Egyptian J. Virol. 1, 355-366 (2004)

Physiological and Histopathological Changes in *Potato Virus X* and *Potato virus Y* - Infected Potato Plants

Hanaa H.A.Gomaa[1], A. F. Moustafa[1], Kh.A.El-Dougdoug[2] A.A.Abou-Zeid[3] and S. Y.M. Mahmoud[4]

[1] Department of Botany. Faculty of Science, Suez Canal University. [2] Department of Microbiology. Faculty of Agriculture. Ain-Shams University, Shoubra El heima, Cairo, Egypt. [3] Plant Pathology Institute. Agriculture Research Center, Giza. [4] Department of Agricultural Botany. Faculty of Agriculture, Souhag. South Valley University

Potato viruses X and *Y* were used for study the effect of the two viruses on the metabolism of potato plants. It was found that detectable decrease of dry matter, total nitrogen, tuber starch, number of starch granules and total protein. The nucleic acid content of infected plants increased than healthy ones. The investigated hydrolytic enzymes of amylase and protease were reduced in infected potato plants while the level of polyphenol oxidase and peroxidase was increased. PVX and PVY-infected potato plants contained less auxins and gibberellins and the pattern of the growth promoters and growth inhibitors was altered. Cytokinin was also inhibited as a result of virus infection. Examination of epidermal strips of infected potato plants revealed the presence of cytoplasmic inclusions. Many cytopathic effects showed in potato leaves such as. presence cytoplasmic vesicles. abnormal appearance of grana and change of mitochondria.

INTRODUCTION

Potato (*Solanum tuberosum*.L) is one of the most economic important crops and is used for human consumption in Egypt. The potato plants are usually subjected to numerous diseases caused by viruses, fungi, bacteria, phytoplasma, and viroids. Viral disease are mainly responsible for the majority of losses caused in potato production more than 25 different plant viruses are pathogenic to potato plants (Salazar, 1996). The viruses infecting potatoes have been an object of intense and careful research. Alterations of physiology and histopathology in virus-infected potato plants have raised poor interest. The alteration in plant physiology and histopathology should be the basis for understanding the effect of viruses on the life cycle of cultivated plants (Pennazio and Roggero, 1999). The purpose of histopathological , cytopathological and phisological studies were to determine, what tissue. cell organelles showed only abnormalities or deformations. As well as determine the changes in amount of some contents and activities of some enzymes.

MATERIALS AND METHODS

For such study Alpha potato cultivar was selected since it is found more susceptible than the other tested cultivars. Two groups of healthy potato seedlings (with 4-5 leaves) were mechanically inoculated with clarified sap infected with two isolates PVX and PVY separately (Kindly provided by virology Lab., Faculty of Agric. Ain – shams Univ.). Seedlings were kept in a greenhouse at 25±2°C and examined for external symptoms up to three weeks.

I. Physiological changes

1. Determination of enzymes

Assay of Amylase enzyme

The activity of amylase was determined as described by (Huth, 1973). Enzyme mixture reaction containing; 1ml of enzyme extract; 1ml of soluble starch solution (10 mg / ml) into 0.5M sodium acetate buffer solution (pH 5.5). The amylase activity is expressed as µg starch / 15 min., µg soluble protein.

Assay of peroxidase activity

The colorimetric assay of the total peroxidase in extracts was conducted as recommended by Seevers *et al.* (1971). The reaction mixture consisted of 1.8 ml of 20 mM sodium acetate buffer (pH 5.0). 0.05 ml of O-dianisidine, 0.2 ml of 30 mM of H2O and 0.1 ml of enzyme extract. The peroxidase activity was expressed in a unit of increase of absorption at 470 nm.

Assay of polyphenol oxidase

The activity of polyphenol oxidase was determined as described by Palmer (1971). Enzyme mixture reaction containing 2 ml of 6 mM catechol and 0.2 ml of acetate buffer (pH 5) was incubated for 5 min. and absorbance was measured at 480 nm.

Assay of protease activity

For such purpose, gelatin cup plate clear zone assay technique was used. Such technique is a device of that used by Salle (1967).

