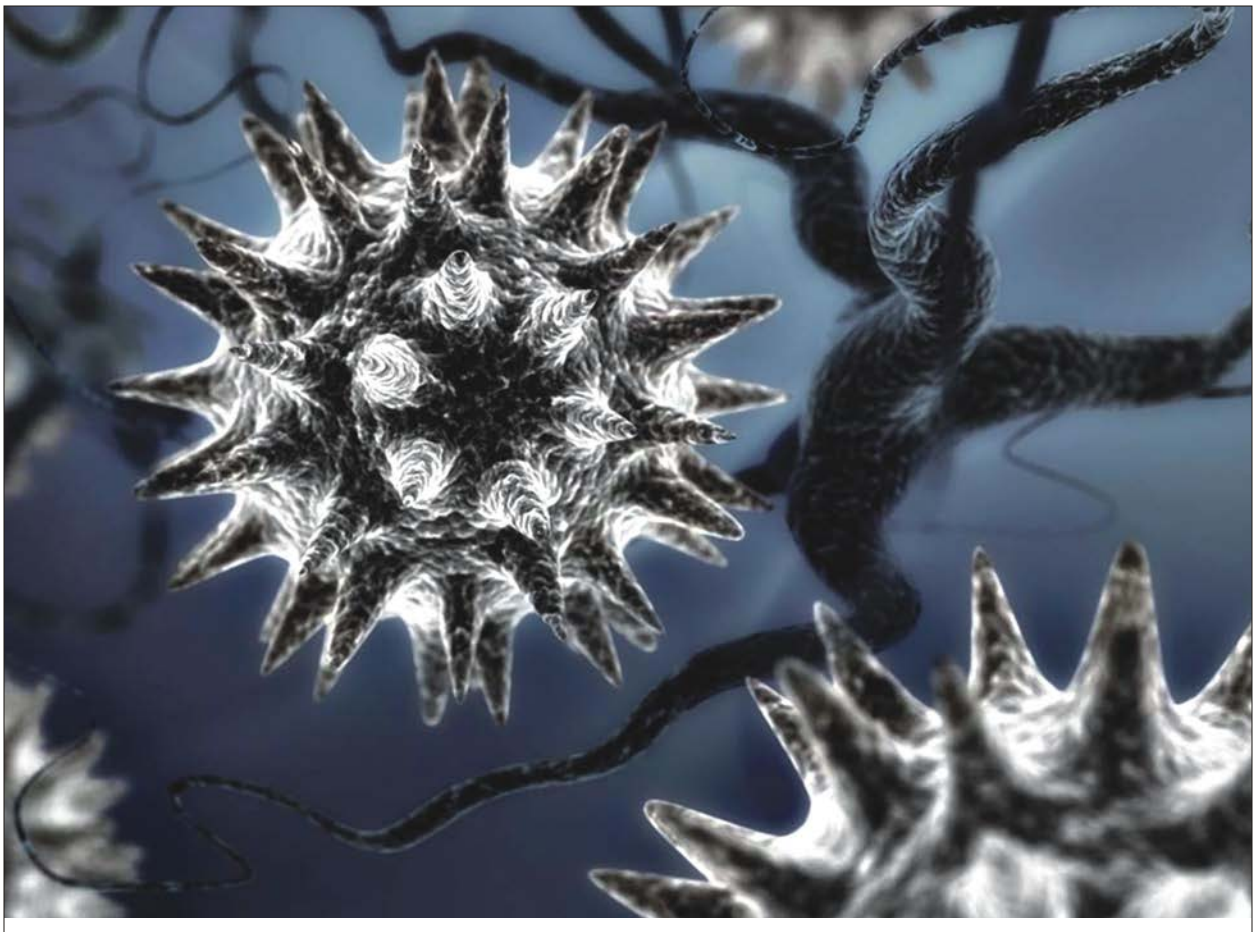

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 11 (3): 123-132, 2015
ISSN 1816-4900 / DOI: 10.3923/ijv.2015.123.132

Observation of Mitotic and Cytological Effects Induced by *Bean Yellow Mosaic Virus* in Affecting Leguminous Plants

[1,2]Eman A. Kahattb, [2,3]Maissa Morsy and [2]Ibtesam M. Al-shamran
[1]Research Department of Virus and Phytoplasm, Research Institute of Plant Pathology, Agricultural Research Center, Giza, Egypt
[2]Department of Biology, Faculty of Science, Taif University, Kingdom of Saudi Arabia
[3]Department of Botany, Faculty of Women for Art, Science and Education, Ain Shams University, Egypt

Corresponding Author: Eman A. Kahattb, Research Department of Virus and Phytoplasm, Research Institute of Plant Pathology, Agricultural Research Center, Giza, Egypt

