 to 41 days after the placement of the dodders from the source plants to the test plants. From these results, it was concluded that the little-leaf pathogens of brinjal, *Jatropa* and *Datura* are not the same.

Detection

Mycoplasma-like pathogens can usually be detected by several simple or more sophisticated techniques. Tetracycline sensitivity and various staining techniques, particularly Dines' strain (Deeley *et al.*. 1979), are often used for routine detection of mycoplasma like diseases. In a sophisticated way the mycoplasma like pathogens are detected by using serological techniques and electron microscopic methods. To detect the mycoplasmal pathogens of witches' broom-like diseases in West Bengal, a few simple tests were performed including tetracycline sensitivity and 2-3-5 tertrazolium reaction.

Tetracycline sensitivity

The tetracycline sensitivity test was performed with brinjal little-leaf, *Jatropa* little-leaf, and *Datura* little-leaf. Tetracycline hydrochloride was used at a concentration of 1000 ppm. Four sprays were applied to the diseased plants at an interval of 10 days for a period of 40 days. The treated plants were kept under observation for 60 days. In case of brinjal 80% of treated plants showed recovery within 46 days. In case of *Datura* 7% plants showed recovery within 40 days whereas no recovery could be found in *Jatropa* plants. Thus the differential sensitivity to tetracycline again may be an indicator for the biological diversity of the mycoplasma-like pathogens.

Temporary remission of mycoplasma-like diseases with tetracycline has been reported by different workers (Appendix – II) but degree of comparative sensitivity of such reactions are not available for the differentiation of the pathogens.

Tetrazolium sensitivity

To perform the tetrazolium sensitivity test, 0 1%2 3-5 triphenyl tetrazolium chloride in 0.05 M phosphate buffer at pH 7.8 was used. Equal volumes of the reagent and the sap from the healthy and diseased plants were mixed thoroughly and kept for 24 h for colour development. Positive reactions were found with respect to *Datura* little-leaf and *Zizyphus* witches' broom and the reaction appeared to be negative with respect to *Jatropa* little-leaf. Thus tetrazolium sensitivity may also have some implications for differentiating the biological diversity of the mycoplasma-like pathogen. Staining (Dienes' strain) and chromatographic detection, fluorescent microscopy, sero-diagnostic techniques and electron microscopy, could perhaps provide more precise informations on the biological diversity of the causative pathogens.

Morphological and cultural features

Ploaie 1981) differentiated mycoplasma-like organisms (MLO) into 6 categories on the basis of their morphology. The first group contains very small particles measuring 50-100 nm in diameter which stain heavily and are highly electron opaque. The second group contains particles 100-300 nm in diameter, spherical or oval in shape with visible unit membrane. The particle diameter of the third group is 400 nm-1 μm which are ovoid bodies with electron dense areas with nuclear like and not like structure and ribosomes. The fourth group contains filaments or segmented filaments of 1-2 μm long and 0.08 0.03 μm wide. The fifth group cantains irregular large bodies which comprises of 2-3 cells on a common support and the sixth group is composed of large cells having dense inclusions or numerous buds on their surface. Hirumi and Maramorosch (1970) demonstrated that all these categories can be observed when more than 2000 thin sections from a single *Nicotiana rustica* plant, infected with a single MLO pathogen (aster yellows) were studied. Thus electron microscopy can not be used to distinguish different MLO agents.

The most reliable method for establishing biological diversity of mycoplasma-like pathogens would be by culturing the organisms and by physiological, chemical and biological characterization of the cultural organisms. Until now, it has not been possible to culture any of the plant pathogenic MLOs, and only spiroplasmas have been cultured successfully. Improvement of technology and standardization of methodologies are necessary for culturing and characteriz ng the MLO pathogens and for appropriate understanding of the biological diversities and specialities of these unique pathogens.

DISCUSSION

Witches' broom like diseases of plants are often associated with mycoplasma-like pathogens. The diseases are usually denoted by English common names of genetic

names of the plant in Latin and the word witches' broom or a wood to specify the allied symptoms. These diseases are mostly referred to as MLOs diseases, based on tetracycline sensitivity tests, staining reactions or electron microscopic observations. The wide range of hosts of these diseases suggest their biological diversity. In a preliminary study made with four witches' broom and little leaf diseases which commonly occur in West Bengal, using dodder transmission, tetracycline and tetrazolium sensitivity tests, indications have been obtained of their biological diversities. Electron microscopic studies of other mycoplasmal diseases have been made, but such studies could hardly differentiate the pathogens. No other information is available on the differential characteristics of different witches' broom or mycoplasmal pathogens. It may be that the same pathogen may affect different hosts. Further research is required to characterize the different witches' broom or mycoplasmal pathogens.