2. Dry matter, total nitrogen and protein contents

Total nitrogen content was determined by microKjeldahl method as described in A.OA.C. (1960)* in healthy and infected plants.

3. Starch content and total starch granules

Starch content was colormetrically determined in potato tubers using the method described by Trevelyan and Harrison (1956). The total count of starch granules were obtained by the direct microscope containing using Breed's slide.

4. Nucleic acid content

Nucleic acids were extracted according to the modified method used Van-Parijs (1976). RNA was determined by orcinol reaction (Omurg and Rosen, 1950). The reaction of diphenylamine according to Burton (1955) was used for the estimation of DNA.

5. Endogenous growth regulators

Gibberellin-like substances (GLS), auxin-like substances (ALS), cytokinin-like substances (CLS) and inhibitors-like substances (ILS) were extracted according to the technique described by El-Antably (1976). Lettuce hypocotyl bioassay was used for testing gibberellins activity (Franckland and Wareing, 1960). Wheat coleptile section was used in straight growth assay technique described by Bently and Hously (1954) for the auxin activity. The determination of cytokinin activity was based on induction of chlorophyll formation in cucumber cotyledons (Fletcher and McCullagh, 1971). The experimental data were statistically analyzed by short cut procedure described by Tukey (1953).

II. Histopathological studies

I –Cytoplasmic inclusion bodies

Epidermal strips obtained from PVX -infected *D. stramonium* and PVY - infected *N. tabacum* cv. White Burley plants were mounted on glass slide and examined with light microscope for amorphous and crystalline inclusions

* Association of Offecial Agricultural Chemists

according to Jordan and Baker (1995). The ultrathin sections were prepared using 2-3 mm^2 of PVX infected *D. stramonium* and PVY infected *N. tabacum* cv. White burley and stained with uranyl acetate. The sections were viewed in Philips 301 electron microscope (unite of Electron Microscopy, Ain Shams university, Specialized Hospital). The method described by Kim (1977) was used.

II – Cytopathological effects

A – By light microscopy

Virus infected and healthy plant tissues were fixed and preserved in formalin acetic acid (FAA) and ethanol 70% according to Johansen (1940). Sections were prepared using hand microtome and double staining with safranin and light green according to Corgan and Widmoyer (1971), and then examined under light microscope.

B – By electron microscopy

Sections were prepared according to Kim (1977) as mentioned before, then used to study the changes in virus - infected tissue compared with healthy ones.

Statistical analysis

Data were analyzed statistically using 't' test.

RESULTS

I. Physiological changes

The alteration of polypeptides of PVX and PVY increased potato plants was determined by assay of enzyme activities. The obtained data in Table (1) show that the level of peroxidase, polyphenol oxidase are found to be considerably higher in infected leaves than healthy ones. The activities of these enzymes were higher in potato plants (cv. Alpha) infected by PVX and PVY (0.275, 0.295 for peroxidase and 0.250, 0.275 for polyphenol oxidase) than those of healthy plants (0.15 and 0.200 respectively). On the other hand the levels of amylase and protease are reduced in infected plants than healthy ones. The activities of these enzymes were lower in infected potato leaves.

Data represented in Table (2) show that PVX and PVY –I nfected leaves of Alpha cultivar plants inoculated at 45 days post- inoculated, contain significant lower contents of chlorophyll a (0.045, 0.055) and chlorophyll b (0.034, 0.050) than that of the healthy ones.

Data tabulated in Table (3) show that PVY-infected potato leaves show highly significant reduction compared with PVX infection in the contents of nitrogen and protein (4.92, 6.24 % and 3.24, 3.78 % for protein-nitrogen, respectively). No significant reduction observed in dry matter content of PVX-infected leaves while high significant reduction in dry matter of PVY-infected leaves. It was also found that the infected tubers contain significant lower content of starch than healthy ones. The percentages of reduction was 79.829 and 77.436 mg/gm dry weight and the numbers of starch granules were 3.75 x 105 and 2.40 x 105 /ml of tuber suspension for PVX and PVY respectively (fig. 1).