ABSTRACT

Bean Yellow Mosaic Virus (BYMV) causing yellow mosaic and leaf malformation was detected and isolated from naturally infected bean plants collected from Southern region of Saudia Arabia BYMV was transmitted mechanically and identified by serologica1 tests. Light microscopy examinations revealed amorphous cytoplasmic and crystalline inclusions in bean plants infected with BYMV. Ultra structural changes in the deformed chloroplasts and grana were severe affected, chloroplasts were more affected, swollen, irregular, rupture lamella, crash grana and combination with each in the form of amoebic. The nucleus was elongated irregular with spindle like shape. The results suggest that the depressive effect in the mitotic process of *Vicia faba* L. was demonstrated when infected with *Bean yellow mosaic virus*. Remarkably, there was a progressive decrease in mitotic indices and increase in the total percentage of abnormalities due to bean yellow mosaic virus infection. Types of abnormalities revealed the induction of spindle disturbance, stickiness, laggards, fragments, bridges and micronuclei which lead to the loss of genetic material.

Key words: *Bean Yellow Mosaic Virus* (BYMV), transmission, inclusion bodies, ultra structural changes and DBIA, genotoxicity, mitotic indices, abnormalities

INTRODUCTION

Bean Yellow Mosaic Virus (BYMV), exhibits overall stunting, color-breaking, flower distortion and reduced flower, mosaic in gladiolus and bean (Katoch *et al*., 2002; Wylie *et al*., 2008). Choi *et al*. (2013) found that, *Bean yellow mosaic virus* causes severe losses to various legume species and a number of non-legume species, particularly freesia plants in Korea.

Radwan *et al*. (2008) examination of electron microscopic of ultrathin sections of infected leaves with *Bean Yellow Mosaic Virus* (BYMV) demonstrated that most chloroplasts with increased stromal area became spherical in shape and some lost their envelopes, either partially or totally. The internal structures of chloroplast, grana and thylakoids were dilated. Two kinds of inclusions were revealed in BYMV infected leaves: Straight or slightly curved bands sometimes coiled or looped at theend and electron opaque crystals with varied shapes. The BYMV infected cells showed lower chloroplast number in comparison to the control.

There are several reports on cytological changes due to virus infection. It has been found that meiotic abnormalities and pollen sterility due to virus infection in *Capsicum annuum* L.,

Caricapapaya L., *Lablab purpureus* L., *Lycopersicon esculentum* L. and *Solanum melongena* L. were studied by Naz *et al.* (2008), frequencies of chromosomal irregularities, asynapsis, multivalent, lower chiasma frequencies and pollen sterility were high in virus infected plants after the comparison with un affected plants.

Regarding the cytological changes in carrot due to infection of cucumber mosaic virus, the scattered metaphase was observed in the diseased plant cells, mitotic index of the diseased cells was decreased while N/C ratio was increased. Chromatin bridges were also observed at anaphase I and II (Afreen *et al*., 2011).

On the other hand, Yadav *et al*. (2011) studied the behavior of chromosomes during meiosis after virus infection with ChiMV of *Capsicum annuum* L. and demonstrated that aberrations were mainly found at metaphase and anaphase stages. At metaphase prominent aberrations were: scattered metaphase, chromatid separation, fragmentation, clumping and stickiness of chromosome. Anaphase induced chromatin bridges, multipolarity, unequal separation and stickiness, so ChiMV induced chromosomal abnormalities at meiotic division.

The aim of this investigation was undertaken to study the effect of BYMV infection on cell organelles observed by electron microscopy and mitotic cell division observed by light microscopy.

MATERIALS AND METHODS

Isolation and propagation of the virus isolate: Samples of infected bean plants showing typical symptoms of virus infection were obtained from Southern region of Saudia Arabia. Infectious sap was obtained by grinding infected tissue in 0.1 M phosphate buffer, pH 7.0 at a ratio of 1:3 (w/v),was used to inoculate the tested plants (*Phaseolus vulgaris*, *Vicia faba*, *Rosa damascena*, *Chenopodium amaranticolor* and *Vigna unguiculata*) after dusting the first leaves with carborandm and kept in an insect proof greenhouse for symptoms expression.

Serological diagnosis

Dot Blot Immuno Assay (DBIA): The DBIA was applied as described by Lin *et al*. (1990). Healthy and infected samples were ground in phosphate buffer and pressed steadily on the nitrocellulose membranes.

Cytological effects of virus infection

Examination of inclusion bodies by light microscopy: To study the chemical nature of the inclusion bodies, epidermal strips taken from the lower surface of infected bean and faba bean leaves and were treated first with 5% solution of Triton X-100 for 10 min, to disrupt the plastids and facilitate the detection of the inclusions. The differential staining of protein were performed (Christie and Edwarson, 1986).

Effect of virus infection on cell organelles by electronic microscopy: Ultra structures of bean leaves cells were investigated to detect the effect of virus infection using the following procedure, samples were collected from bean leaves infected with the isolated virus after 20 days from inoculation.