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APPENDIX I

List of some witches' broom like diseases of plant found in India and abroad

Name of the disease	*First reported by*
Anisomeles witches' broom	Padmanabhan *et al.*, (1973)
Aster yellows	Kunkel (1926)
Alfalfa Witches' broom	Edwards (1936)
Ash (Arizona) Witches' broom	Plakidas (1949)
Acalypha little leaf	Van velsen (1961)
Ammi sp. proliferation	Ploaie (1969)
Acalypha Witches' boom	Varma *et al.* (1974)
Angelona W. B.	Raju *et al.* (1975)
Allamanda Witches' broom	Ghosh (1977)
Acrostaphylos W. B.	Bremmer (1981)
Brinjal little leaf	Thomas and Krishnaswami (1939)
Broadbean Witches' broom	Nour (1962)
Bidens Witches' broom	Vega (1981)
Butterfly flower W. B.	Ahlawat *et al.* (1983)
Conifir Witches' broom	Tubeuf (1919)
Cassava Witches' broom	Silberschmidt and Compos (1944)
Cantaloupe W. B.	Smith (1949)
Casurina W. B.	Wellman & Grant (1951)
Crotalaria W. B.	Hadiwijaja (1952)
Convolvulus yellows	Amica *et al.* (1972)

Cowpea Witches' broom	Varma (1974)
Clove Little leaf	Karunakaran (1980)
Cocao W. B.	Calle (1982)
Datura little leaf	Padmanabhan *et al.* (1973)
Dodonea little leaf	Padmanabhan (1973)
Nesmodium Witches' broom	Chen *et al.* (1982)
Elm Witches broom	Mokra (1964)
Eucalyptus little leaf	Sastry *et al.* (1971)
Groundnut Witches' broom	Rutgers (1913)
Grapevine W. B.	Dias (1963)
Holodiscus W. B.	Zeller (1931)
Lilac Witches' broom	Lorentz & Brierly (1953)
Longan W. B.	Li (1955)
Laucas little leaf	Padmanabhan *et al.* (1973)
Lupinum W. B.	Ulrychova (1980)
Lucerne W. B.	Edwards (1936)
Mulberry dwarf	Sakai (1937)
Mirabilis Witches' broom	Ghosh *et al.* (1975)
Mungbean W. B.	Sumardiyono (1980)
Opuntia Witches' broom	Maramorosch (1972)
Potato Witches' broom	Hungerford & Dana (1924)
Paulownia W. B.	Tokushige (1951)
Primula W. B.	Kulkarni & Scheffeild (1966)
Passion fruit W. B.	Kitazima *et al.* (1981)
Rice W. B.	Singh (1978)
Rose W. B	Crowe (1983)
Sandal Spike	McCarthy (1903)
Sesamum phyllody	Vasudeva *et al.* (1955)
Shagback hickery W. B.	Stewert (1924)
Sweet potato W. B.	Thung (1947)
Solanum pubesnens little leaf	Padmanabhan *et al.* (1973)
Styllosanthes W. B.	Chen *et al.* (1982)
Strawberry W. B.	Zeller (1927)
Tuna Witches' broom	Mukhopadhyay (1982)
Winged beans Witches' broom	Sumardiyonoo (1980)
Zizyphus Witches' broom	Cicearone (1957)

APPENDIX II

This list is a modification of McCoy's record (McCoy, 1982)

1. Western X disease (Amin *et al.*, 1971).
2. Aster yellows disease (Davis *et al.*, 1968, 1970).
3. Potato witches' broom (Barrak *et al.*, 1969).
4. Egg plant little leaf disease (Anjaneyulu *et al.*, 1969, 1972 ; Varma *et al.*, 1975).
5. Yellows and witches's broom disease of Strawberry (Dianne *et al.*, 1971).
6. Clover phyllody and aster.yellows disease (Chiykowski 1972, 1973).
7. Citrus likubin disease (Chiu *et al.*, 1979).
8. Legume little leaf disease (Bowyer *et al* , 1972).
9. Citrus sutbborn disease (Bowyer *et al.*, 1974).
10. Effects of tetracyclines on mycoplasma like agents (Asuyama *et al.*, 1973).
11. Opuntia subulata montrosity disease (Delay *et al.*, 1977 .
12. Apple proliferation disease (Deubek *et al.*, 1977).
13. Lethal yellowing in coconut palm (Hunt *et al.*, 1974).
14. Citrus stubborn disease Igwegbe *et al.*, 1973).
15. Witches' broom diseases jujuba (La *et al.*, 1976).
16. Witches's bromm of Opuntia tuma (Maramorosch *et al.*, 1972).
17. Citrus greening (Nariani *et al.*, 1971).
18. Pear decline (Nyland *et al* , 1973).
19. Peach X Disease (Pearson, 1977).
20. Peach X Disease (Rosenberger *et a/.*, 1977).
21. Mulberry dwarf disease (Sudo *et al.*, 1969).