3-Nucleic acid contents

Nucleic acids content differs greatly in leaves infected with PVX and PVY comparingt with healthy ones (Table 3). The concentration of RNA in infected leaves was higher than those of healthy ones. It was found 90.542-µg/gm dry weights in healthy. While it was 102.18, 111.450 in infected leaves with PVX and PVY respectively. In addition, it was found that infected leaves contain highly significant increases of DNA content

in comparison with healthy ones. It was found 27.75, 30.156 and 22.47 µg/gm dry weight of infected leaves PVX, PVY and healthy ones respectively.

4-Growth regulators

The effect of PVX and PVY infections on content of gibberellins (assayed by lettuce hypocotyls growth), auxins (determined by the straight growth of wheat coteoptile section) and cytokinins activities (tested by green test of the excised cucumber cotyledons assay) in leaves are illustrated in Fig. (2).

The extracts of healthy leaves contain significant lower levels of gibberellin-like substances of Rf 0.1-0.3. On the contrary, the infected leaves show no gibberellins except at Rf 0.6-0.7 and 0.9-1.0 which non-significant levels are observed in infected leaves with PVY and PVX respectively, Fig. (2). On the other hand both healthy and infected leaves show highly significant levels of gibberellins-like inhibitors at Rf 0.3-0.6 for healthy leaves; Rf 0.0-0.6; 0.5-0.7 and 0.8-1.0 for leaves infected with PVX and 0.0-0.9 for infected leaves with PVX. However, leaves infected with PVY show the highest significant levels of gibberellin-like inhibitors followed by the levels of leaves infected with PVX. Concerning the auxin-like substances, it was found that the extraction from healthy leaves show significant levels at Rf 0.6-0.7 while they are at Rf 0.0-0.1 and 0.7-0.8% for leaves infected with PVY and PVX, respectively (Fig. 16). At Rf 0.0-0.1 and 0.9-1.0 in both healthy and leaves infected with PVX non-significant levels of auxin-like inhibitors was observed. In addition, auxin-like inhibitors are found at Rf 0.4-0.5 and 0.6-1.0 in leaves infected with PVY and Rf 0.2-0.7 in leaves infected with PVX.

Results in Table (2) indicate that infected potato leaves contained higher activities of cytokinins based on induction of chlorophyll formation (0.045and 0.034 for PVX and 0.055, 0.050 for PVY) than that observed in healthy ones (0.067 and 0.057 mg/L) of chlorophyll a and b respectively.

II. Histological study

1-Inclusion bodies

Amorphous inclusions for PVX and PVY were stained in red color, while the nucleus were stained blue (Fig 3). Also, crystalline inclusions were observed in epidermal cells of infected tissue. On the other hand, the electron microscope examination showed cytoplasmic inclusions in PVX–infected *D. stramonium* plants. Whereas PVY-infected tobacco leaf cells showed pinwheels and laminated aggregates (Fig. 4).

2- Anatomical features

The careful microscopic examination revealed several changes in both petiole and blade due to PVX and PVY infections. The mesophyll cells are differentiated with fewer chloroplasts and fewer intercellular spaces when infected with PVX. Also, deformation in the phloem elements was observed compared with healthy ones. On the other hand, in potato leaves infected with PVY the mesophyll cells were less differentiated with fewer chloroplasts. The cortex of leaf petiole gained larger thickness but less cellular tires, that means cortical cells were larger in size than those of healthy ones. The examination of leaf blade revealed that palisade tissue was injured by infection. The healthy blade contained two palisade layers, while infection diminished it to one layer compared with healthy one (Fig. 5). By electron microscopy, many major cytopathic changes were detected such as the abnormal grana and thylakoid

membrane of chloroplast. The thylakoid does not stock up uniformly and appears to be destroyed. In the normal cell the thylakoids are uniformly stocked up (fig. 4).