Then prepared for ultrathinsections according to the procedure described by Sadik (1994). Finally, the ultrathin sections were examined under the electron microscope 100 S×11, in the Electron Microscope Unit, Faculty of Science, Taif University.

Mitotic study: Seeds of *Vicia faba* L. from healthy and infected plants with BYMV of equal size and weight about (100 g) were germinated on moist filter paper in petri dishes. Radicals of 1.5-2 cm were cut, fixed with Carnoy's fixative for 24 h, repeat washed in water, then Feulgen squash technique was carried out. Five temporary slides were prepared, approximately 1000 cells per slide were examined. Recorded data included: the frequency of Mitotic Index (MI) and numbers and types of abnormalities were also examined.

RESULTS

Isolation and propagation of the virus isolate: The virus isolate was purifed biologically by successive local lesion on *Chenopodium amaranticolor* and propagated in *Phaseolus vulgaris*, *Vigna unguiculata* and *Vicia faba*. The virus isolate was identified as BYMV on according to symptomatology, Dot Blot Immuno Assay (DBIA) and inclusion bodies. The infected plants BYMV isolated from Taif governorate of Saudia Arabia induced local lesion on *Chenopodium amaranticolor* while systemic mosaic, malformation on *Phaseolus vulgaris, Vicia faba vigna unguiculata* and yellow mosaic, colour breaking on flower *Rosa damascena* and *Vigna unguiculata*, while induced local lesion on *Chenopodium amaranticolor* (Fig. 1a-d).

Serological diagnosis: The BYMV was readily detected immunologically in tested plants using DBIA techniques. Positive reaction was indicated by the develop color produced a purplish-blue color whereas in negative reaction, tissues from healthy plants remain green (Fig. 2).

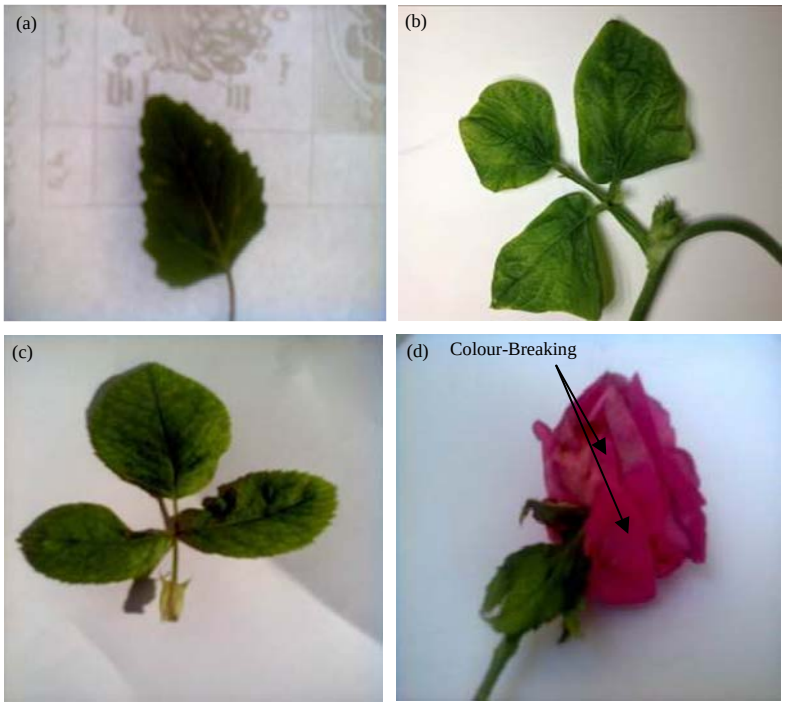

Fig. 1(a-d): (a) Chlorotic local lesions produced by BYMV on *Ch. amaranticolor*, (b) Sever mosaic and malformation of BYMV infected leaves of *Phaseolus vulgaris* cv. Giza3, (c) Yellow mosaic and (d) Colour-Breaking on flowers produced by BYMV on *Rosa damascena*

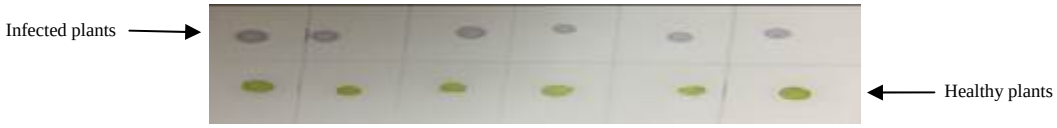

Fig. 2: Detection of BYMV by Dot Blot immunoassay (DBIA)

