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Distribution, epidemiology and molecular variability of the begomovirus complexes associated with yellow vein mosaic disease of mesta in India

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ABSTRACT

Yellow vein mosaic disease of mesta (*Hibiscus* spp.) poses a serious threat to the cultivation of this crop in India. The disease was found to be associated with two different whitefly-transmitted monopartite begomoviruses, *Mesta yellow vein mosaic virus* and *Mesta yellow vein mosaic Bahraich virus*, together with two betasatellite species, *Cotton leaf curl Multan betasatellite* and *Ludwigia leaf distortion betasatellite*. These begomovirus complexes were detected in different combinations throughout the mesta growing regions of India. All the eight cultivars tested were highly susceptible to the disease. The effect of the disease in terms of loss in fibre yield was greatest (around 70%) in plants that were inoculated at an early stage of growth. A regression approach was adopted to consider the relationship of whitefly vector populations with weather conditions and disease spread which explained that different conducive weather factors facilitated the build up of whitefly populations and contributed to the spread of the disease.

1. Introduction

Mesta is second in importance to jute as a fibre crop in India. It is an herbaceous annual ligno-cellulosic bast fibre plant of the family Malvaceae. There are two cultivated species namely *Hibiscus cannabinus* (kenaf) and *Hibiscus sabdariffa* (roselle). Besides providing traditional packaging material in the fibre industry, both the cultivated species are also used as a leafy vegetable. Their seeds are rich in oil which has a low proportion of unsaturated fatty acids and they have potential medicinal value (Duke, 1986; Pal and Jain, 1998). Mesta, particularly *H. cannabinus*, is receiving increasing global attention as an alternative source of good quality paper pulp due to an increasing shortage of hardwood sources. Both species are more adaptable than jute (*Corchorus* spp.) under diverse agro-climatic and soil conditions. Generally, mesta grows best in the warmer regions between the latitudes of 30°N and 30°S with rainfall of at least 100 mm or more per month and a fairly uniform temperature during the crop season (Anonymous, 2007). In India, mesta is cultivated for fibre and seed in different states either as sole crops (eastern and southern India), or together with maize and sorghum as mixed crops (northern India) under rain-fed agro-ecological condition during April–July and June–October. With the recent increase in awareness of the biodegradable nature of bast fibres and their utilization in different value-added diversified products, demand for such bast fibre crops is increasing remarkably. In India, the productivity of mesta crops is less than that of other mesta producing countries. Different diseases of mesta crops and particularly those caused by viruses are considered to be one of the factors contributing to the low-average yields of these crops across India (Brunt et al., 1996). Among these diseases, mesta yellow vein mosaic disease (MeYVMD), characterized by yellowing of leaf veins followed by complete chlorosis of the leaves, is spreading rapidly in many parts of eastern and northern India (Chatterjee et al., 2005; Ghosh et al., 2007) and thus has become a serious problem in the cultivation of these crops. The height of diseased plants is reduced significantly and thus adversely affects the bast fibre yield (Chatterjee et al., 2008).

In eastern India, MeYVMD is associated with a recently described begomovirus, *Mesta yellow vein mosaic virus* (MeYVMV), and an isolate of *Cotton leaf curl Multan betasatellite* (CLCuMB) (Chatterjee and Ghosh, 2007a,b). Symptomatic samples obtained from northern India showed the association of another recently described species of begomovirus, *Mesta yellow vein mosaic Bahraich virus* (MeYVMBV) and an isolate of *Ludwigia leaf distortion betasatellite* (LuLDB) (Das et al., 2008a,b). These begomovirus complexes have been shown to be transmitted efficiently by a whitefly (*Bemisia tabaci*) and they have a very narrow host range (Chatterjee et al., 2008; Das et al., 2008a). The complexes have been char-

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acterized extensively both biologically and molecularly. However, information regarding the relationship of different epidemiological factors including the molecular variability of the associated begomovirus complexes is yet to be understood and this has become crucial for developing an effective management strategy for the disease. Hence, with an aim to bridge these gaps, we report here the distribution of MeYVMD with special reference to its incidence and severity in relation to different aspects of epidemiology including crop age, weather factors, whitefly vector populations and the molecular variability of the associated begomovirus complexes occurring in India.

