Anatomical and cytological changes in bean leaf cells infected with *Bean common mosaic virus*

A. B. Badr[1] ; M. A. S. El-kady[2]; and Kh. E. A. Saker[2]

[1]-Department of Botany, Al-Azhar University, Nasr City, Cairo, Egypt.[2]-Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

Bean common mosaic virus (BCMV) infection has bean shown to influence the anatomical and cytological characters of infected bean plants. The thickness of leaflet blades, palisade and spongy tissues were increased than the healthy plants. In contrast the thickness of upper and lower epidermis layers were reduced. Also the thickness of midrib zone was slightly reduced. Moreover, vascular tissues lost their normal shape and arrangement as xylem arms. The most interesting finding, lengths of both , protoxylem and metaxylem were reduced while their widths were increased. Ultrathin sections of symptomatic leaf tissue showed several cytological changes characteristic of Potyviruses. The thlakoidal structure of the chloroplastes becomes disorganized, abnormally distributed and dilated . The number of plastoglobulis increased and clumped together, their starch content was decreased. Clumping of mitochondria , chloroplastes and degeneration of grana were also observed. Pinwheel inclusions and associated virus particles are frequently found in the cytoplasm of infected leaf cells.

Keywords: *Bean common mosaic virus,* Ultrathin sections**,** Anatomical, Cytology, Mitochondria, Chloroplasts, Pinwheel inclusions.

INTRODUCTION

In nature BCMV has a limited host range and depends upon aphid and seed transmission for its survival. The virus is known to be seed-borne and there is evidence that it is carried in the embryo (Hoch and Provvidenti, 1978, Marvic and Sustar-Vaslic, 2004 and Nalini *et al*., 2006).

External symptoms are reflections of disturbed cell metabolism leading to modification in tissues, cell and cell organelles (Dijkstra and De-Jager, 1998). Some viruses of *Potyvirus* group have been reported to affect chloroplast and mitochondrion structures. They cause changes in number, size, shape and clumping pattern of chloroplasts and mitochondria in infected leaf cells (Hinrichs-Berger *et al*., 1999, Zechmann *et al*., 2003 and Zielinska *et al*., 2012). Besides these anatomical and cytological abnormalities, BCMV-infected plants often show abnormal intracellular structure , such as pinwheel inclusions in the form of scrolls or tubes characteristic of members of *Potyvirus* group (Morales *et al*., 1990, El-Nagar, 2007 and Owolabi *et al*., 2011).

In this investigation, we described the histological and cytological effects of BCMV infection in bean (*phaseolus vulgaris*).

MATERIALS AND METHODS

1-Anatomical studies:

The anatomical studies were carried out only in the second season to investigate the changes occurred in bean plants infected with BCMV (characterized previously by El-Kady *et al*.(under – publication).

Samples were taken from the terminal leaflet of fourth leaf developed on the stem. In laboratory, the sections of samples were prepared by the method suggested by Sass (1958). Sample of one centimeter square from the terminal leaflet of bean plants was taken. Plant materials were immediately kept and preserved on F.A.A. solution. Samples were dehydrated in series of solutions of ascending concentrations of ethyl alcohol varying from 50% to 100% ethyl alcohol. The samples were then embedded in paraffin wax m.p.58- 61c using xylol as solvent. By using rotary microtome, sections were cut at the thickness of 15 microns and then mounted on slides with the aid of egg-albumin as an adhesive. Wax dissolved in xylol and the slides were passed through descending series of ethyl alcohol solutions varying from 100% to 50% ethyl alcohol concentrations in descending order.