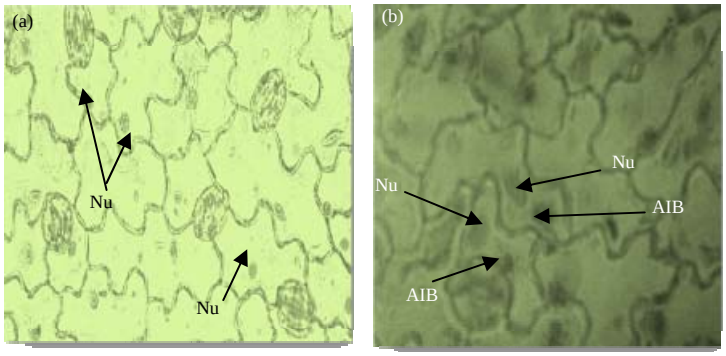

Fig. 3(a-b): Light microscopy of epidermal strips of bean leaf infected with BYMV, 14 days post- inoculation showing cytoplasmic inclusions. Magnification (X100) (a) Healthy cell and (b) Infected cell, Nu: Nucleus, AIB: Amorphous cytoplasmic inclusion

Cytological effects of the virus infection

Virus induced inclusions by light microscopy: Examination of epidermal strips of faba bean (*Vicia faba* L.) and bean (*Phaseolus vulgaris* L.) leaf infected with BYMV 14-24 days after inoculation with light microscopy revealed amorphous cytoplasmic inclusions, whereas inclusions were not observed in healthy plants (Fig. 3).

Effect of virus infection on cell organelles: Electron microscopic examination of ultrathin sections of infected bean leaves showed changes in the deformed chloroplasts and grana were severe affected. The chloroplasts were more affected in this stage as well as swollen, irregular, rupture lamella, crash grana and combination with each in the form of amoebic (Fig. 4) . Cylindrical cytoplasmic inclusions were appeared as pinwheels (Fig. 5). The nucleus was elongated irregular with spindle like shape (Fig. 6) and the chromatin was separately in on side.

Mitotic studies: *Bean Yellow Mosaic Virus* (BYMV) induced cytological variation in *Vicia faba* L. plants. The infected plants showed very important characters in the cells which were specific host virus combination. It is evident from Table 1 that the Mitotic Indices (MI) of healthy and infected plant cells of *Vicia faba* L. were 8.78 and 4.00, respectively. The decrease in mitotic indices was significant at 0.01 and was accompanied by an accumulation in prophase stage and documented in Table 2. These results demonstrated that mosaic virus inhibits cell division and can also inhibit growth and development of *Vicia faba* L. plant. The percentage of prophase increased with a corresponding decreased in the percentages of the other stages after the infection with BYMV.

Cytological analysis of cells in the course of mitosis revealed a universal increase in the percentage of abnormalities of *V. faba* L. roots after virus infection reaching (35.41%) as compared to their respective control Table 1.

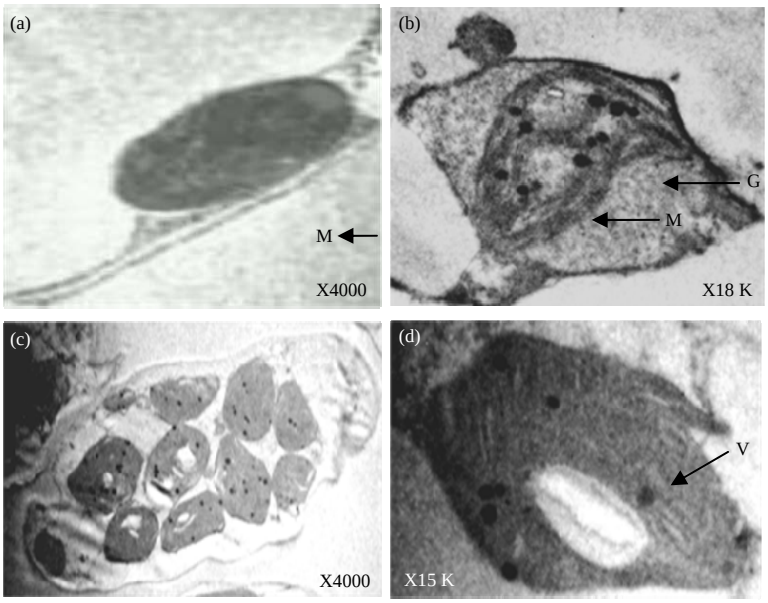

Fig. 4(a-d): Ultrathin section prepared from bean leaf appeared yellow mosaic after 20 days from inoculation with BYMV (a) Healthy chloroplasts, (b and d) Shown rupture Lamelland crash Grana, destroyed irregular and swollen chloroplast, (c) Chloroplasts showing combination with each in the form of amoebic in the cells Infected, Ch: Chloroplast, G: Grana, V: Vacuole, M: Membrane of chloroplast