2. Materials and methods

2.1. Disease survey and collection of symptomatic samples

A detailed field survey was conducted during the cropping seasons of 2005, 2006 and 2007 on the incidence and severity of MeYVMD so as to understand the distribution of the disease throughout the mesta-producing regions of eastern, northern and southern India. The data on incidence and severity were recorded from a total of 137 farmers' plots during the later phase of vegetative growth (100–120 days from planting) of mesta crops grown under the different agro-ecological situations of northern and southern West Bengal (East India), eastern Uttar Pradesh (North India) and north-eastern Andhra Pradesh (South India) (Fig. 1). Disease incidence was expressed as the number of symptomatic plants as a percentage of the total number of plants assessed. Disease severity was calculated based on the number of symptomatic leaves out of the total number of leaves on individual plant and averaged from the data on 100 symptomatic plants observed within a particular location.

Leaves from diseased plants, showing typical yellow vein mosaic symptoms, were collected during 2005–2007 from the cultivated fields of different locations under survey. Twenty symptomatic leaf samples were collected randomly from each of these locations for molecular analysis.

2.2. Whitefly transmission

All the whitefly transmission experiments were carried out in controlled glasshouse conditions at the Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, India. The symptomatic disease samples collected from Haringhata location (southern West Bengal) were used as the initial source of virus inoculum. A virus-free stock of whiteflies (*B. tabaci*) was reared on healthy tobacco plants (*Nicotiana tabacum*) in an insect-proof wood-framed cage. The adult whiteflies, which emerged from nymphs present on tobacco, were used for transmission studies. Healthy mesta plants (5 seedlings per pot) were raised under glasshouse condition. Twenty non-viruliferous whiteflies were collected using an aspirator and put into polyvinylchloride bottles containing the symptomatic leaf samples and allowed access for 12 h to acquire the virus. The whiteflies were then released onto each pot having healthy mesta plants and covered with a small plastic cylindrical cage. After 12 h the seedlings were sprayed with 0.2% dimethoate and kept in an insect-proof rectangular cage for disease development. Similar numbers of healthy plants, infested with non-viruliferous whiteflies in each case, served as control.

2.3. Varietal response

Two cultivars of *H. cannabinus* (HC-583, AMC-108) and 6 cultivars of *H. sabdariffa* (HS-4288, HS-7910, AMV-1, AMV-2, AMV-3 and AMV-4) were grown separately in earthen pots (5 seedlings per pot) with 10 replications under glasshouse conditions and whitefly transmission in each case was carried out as described earlier. Progress of the disease was monitored following the methodology of Chatterjee (2007). Briefly, the disease progression was recorded in each leaf based on (*A*) development of foliar symptom, (*B*) nature of discolouration of leaf lamina, (*C*) extent of yellowing of veins, (*D*) area of discolouration and also in individual plants with respect to (*E*) extent of stunting. For each of these parameters different categories were distinguished and numerical values were assigned using a scale of 0, 5, 10, 15, 20 (Table 1). Data on each category were recorded chronologically at 7-day intervals from 10 to 52 days post-inoculation (dpi). Numerical values for each leaf of individual plants were recorded separately and as reported by Chatterjee (2007) a disease intensity index was calculated according to the following formula:

$$\text{Disease intensity index} = \frac{\left[\sum_{s=A}^{D} L_{1s} + \sum_{s=A}^{D} L_{2s} + \sum_{s=A}^{D} L_{3s} + \cdots + \sum_{s=A}^{D} L_{ns}\right]}{T_N} + G$$

L_1, L_2, L_3, L_n = number of affected leaves on a plant ranging from 1 to n; s = score of a diseased leaf varying from A to D; L_{is} = total score on diseased leaf of a plant in a particular stage/period ($i = 1/2/3/\ldots/n$); G = growth habit of infected plant (i.e. extent of stunting = E); T_N = total number of leaves on infected individual plant.

Areas under the disease severity curves (AUDSCs) were calculated by trapezoidal integration in accordance with the intensity at 7-day intervals throughout crop growth, using the formula of Campbell and Madden (1990):

$$\text{AUDSC} = \sum_{i=1}^{n-1} \frac{(y_i + y_{i-1})}{2 \times (t_{i+1} - t_i)}$$

Table 1
Numerical scale values for different grades of symptom expressions of mesta yellow vein mosaic disease.

Category	Severity
(A) Type (development) of foliar symptom (discolouration)	
No symptom	0
Appearance from one half	5
Appearance from both halves	10
Erratic appearance	15
Complete foliar discolouration	20
(B) Nature of discolouration	
No discolouration	0
Greenish yellowing	5
Yellow mosaic	10
Chlorotic yellowing	15
Complete chlorosis	20
(C) Extent of yellowing of veins	
No symptom	0
Appearance of small chlorotic flake	5
Appearance of yellowing of veins	10
Appearance of yellow netting	15
(D) Area of discolouration	
Nil	0
25%	5
50%	10
75%	15
100%	20
(E) Extent of stunting (growth habit)	
Nil	0
Mild	5
Moderate	10
Severe	15