Table (1): Effect of PVX and PVY on some enzymes of infected potato plants

Enzymes / Potato plant samples	Peroxidase		Polyphenol oxidase		Amylase		Protease	
	Activity	%	Activity	%	Activity	%	Activity	%
Healthy	0.150	100	0.200	100	450.25	100	520.75	100
PVX-infected	0.275 HS	83.3+	0.250 HS	20+	185.25 HS	85.8-	375.25 HS	27.90-
PVY-infected	0.295 HS	96.7+	0.275 HS	37.5+	225.50 HS	49.9-	420.25 HS	19.29

(%) = relative activity HS= Highly significant (-)= decrease than healthy (+)= increase than healthy

Table (2): The effect of PVX and PVY infection on cytokinin activities in potato leaves

Virus / Chlorophyll	Healthy	PVX	PVY
A	0.067	0.045	0.055
B	0.057	0.034	0.050

Each number was an average of 3 replicates.

Table (3): Effects of PVX and PVY on some chemical content in potato plants cv. Alpha

Viruses / Some Chemical content	Healthy plants	PVX-infected plants	PVY-infected plants	L.S.D. 5%	L.S.D. 1%
Dry matter (gm)	13.65	13.41	12.48	0.95	1.30
Total nitrogen (%)	4.38	3.78	3.21	0.75	1.05
Total protein (%)	7.44	6.24	4.92	0.85	1.25
Tuber starch content	90.492	79.829	77.436	3.75	5.35
No. of starch granules in tuber	5.85×10^5	3.75×10^5	2.40×10^5	-	-
Nucleic acids RNA	89.547	102.18**	111.450**	4.55	6.25
DNA	22.47	27.75*	30.156**	2.55	3.45

RNA and DNA content were calculated as mg /gm protein

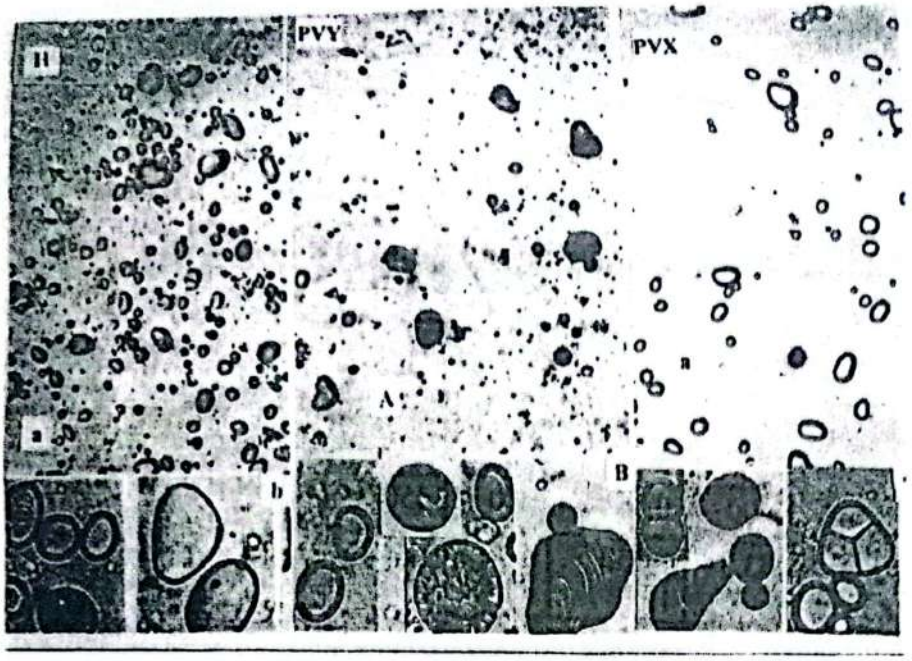

Fig. (1): Appearance of starch granules from (H) healthy, (PVY) and (PVX) infected potato tubers. (X 400) (a) represent number of starch granules. (b) represent shape of starch granules.