The sections on the slides were stained with safranin and then the colored sections (light green) were kept as permanent preparations on the slides with Canada balsam as mounting medium. Sections in such cases were microscopically explored for the different microphotographs, which can be explored for the different tissues and components. All micrographs were prepared by Nikon Camera on a Carl Zeiss Jena microscope

2-Cytological studies:

Bean leaf tissues infected with BCMV were examined using thin-sections by transmission electron microscope (TEM). The infected leaves were cut into small pieces about 1-2 mm., fixed in 2% Glutralhyde in 0.1 M Na-Cacodylate buffer, pH 7.2 and subjected to a vacuum for 1-4 min every 15 min for 2 hours on ice. Prior to vacuum treatment, floating samples were poked under the buffer surface with pointed metal pokers. Rinsing took place in 0.1 M Na-Cacodylate buffer, pH 7.2 for 45 min., with buffer changes at 15 min.with pointed metal pokers. Rinsing took place in 0.1 M Na-Cacodylate buffer, pH 7.2 for 45 min., with buffer changes at 15 and 30 min. Further fixation in 1% Osmium Tetraoxide in Na-Cacodylate buffer, pH 7.2, under intermittent vacuum and poking, took place for 1.5 hours (Osmont and Freeling, 2001). The samples were rinsed in the Na-Cacodylate buffer then, dehydrated through an ethanol series in buffer (35 - 50 - 70 - 80 - 95 - 100%) for 60 min. and then infiltrate with the resin mixture through a graded serics in glass vials with polypropylene caps. Semi-thin sections were prepared on glass slides through cutting at 1µ using the ultra-microtome. They were cut and stained with Toludine blue for 5 min. and examined by light microscopy model Olympus UC 30 BX 53. Both semi and ultrathin -sections were cut using ultra-microtome (Leica model EM-UC6) at thickness 90 nm, mounted on copper grids (400 mish). Ultra-thin sections were stained with double stains(Uranyl acetate 2% 10 min., followed by lead citrate for 5 min and examined by transmission electron microscope (Al-Azhar Univ. JEOL (JEM-1400) at the candidate magnification. Electron micrographs were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel format as side mount configuration.

RESULTS

Histological and cytological Studies:

1-Histological studies:

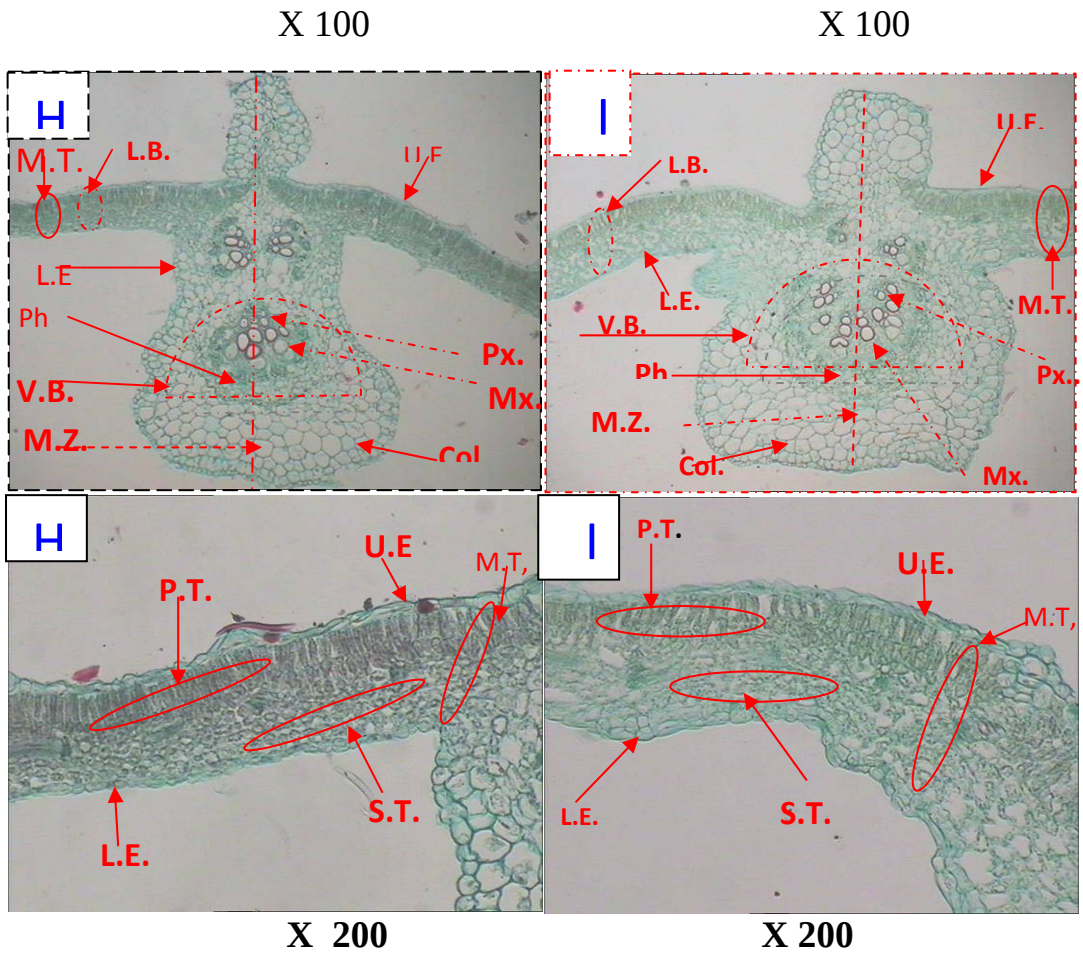
The presented data in Table (1) and Fig(1-H) show that the epidermal cells of healthy plant (Fig 3) do not contain any intercellular spaces neither do they contain chloroplasts. Thus, the palisade mesophyll commonly occurs on the adaxial (upper) side of the healthy leaf and contain abundant chlorophyll and have narrow intercellular space between