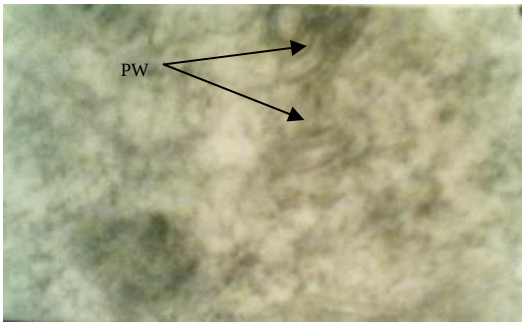

Fig. 5: Ultrathin section prepared from a leaf bean plant showed cylindrical inclusions appeared as pinwheel in the cytoplasm (20000x), PW: Pin Wheel

The effect of mosaic virus infection on *V. faba* L. induced some abnormalities in all the stages of mitosis: irregular prophase, spindle disturbance, stickiness bridges, laggards, fragments and micronuclei (Fig. 7, Table 3).

DISCUSSION

Light microscopy is still important in the study of cytological abnormalities as much grater areas of tissue can be scanned for presence or absence of inclusion bodies (Matthews, 1991). In the present work , amorphous cytoplasmic inclusions induced by BYMV was observed in infected

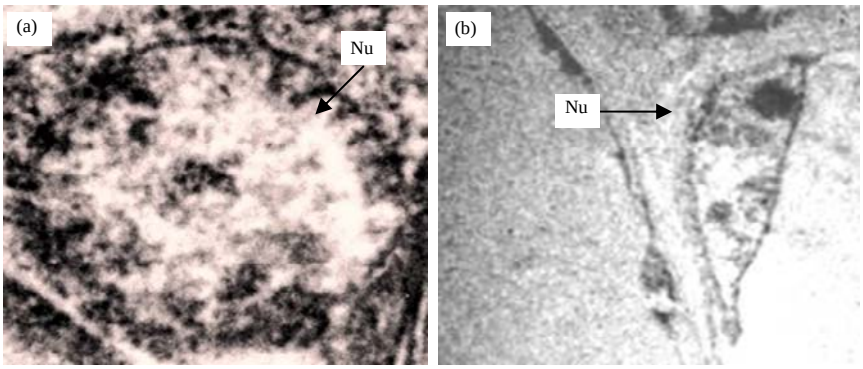

Fig. 6(a-b): Ultrathin section prepared from bean leaf appeared yellow mosaic after 20 days from inoculation with BYMV, shown hypertrophy of nucleus and irregular nuclear membrane and elongated shaped nucleus (a) Healthy nucleus and (b) Infected nucleus, Nu: Nucleus

Table 1: Mitotic index and total percentage of abnormalities of *Vicia faba* L. root tip cells infected with mosaic virus

	Total No. of cells		Mitotic index±SD		Total percentage of abn.±SD	
Slide No.	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	1010	1000	7.62	4.1	11.68	24.39
2	1035	1000	9.46	4.5	8.16	28.88
3	1026	1010	11.2	3.36	12.17	32.35
4	1060	1005	9.24	3.28	10.2	27.27
5	1000	1000	6.4	4.8	10.93	35.41
Mean	1026.2	1003	8.78±19.98	4.00±6.61**	10.62±2.70	29.66*±3.16

**Significant from control at 0.01 level (t-test), abn: Abnormalities

Table 2: Frequency and percentage of abnormalities of mitotic phases in *Vicia faba* L. root tips after infection with mosaic virus

	Prophase				Metaphase				Anaphase-Telophase			
	Phase (%)		abn. (%)		Phase (%)		abn. (%)		Phase (%)		abn. (%)	
Slide No.	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	72.72	56.25	44.44	58.82	10.38	18.75	22.22	17.64	16.88	25.00	33.33	23.52
2	78.57	57.77	62.50	46.15	8.16	15.55	0.00	23.07	13.26	26.66	37.50	30.76
3	78.26	44.11	50.00	45.45	6.95	23.52	14.28	27.27	14.78	32.35	35.71	27.27
4	82.65	63.63	60.00	66.66	6.12	18.18	10.00	11.11	11.22	18.18	30.00	22.22
5	81.25	41.46	71.42	20.00	3.12	21.95	0.00	30.00	15.62	36.58	28.57	50.00
Mean	78.69±16.45	52.64**±5.31	57.67±1.14	47.41±2.86	6.94±2.60	19.59±1.30	9.30±1.00	21.81*±0.89	14.35±2.68	27.75±3.27	33.02±1.09	30.75±1.14

*Significant from control at 0.05 level (t-test), **Significant from control at 0.01 level (t-test), abn: Abnormalities

Table 3: Percentage of different types of abnormalities in *Vicia faba* L. roots using after mosaic virus infection

		Types of abnormalities (Mean)							Interphase
Treated plant	Mean of total percentage of abn.	Disturb.	Irr. prophase	Stick.	Bridge	Lagg.	Frag.	Macro-nucleus	Macro-nucleus
Healthy	10.62	1.99	3.90	2.80	1.99	0	0	0	0
Infected	29.66	7.42	18.90	8.40	1.48	1.48	0.99	0.99	0.74

abn: Abnormalities, Stick: Stickness bridge, Lagg: Laggards, Frag: Fragments

epidermal strips of *Phaseolus vulgaris* L. These result was in conformity with those obtained by Brunt *et al.* (1996), Matsumoto *et al*. (1999), Khattab (2002), Bellardi and Bianchi (2003) and Al-Sama'a and Ramadan (2005).