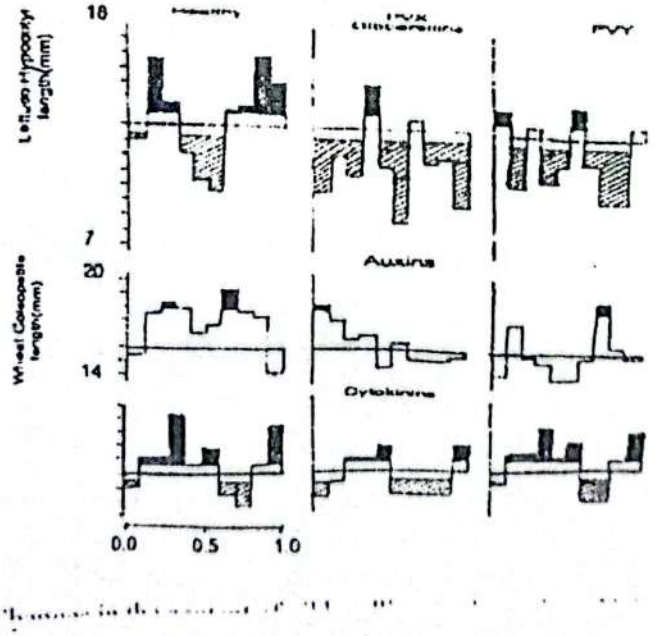

Fig. (2): Changes in the content of gibberellins, auxins and cytokinins in potato leaves as a result of PVX and PVY infection

significant promotion significant inhibition

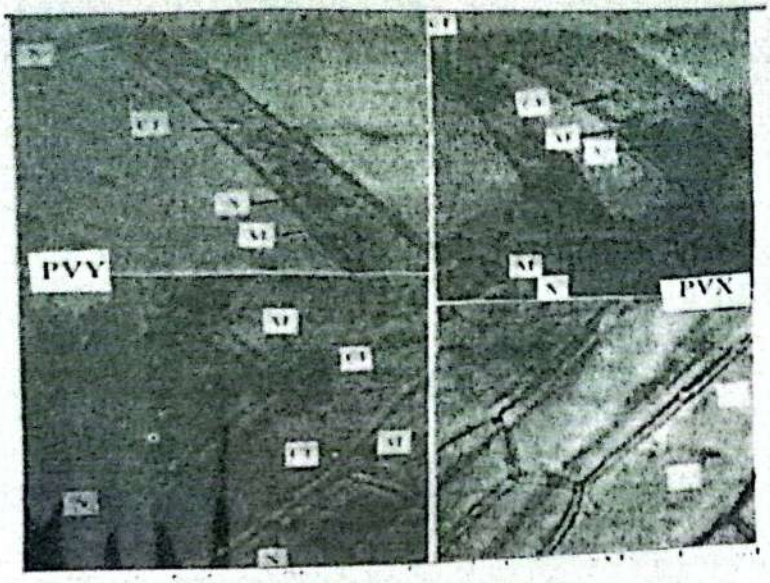

Fig. (3):Crystalline inclusions (CI), amorphous inclusions (AI) and granules near nucleus induced by PVX and PVY (30 days post-inoculation) in hair (A) and epidermal cells (B) of infected leaves of *D. stramonium* and *N. tabacum* cv. White Burley respectively. Inclusions stained with 0.5% mixture of methyl green and pyronine Y. Amorphous inclusions (AI) stained red while nucleus (N) stained blue. (Magnification = 400X)