them .Whereas, leaflets blade thickness in the infected cells (Fig.1-I) was increased by +30.79% more than in the healthy plants. This increment was corresponding to increase of palisade and spongy tissues. On the other hand thickness of upper epidermal layer reduced by-12.42% as comparing to the healthy plants (control) , also, thickness of lower epidermal layer reduced by -31.63% less than the control. In the sametime , thickness of palisade and spongy cells were increased (+37. 49% and +45.21%, respectively) comparing with the control healthy plants (Fig. 3 H).On the contrary, The thickness of midrib zone was slightly reduced by -5.14% less than the uninfected plants.

Such response in anatomical structure of bean leaflets was mainly correlated with the reduction of epidermis either upper or lower epidermal layers, also, this effect was associated with evident changes on the main vascular bundle structures as comparing to the healthy plants (-33.33%). This effect was corresponding with clear developed and differentiated of vascular bundles especially protoxylem vessels. Furthermore, the main vascular bundle of midrib zone tended to be incomplete circular on shape and lost it's normal shape and it's arrangement as xylem arms as comparing to the untreated plants. The same was happened with the vascular tissue . Length of protoxylem vessel was reduced by -23.69%, while, it's width increased by+19.92% more than the healthy plants. Also, length of metaxylem vessel was reduced by -13.98% but it's width enhanced by +9.26% more than the healthy plants.

Table (1): Effect of BCMV infection on some anatomical characteristics:

Treatments	Healthy plants	Infected plants	
Characteristics (µm.)	Average of absolute values µm	Average of absolute values µm	% ± of control
Thickness of leaflet blade	140.95	184.35	+30.79
Thickness of upper epidermal layer	16.42	14.38	-12.42
Thickness of lower epidermal layer	10.56	7.22	-31.63
Thickness of palisade tissue	35.61	48.96	+37.49
Thickness of spongy tissue	78.36	113.79	+45.21
Thickness of midrib zone	1266.74	1201.61	-5.14
Number of differentiated vascular bundles	3.00	2.00	-33.33
Length of protoxylem vessel	22.54	17.20	-23.69
Width of protoxylem vessel	19.03	22.82	+19.92
Length of metaxylem vessel	46.03	39.64	-13.98
Width of metaxylem vessel	32.08	35.05	+9.26



Figs. (1) : Light micrographs showing cross-sections of healthy leaflets**(H)** and BCMV-infected **(I)** bean plants *cv. Balady*.

Abbreviations :.