Electron microscopic examination of ultrathin sections of infected bean leaves showed changes in the deformed chloroplasts and grana were severe affected, the chloroplasts were more affected in this stage as well as swollen, irregular, rupture lamella, crash grana and combination with each in the form of amoebic. Cylindrical cytoplasmic inclusions were appeared as pinwheels. The nucleus

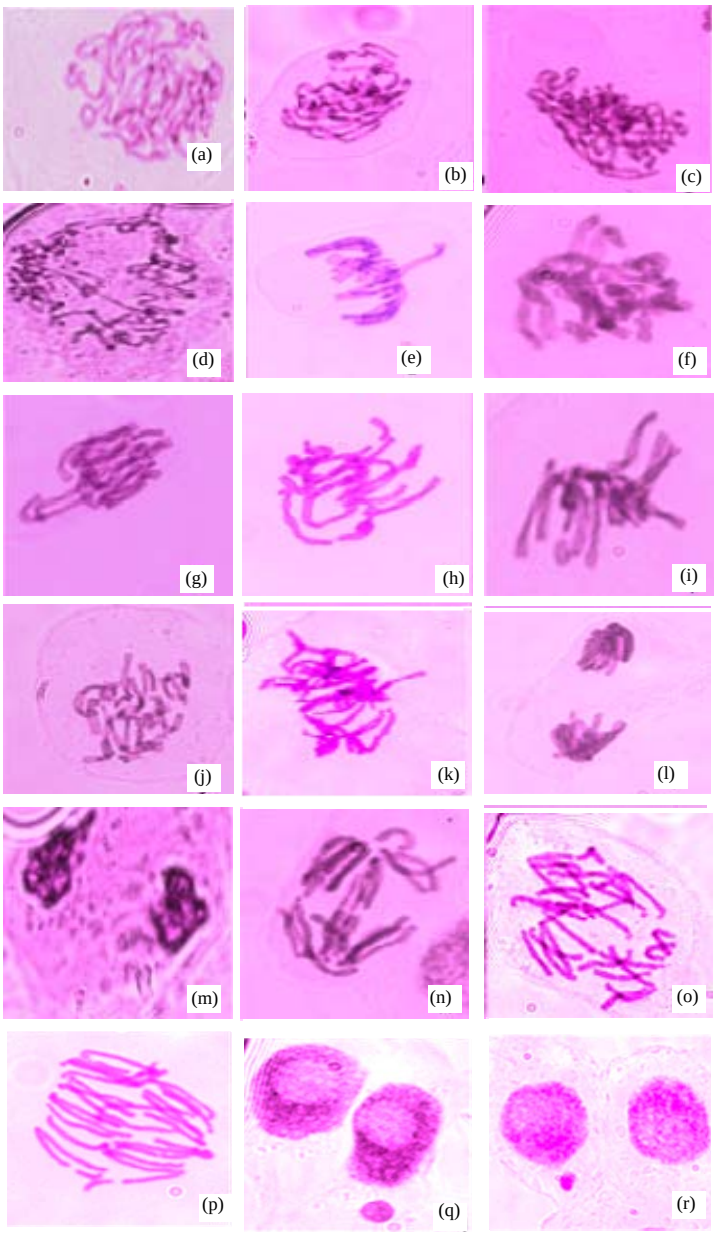

Fig. 7(a-r): Different types of chromosome aberrations of Vicia root meristems induced by virus infection: a-d: Irregular prophase, e-h: Disturbed metaphase and stickiness, i, j: disturbed metaphase with fragment, k: Disturbed anaphase and stickiness, l-m: Severe sticky anaphase, n-p: Disturbed and light sticky anaphase with multibridge and laggards q, r: Interphase with micronucleus

was elongated irregular with spindle like shape and the chromatin was separately in on side. These result is in harmony with those obtained by Bos (1970), Allam *et al*. (1986), Khattab (2002) and Radwan *et al*. (2008).

The mitotic cell division in the infected plant cells were very low, thus, the mitotic index of the infected cells of *Vicia faba* L. was decreased significantly in comparison to healthy plant cells.

