MATERIALS AND GENERAL METHODS
Test Plant

_Cucurbita maxima_ L. has been selected for the present study. Seeds of different varieties were obtained from Germplasm Exchange Division, N.B.P.G.R. (ICAR), Pusa Campus, New Delhi, as well as from the local seed companies. Variety IC 33 8908 was found most susceptible and used as test plant throughout the entire study.

Experimental conditions

All the experiments in the present study were carried out in an insect free glass house, where usual precaution was taken to keep the plants free from fungal, bacterial and nematode infection. All the glassware were used in the experiments were thoroughly washed with detergents and then sterilized in an oven at 180°C for 24 hrs.

Pot culture of test plant

Experimental plants were grown in 25 cm diameter earthenware pots containing a mixture of sandy loam soil and compost (1:1). Pots were daily irrigated with tap water. Seeds of same size were selected for sowing. Five seeds of the host were sown in each pot. When seedling came out they were inoculated with different strains of watermelon mosaic virus. Plants of pot culture were taken for study.

Virus culture

The culture of three different strains of WMV are already identified and referred to as WMV-vb, WMV-mm and WMV-c were taken from the culture collection already maintained in this laboratory. The culture of these virus strains were maintained by inoculating it regularly to healthy _Cucurbita maxima_ plants.
**Virus inoculum**

Young infected leaves showing prominent symptoms were macerated to pulp with simultaneous addition of requisite amount (1:1, w/v) of phosphate buffer (0.1 M, pH 7) each strain separately. Slurry was squeezed through double fold of muslin cloth. Sap was centrifuged at 10 rpm for 5 minutes and the supernatant thus obtained was used as inoculum for standard inoculation.

**Inoculation**

Mechanical inoculation was done by gently rubbing the upper surface of cotyledonary or primary leaves of healthy host plants with forefinger dipped in infected sap (i.e. inoculum). Carborundum powder of 600 mesh was used as an abrasive before rubbing. Care was taken to have uniformity of pressure and spread of inoculum while inoculating the leaves. The inoculated leaves were washed gently with distilled water. Control plants were treated similarly using the phosphate buffer (pH 7.0, 0.1 M) in place of inoculum.

**Histopathological and cytological studies**

For histological studies samples of root, stem and leaves from plants infected by three different strains and healthy plants were collected at different intervals of growth separately. The samples were fixed following the method prescribed by Johnson (1940) in FAA and were softened with 50% aqueous solution of hydrochloric acid for the purpose of hand-cut section. The sections were dehydrated in different grades of alcohol ranging from 30 to 90% and absolute alcohol. For microtechnical preparation chips of paraffin wax added successively to the medium of pure butanol having the samples until the medium reached a saturation point at
microtechnical preparation chips of paraffin wax added successively to the medium of pure butanol having the samples until the medium reached a saturation point at room temperature and later under table lamp (40 watts). Finally the materials were given changes with molten pure paraffin at 60°C in an oven. Thus, replacing the last traces of butanol with paraffin. The materials were subsequently embedded in the paraffin wax employing paper boat technique. Blocks were prepared and serial sections of 10 μm were cut with ERMA rotary microtome taking care to get uniform thickness of section. The ribbon so prepared were stained employing usual methods of staining and sections were mounted in DPX mountant. The photographs were taken with the help of photomicrographic attachment on Nikon trinocular microscope. Stage and ocular micrometer were employed to measure the length and breadth of stomata as well as frequency of distribution of stomata from epidermal peeling of fresh healthy and diseased leaves.

At the time of blooming flower buds of appropriate age were collected at random separately from healthy and infected plants by three strains of the virus. Collection of buds was done between 5 a.m. to 7 a.m. and were fixed in carnoys fluid (Swaminathan et al. 1954). Meiosis was studied in squashes of 500 pollen mother cells of the same age in healthy and infected plants. The slides were permanently fixed using butyl alcohol schedule by Bhaduri and Ghosh (1954). Pollen sterility was calculated out of 2000 pollen grains for each healthy and infected plants on the basis of stainability with Alexander’s stain (1969). Five flowers were selected from each plant for estimating pollen fertility.
Statistical Analysis

Variation is a feature of all experimental work. The objective of statistical analysis is to separate the variation due to experimental treatment from other factors. When the variation due to treatment determined, logical, deduction can be made about the experimental results (Brockwell, 1980).

Analysis of variance

The data collection in the present work is summarized in an analysis of variance (ANOVA). Values have been calculated by the method of Bailey (1959).