L.B. = Leaf blade, **L.E.** = Lower epidermal layer, **M.T.** = Mesophyllic tissue, **Mx.** = Metaexylem, **M.Z.** = Midrib zone, **P.T.** = Palisade tissue, **Px.** = Protoxylem, **S.T.** = Spongy tissue, **U.E .**= Upper epidermal layer, **Ph**. = Phloem and **Col. =** Collenchyma

2: Cytological studies:

Electron microscopic comparisons of infected and healthy bean leaf tissues revealed significant differences.

A- Examination of ultrathin section of healthy leaf (Fig 2) showed normal cell wall (CW) . Whereas, the chloroplasts (Ch) are typical lens – shaped and bounded by an envelope , enclosing the stroma (S). Grana arranged in regular rows and connected to each other by a system of flatted tubes, the intergranum lamellae (L) , a system known as thylakoids. The mitochondria (M) are slightly ovoid and somewhat more viscous and dense than cytoplasm. Regarding the nucleus (N), it is usually a spheroidal or elliposidal protoplasmic body enclosed within the cytoplasm and bounded by the nuclear membrane , its viscosity is higher than that of the cytoplasm.

B- Examination of ultrathin section of BCMV – infected cells revealed several morphological changes . These features were not observed in comparable cells of healthy plants :

1- cell wall: Different abnormalities have been observed in or near the walls (Figs 3,4 and 7) of virus- diseased cells

.One of the marked differences , due to the deposition of electron dense material between the cell wall and the plasma membrane, it may also occur in association with plasmodesmata (P1) as shown in Fig (7). Moreover, degradation of the cell wall may also observe.

2- Chloroplasts : They have a complex internal structure. An outer, double membrane surrounds a finely granular mass , the stroma (S) . BCMV infection can cause many cytological changes in the chloroplasts (Ch) . In the inoculated leaves , the chloroplasts (Figs 3, 4,5 and 7) become rounded and clumped together with irregular rows of the grana , which may be degenerate , the reduction of grana stack height have been also observed at the later stage of degreneration of the chloroplasts, electron-lucent material is replaced by virus particles (Fig. 6).

3- Mitochondria : Destruction and aggregation of mitochondria (M) have been noticed in thin sections of infected leaf cells (Figs 3,4,5 and 6).

4- Nuclei : No detectable cytological changes on nuclei except that occasionally , the nucleus is become elongated than normal and is not centrally located (Fig. 7) .

5- Inclusion bodies : The most characteristic internal symptoms in viral infection is the occurrence of inclusion bodies (Figs. 5and 7). They may contain virus related materials. Amorphous inclusions which are aggregates of single small rounded particles , also pinwheel inclesions (Pw) in the form of scrolls or tubes have bean shown in the ultrathin sections of infected cells (Figs. 4 and 7).

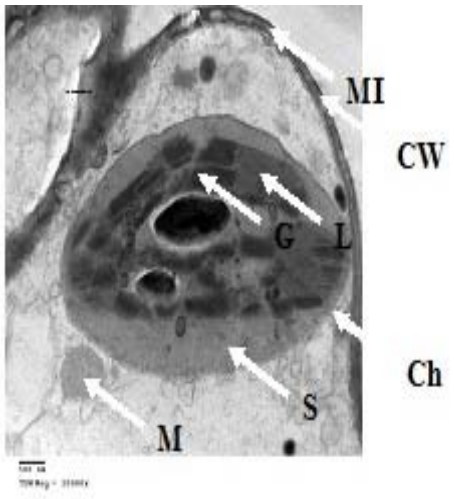


Fig.(2): Ultrathin section of healthy bean leaf cells revealed normal cell wall (CW) and normal chloroplast (Ch) with normal grana (G) and intergrana (L) (Magnification X 20,000).