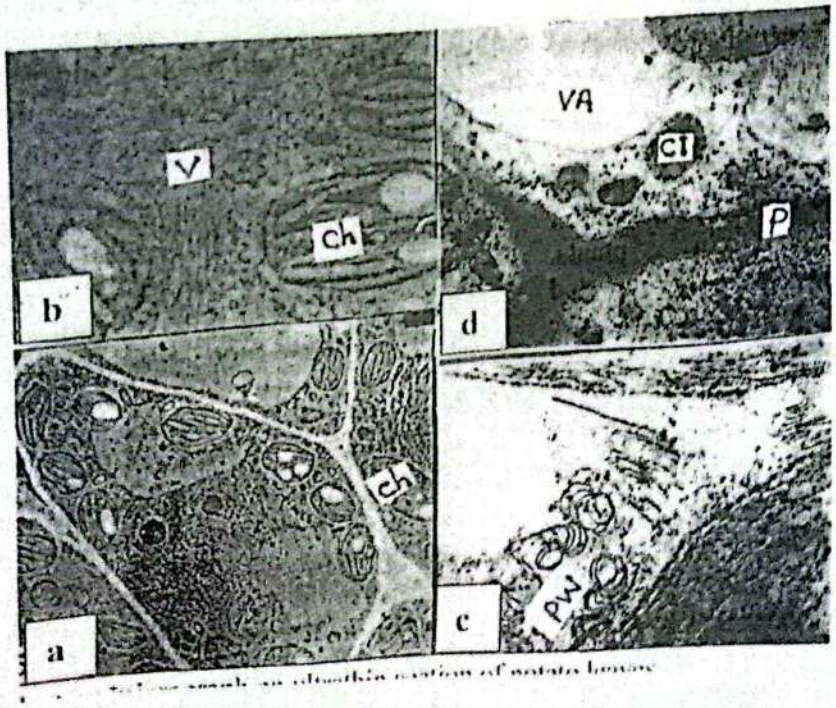

Fig. (4): Electron micrograph on ultrathin section of potato leaves.
(a) Chloroplast (Ch) of healthy potato cell (X 25000). (b) PVX-infected leaf showing viral particles (V), cytoplasmic inclusion (CI), swollen chloroplast (Ch) and scattered vesicles (VS) (X 30000). (c) PVY-infected leaf showing pin-wheal inclusion bodies (PW) (X 36000). (d) PVX-infected leaf showing plasmodesmata (P), inclusion bodies (CI) and vacuoles (VA) in cytoplasm and irregularity of cell wall thickness (X 40000)

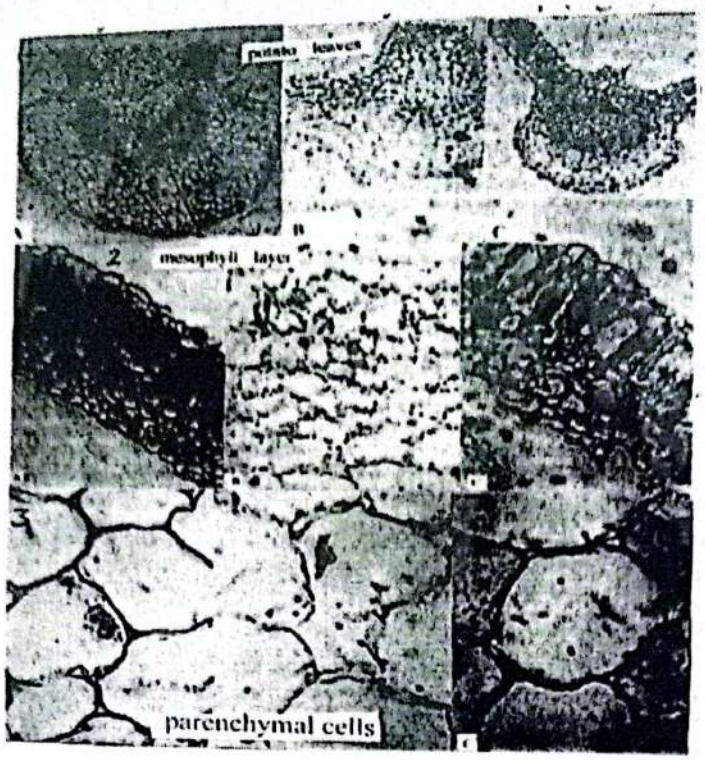

Fig. (5): Transverse section of (1) potato leaves (X 400), (2) mesophyll layer (X400), (3) parenchymal cells (X 520). (A) Healthy leaf (B)PVX-infected leaf (C) PVY-infected leaf

DISCUSSION

The enzymatic pools and their metabolic pathways are the most important factors affecting pathogenicity and especially with viruses. Increase in oxidative enzyme activities can be applied as a tool for virus detection in plants. Hammeshmidtt *et al.* (1982) reported that the increase in these enzyme activities have been detected after infection by pathogens in different host-pathogen conditions. Results shown that the levels of peroxidase and polyphenol oxidase are increased in infected plants than healthy plants. On the other hand, the levels of amylase and protease are decreased in infected plants than healthy plants.