The lowering of MI might have been achieved by the inhibition of DNA synthesis at S-phase after the use of some food additives (Sudhakar *et al*., 2001). An explanation after the use of some medicinal plants as *Nigella sativa* L. which contains antimitotic constituents that can stop the mitosis in anywhere of the cell cycle, furthermore these constituents probably affects the cytoskeleton or tubulin polymerization or degradation (Ozmen *et al*., 2007). However, (Mahakhode and Somkuwar, 2013) demonstrated that Glyphosate inhibited cell division due to mitodepressive and toxicity of herbicide on cell and cell cycle, the same effect was found after virus infection.

The infection conducted with *Bean yellow mosaic virus* showed an increase in the percentage of prophase with a corresponding decrease in the percentage of other stages. The same result occurred by Ilbas *et al*. (2012) after the use of *Aloe vera* gel extract on *Allium cepa* L., this may attributed to the blocking of cell division by virus at the end of the prophase stage. In this case, the virus may be accepted as premetaphase inhibitors.

An increase in the percentage of abnormalities of *V. faba* L. roots occurred after virus infection reaching (35.41%) as compared to their respective control. The chromosomal aberrations noticed in this study indicate that exposure of plants to virus infection can lead to mutation or can act as clastogene, the same result reported by (Njoku *et al*., 2011), who found that altered chromosomes may possible have altered DNA and gene sequences.

Some abnormalities in all the stages of mitosis induced, irregular prophase, spindle disturbance, stickiness bridges, laggards, fragments and micronuclei after the effect of *Bean yellow mosaic virus* on *V. faba*.

Disturbance may be caused by inhibition of DNA synthesis at S-phase of cell cycle, Kumari *et al*. (2011). As noted by Mahakhode and Somkuwar (2013), various structural disturbances in cell organelles translated into physiological and metabolic events leading to the slowing down of the growth of the plant and finally to its death.

In this study, chromosome stickiness was the other major chromosomal aberrations and was recorded in a considerable percentage. The presence of chromosome stickiness is an indication of virus affecting organization of chromatin and suggests a possible role by which this cytotoxic agent may impact the physical and chemical properties of DNA, protein, or both, ultimately leading to improper folding of chromatin (Teerarak *et al*., 2010).

This led to the conclusion that the disturbance in the enzyme system might be resulting in disturbance of the homeostasis of the cell by genetic variability induced by stickiness which result in chromosomal aberrations and decrease in the rate of cell division (Mahakhode and Somkuwar, 2013).

The bridge formation due to chromosomal stickiness or due to chromosomal breakage and reunion stated by Kumari *et al*. (2011).

The occurrence of chromosome laggards was due to the failure of the chromosomes or acentric chromosome fragments to move to either of the pole at anaphase stage (Liman *et al*., 2012).

Micronuclei are the results of acentric fragments or lagging chromosomes that fail to incorporate in to either of the daughter nuclei during telophase of the mitotic cells (Krishna and Hayashi, 2000). Turkoglu (2009) found that micronucleus formation implies loss of genetic materials. Moreover, micronucleus formation is considered to be one of the most economical, quickest and most effective ways in determining genotoxicity of different chemicals (Hamedo and Abdelmigid, 2009).

CONCLUSION

In conclusion, *Bean yellow mosaic virus* infection caused harmful effects on the root tip cells of *Vicia faba*. The reduction in mitotic activity, the high percentage of chromosome and nuclear irregularities in the meristematic cells of *Vicia faba*, suggest the presence of certain cytotoxic/genotoxic substances in the tested virus.

REFERENCES

Afreen, B., M. Gulfishan, G. Baghel, M. Fatma, A.A. Khan and Q.A. Naqvi, 2011. Molecular detection of a virus infecting carrot and its effect on some cytological and physiological parameters. Afr. J. Plant Sci., 5: 407-411.

Al-Sama'a, M.S. and N.A. Ramadan, 2005. Microscopical study of internal symptoms of *Bean yellow mosaic virus* in infected Faba bean plants. Rafidain J. Sci., 16: 62-68.

Allam, E.K., A.A. Kishtab, S.A. Eid, A.M. Abdelsalam and M.A.S. El-Kady, 1986. Type and chemical nature of inclusion bodies induced by two Egyptian isolates of bean yellow mosaic virus. Egypt. J. Phytopathol., 18: 55-58.

Bellardi, M.G. and A. Bianchi, 2003. First report of *Bean yellow mosaic virus* in *Aegopodium podagraria* L. J. Plant Pathol., 85: 135-135.

Bos, L., 1970. Bean yellow mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 40, Commonwealth Agricultural Bureaux.

Brunt, A.A., K. Crabtree, M.J. Dallwitz, A.J. Gibbs and L. Watson, 1996. Viruses of Plants. Descriptions and Lists from the VIDE Database. 2nd Edn., CAB International, Wallingford, UK., ISBN: 0-85198-794-X, Pages: 1484.