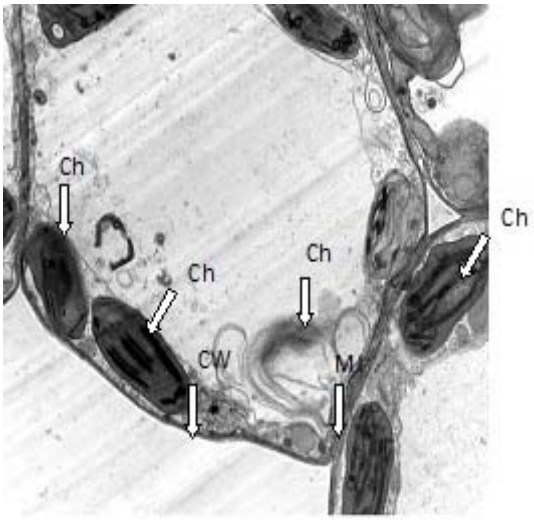


Fig. 3: Electron micrograph of ultrathin section of infected bean leaf cells showing elongation and destruction of chloroplasts (Ch) cells. (Magnification X 8,000).

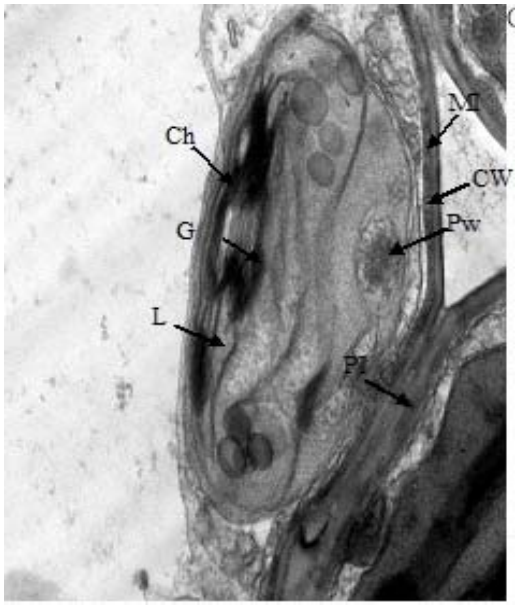


Fig.(4): Ultrastructure of bean leaf cells infected with BCMV, showing abnormal thickening of the cell wall (Cw) , fragmentation of chloroplasts (Ch) , reduction in granal (L) stack height , aggregation of mitochondria (M) and pinwheel inclusions (Pw) (Magnification X 30,000).

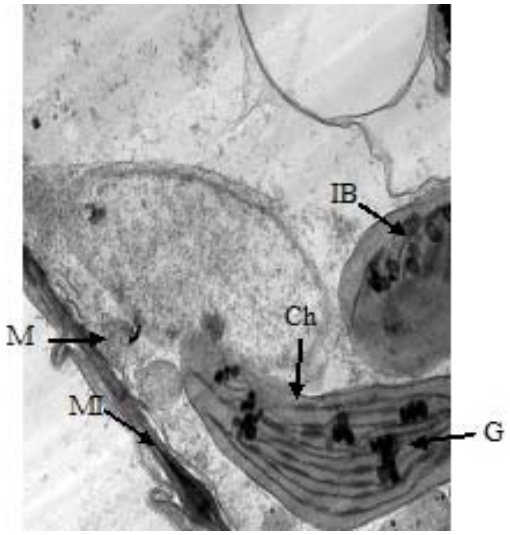


Fig.(5): Cytological alternations included by BCMV in bean leaf cells: Chloroplast (Ch) contains numerous osmiophilic inclusions (IB), reduction in granal stick height (L) and distraction of mitochondria (M) .(Magnification X 20,000).

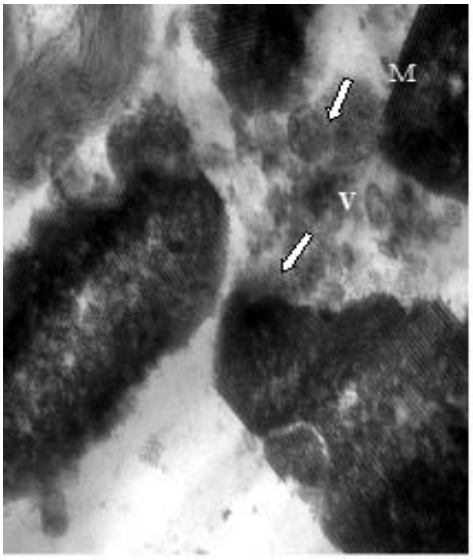


Fig.(6): Ultrathin section of infected bean leaf cells showing aggregated virus particles (V) and destruction of mitochondria (M) (Magnification X 50,000).