It is thought from research on potyviruses that a cellular protease prevents viral spread and propagation in meristems and in embryonic tissues. Embryonic cells may transport a protease to the outer plasma membrane Thus preventing plasmodesmata formation (Salomon, 1989).

Peroxidase activity was enhanced only in leaves infected by veinal necrotic strain (PVY^N) and reached values up to 10 times higher than those of the control during necrotic symptom expression (Montalbini *et al.*, 1991).

The total protein content was decreased non-significantly in infected plants than healthy plants. (At 45 days post-inoculation). The same results were obtained by virus infection whereas Henke (1957) stated that virus-diseased leaves generally contain less protein-nitrogen, but always more soluble nitrogen than healthy ones. Comparable with healthy potato plant tissues, DNA content of infected ones was found to increase at 45 days post-inoculation. This finding is in full agreement with that obtained by Allam *et al.* (1989). RNA content in infected potato leaves with PVX and PVY was increased over the healthy ones. Such increases may be due to the presence of virus particles inside the infected tissues of leaves (Allam *et al.*, 1989 and Matthews, 1992).

Infected leaves of potato Alpha plants showed a significant decrease in levels of gibberellin-like substances, non-significant level in auxin-like substances and low significant levels in cytokinins activation. These results are in agreement with those obtained by Pennazio and Roggero (1999). On the contrary, infected potato plants with PSTVd showed a significant decrease in levels of gibberellin and auxin-like substances and high significant increase in cytokinin activation (Rodriguez *et al.*, 1978 and Allam *et al.*, 1989). Woolley and Woreing (1972) indicated that gibberellins may act by suppressing cytokinins production, the possibility of gibberellins mediated feedback inhibition of cytokinin most be recognized.

The number of starch granules was decreased in virus-infected than healthy potato plants. Matthews (1992) concluded that the translocation of starch content is distributed in leaves from diseased plants while it was normal in healthy ones.

In the present investigation PVX and PVY infected potato plants showed mosaic at 45 days post-inoculation, which contain lower amount of chlorophyll than healthy ones. It was observed that the increase in chlorophyll content in infected leaves is accompanied by low significant levels of cytokinins.

Whereas Fletcher and McCullagh (1971) indicate the relationship between the chlorophyll and cytokinin contents. Allam *et al.* (1989) reported that the increase of chlorophyll content in PSTVd-infected potato plants showing dark green leaves is accompanied by higher significant levels of cytokinins.

It was observed that the virus infection has caused the chlorophyll content (A, B & total) of the infected plants to be decreased than healthy plants. Similarly, Kishtah *et al.* (1983) reported that the virus decreased the concentration of chlorophyll a in leaf samples, increased respiration rate during symptom development but did not affect permeability. Some alterations were observed in the ultrastructure of mitochondria, chloroplasts and cell walls.

PVY caused a decrease in chlorophyll A of Datura leaves in the period 15-30 d after inoculation, in chlorophyll B from 30 d onward, in total chlorophylls from the 20 d and in carotenoid content at 10-30 d. in tomato leaves, chlorophyll A decreased over 6-30 d, chlorophyll B 14-30 d, and total chlorophylls after 10 d, carotenoids remaining unchanged even after 30 d (Ismail & Eskarous, 1984).

The effect of PVY infection on the photosynthesis of tobacco was evaluated under relatively steady laboratory conditions. The results showed that the content of chlorophyll, net photosynthetic rate (Pn), evaporative rate, stomatal conductance (Gs) and the activity of the Hill reaction decreased, while intercellular CO2 concentration (Ci) increased in comparison with that of the healthy plants. The virus infection also resulted in changes in chloroplast ultrastructure. The ultrastructural analysis of leasves showed that choloroplast structure was destroyed by PVY infection (Guo *et al.*, 2000).