Choi, S.H., J.Y. Yoon, K.H. Ryu and S.K. Choi, 2013. The complete nucleotide sequence of a Korean isolate bean yellow mosaic virus from *Freesia* sp. and comparison to other Potyviruses. Res. Plant Dis., 19: 77-83.

Christie, R.G. and J.R. Edwarson, 1986. Light microscopic techniques for detection of plant virus inclusions. Plant Dis., 70: 273-279.

Hamedo, H.A. and H.M. Abdelmigid, 2009. Use of antimicrobial and genotoxicity potentiality for evaluation of essential oils as food preservatives. Open Biotechnol. J., 3: 50-56.

Ilbas, A.I., U. Gonen, S. Yilmaz and M.Y. Dadandi, 2012. Cytotoxicity of *Aloe vera* gel extracts on *Allium cepa* root tip cells. Turk. J. Bot., 36: 263-268.

Katoch, M., M.Z. Abdin, R. Ram and A.A. Zaidi, 2002. An overview of diagnostics for viruses infecting *Gladiolus*. Crop Prot., 22: 153-156.

Khattab, E.A.H., 2002. Recent techniques to study some broad bean viral diseases. Ph.D. Thesis, Faculty of Agriculture, Zagazig University, Egypt.

Krishna, G. and M. Hayashi, 2000. *In vivo* rodent micronucleus assay: Protocol, conduct and data interpretation. Mutat. Res./Fundam. Mol. Mech. Mutagen., 455: 155-166.

Kumari, M., V.K. Sinhal, A. Srivastava and V.P. Singh, 2011. Cytogenetic effects of individual and combined treatment of Cd^{2+}, Cu^{2+} and Zn^{2+} in *Vigna radiata* (L.) wilczeck. J. Phytol., 3: 38-42.

Liman, R., U.G. Gokce, D. Akyil, Y. Eren and M. Konuk, 2012. Evaluation of genotoxic and mutagenic effects of aqueous extract from aerial parts of *Linaria genistifolia* subsp. *genistifolia*. Revista Brasileira de Farmacognosia, 22: 541-548.

Lin, N.S., Y.H. Hsu and H.T. Hsu, 1990. Immunological detection of plant viruses and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. Phytopathology, 80: 824-828.

Mahakhode, R.H. and S.R. Somkuwar, 2013. Mitotic abnormalities induced by glyphosate in *Psoralea corylifolia* L. Int. J. Curr. Pharmaceut. Res., 5: 46-48.

Matsumoto, J.I., T. Maeda and N. Inouye, 1999. Some properties of bean yellow mosaic virus isolated from *Calanthe* sp. (Orchidaceae) in Japan. Bull. Res. Inst. Bioresour. Okayama Univ., 6: 43-51.

Matthews, R.E.F., 1991. Plant Virology. 3rd Edn., Academic Press Inc., London, UK., ISBN-13: 9780124805538, Pages: 835..

Naz, A., G. Kabir and M.M. Ud-Deen, 2008. Cytological effect of virus infection in five crop species. Bangladesh J. Bot., 37: 207-209.

Njoku, K.L., M.O. Akinola and O.R. Oshodin, 2011. Phytotoxicity assay of crude oil using different accessions of *Sorghum bicolor*. Iranica J. Energy Environ., 2: 235-243.

Ozmen, A., G. Basbulbul and T. Aydin, 2007. Antimitotic and antibacterial effects of the *Nigella sativa* L. seed. Caryologia, 60: 270-272.

Radwan, D.E.M., G. Lu, K.A. Fayez and S.Y. Mahmoud, 2008. Protective action of salicylic acid against bean yellow mosaic virus infection in *Vicia faba* leaves. J. Plant Physiol., 165: 845-857.

Sadik, A.S., 1994. Studies on some viruses affecting banana in Egypt. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Sudhakar, R., K.N.N. Gowda and G. Venu, 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia, 66: 235-239.

Teerarak, M., C. Laosinwattana and P. Charoenying, 2010. Evaluation of allelopathic, decomposition and cytogenetic activities of *Jasminum officinale* L. f. var. *grandiflorum* (L.) Kob. on bioassay plants. Bioresour. Technol., 101: 5677-5684.

Turkoglu, S., 2009. Genotoxic effects of mono-, di- and trisodium phosphate on mitotic activity, DNA content and nuclear volume in *Allium cepa* L. Caryologia, 62: 171-179.

Wylie, S.J., B.A. Coutts, M.G.K. Jones and R.A.C. Jones, 2008. Phylogenetic analysis of *Bean yellow mosaic virus* isolates from four continents: relationship between the seven groups found and their hosts and origins. Plant Dis., 92: 1596-1603.

Yadav, P.V., M. Kumari and Z. Ahmed, 2011. Occurrence of vivipary in *Capsicum annuum* L. cv. California wonder. Curr. Sci., 100: 1122-1122.