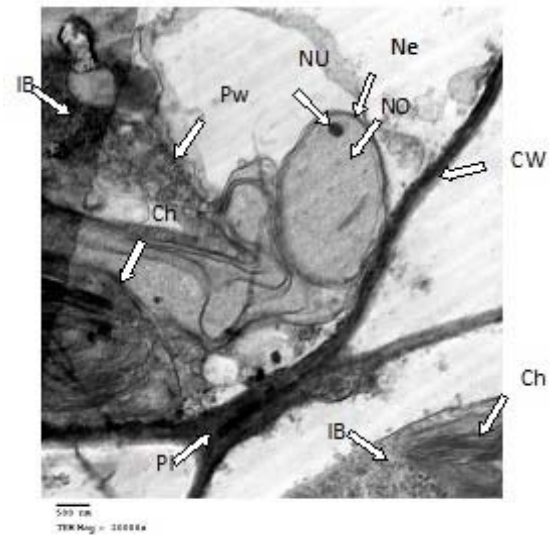


Fig.(7): Electron micrograph of infected bean leaf cells showing elongated nucleus (Nu) and the nucleolus (No) also observed , fragmentation of chloroplast , pinwheel and amorphous inclusions (Magnification X 20,000) .

DISCUSSION

This study cleared anatomical variations between plants infecting with BCMV compared with the healthy plants (control).

Our results showed that thickness of either upper or lower layers and midrib zone were reduced in infected cells than those of healthy ones. In contrast, thickness of leaflet blades, palisade and spongy tissues were increased in infected cells than those of healthy ones. In addation , length of neither protoxylem nor metaxylem were reduced comparing with those of healthy cells. In the contrary the width of whether potoxylem or metaxylem vessels were higher in infected than those of healthy ones. However, It was also, noticed that the number of differentiated vascular bundles were decreased in healthy cells comparing with healthy ones. In this regard Ishak and El-deeb(2004) found that, the most important changes due to sweet potato chlorotic stunt virus (SPCSV) infection were confined to the vein region. Similar results were obtained by Mohamed *et al.* (2012), who reported that, infected phloem tissues showed less active sieve elements, and thickness of phloem radial and secondary phloem fibers were reduced also, the thickness of xylem tissue and vessels diameter were also reduced. Such anatomical and ultra structural variations have been reported earlier in citrus leaves infected by *Citrus psorosis* (Sofy *et al*. 2007). Kunkalikar *et al*. (2007) showed that, papaya ring spot virus brings about histological and histochemical changes in papaya upon infection. In diseased leaves, palisade cells were markedly distorted .The spongy cells lost their normal round shape with complete disintegration.

Ultrathin sections of BCMV-infected plants showed alternations in host cells , either in cytoplasmic component or cell organells.

These abnormalities including cell wall , chloroplasts, mitochondria , nucleus and virus – related inclusion materials. Concerning the cell wall , it was thicker than normal in association with somewhat distorted and extended plasmodesmata. The chloroplasts become rounded and clumped together with irregular rows of the grana. The grana become disorganized and degenerated in infected cells. Reduction in grana stack height was also detected. The faster breakdown and viscous state of lamella system can be contribute to a decline in photosynthetic potential of infected cells compared with the healthy ones (Pome-Novak *et al*, 2001) . Aggregation and destruction of mitochondria have been also observed (Omar *et al.*, 1995 a and b , Nader and Berger, 1997, Khatab , Eman , 2002 and El-Nagar , 2007). No changes were evident in the morphological features of nuclei except that occasionally the nucleus is become elongated than normal and is not centrally located (Matthews, 2002). BCMV infection induced different types of cytoplasmic and pinwheel inclusions in the form cerolls or tubes characteristic of the members of *Potyvirus* group (Morals *et al.*, 1990, El-Nagar , 2007 and Owolabi *et al.*, 2011).

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