Examination of epidermal strips of various infected plants treated with mercuric bromophenol blue using light microscopy revealed presence of cytoplasmic inclusions. This result is accordance with that obtained by many investigators (Zein, 1995 and Amer, 1999). Light and electron microscopy revealed the presence of PVX in palisade parenchyma cells but not in sieve elements or satellite cells. It is concluded that the symptoms caused by virus reproduction in palisade

parenchyma cells resulting in decay of chloroplasts. Grama *et al.* (1990) and Stussi-Garaud *et al.* (1994) concluded the presence of cytopathic effects in potato leaves infected with PVX and PVY. The first was the presence of many cytoplasmic vesicles formed of the plasmalemma (Gibbs, 1979). The second was the presence of inclusion cytoplasmic vesicles (Kozar and Sheludko, 1969). The third major cytopathic change was the abnormal appearance of the grana and thylakoid membrane of the chloroplasts. The fourth effect was the change of mitochondria (Castellano *et al.*, 1995).

REFERENCES

Allam E.K.; El-Afifi, Sohair; Abo El-Nasr M. and El-Dougdoug Kh.A. (1989). Response of some potato cultivars to *Potato Spindle Tuber Viroid* (PSTVd) infection. Proc. 3rd Nat. Conf. Of Pests and Dis. of Veg. And Fruits in Egypt and Arab Countries. Ismailia, Egypt, pp. 736-767.

Amer, M.A. (1999). Copmarative studies on the diagnostic methods for *Potato Virus* Y disease. Msc. Thesis. Faculty Agriculture, Ain Shams University.

Bently, J.A. and Housley, S. (1954). Bioassay of plant growth hormones. J. of Plant Physiol. 7: 405.

Burton, K. (1955). A study of the conditions and mechanisms of Diphenyl Amine reactions for the colorimetric estimation of DNA. Biochem. J. 62: 315-323.

Castellano, M.A.; Abou-Ghanem, N.; Martelli, G.P.; Boscia D. and Savino V.(1995). Cytopathology of two filamentous grapevine viruses and their intracellular identification by gold immunolabeling. Zeitschrift fur pflanzenkrankheitenn und pflanzenschutz 102 (1): 23-33.Rev.of plant path.74:7703.

Corgan, J.N. and Widmoyer, F.B. (1971). The effect of gibberellic acid on flower differentiation, data of bloom, and flower hardiness of peach. J.Amer.Sco. 96 : 54-57.

El-Antably, H.M.M. (1976). Study on the physiology of shedding. 1- Effect of cycocel and role of endogenous auxin and abscisic acid Z. Pflamzephysiol. 8 : 21: 28.

Fletcher, R.A. & McCullagh M.D. (1971). Cytokinin induced chlorophyll formation in cucumber cotyledons. Planta 101: 88-90.

Franckland, B. & Wareing, P.F. (1960). Effect of gibberellic acid on hypocotyl (growth of lettuce seeding. Nature 185: 225-256.

Gibbs, A.J. (1979). *Tobamovirus* group (MI/AAB) Description of plant viruses No. 184.

Grama, D.P.; Kolesnik, L.V.; Parkhomenko, N.I. & Major I.Y. (1990). Studies of the nature of systemic infection of *Potato Virus* X in Datura plants. Mikrobiologicheskii Zhurnal 51(5): 51-56.

Guo, X.Q.; Wen,F.J. & Zhu,H.C. (2000). Effect of PVY infection on photosynthesis of tobacco. Journal of Zhejiang University Agriculture and Life Sciences. 26(1): 75-78.

Hammeschmidtt, R., Nuckies, E.M. & Kuc, J. (1982). Association of systemic resistance to colletorichum lagenarium. Physiol.Plant Pathol. 20: 73-82.

Henke, O. (1957). Studies on the metabolism of leaf roll diseased potato plants. Zd Bokt.Abt. 2, 110, 11-15pp. 361-377.

Huth, W. (1973). Behaviour of some enzymes of virus X infected tobacco plants. Phytopatholo. Z. 77(2): 117-124.

Ismail, M.H. & Eskarous, J.K. (1984). The changes of pigments in Datura

and tomato plants infected with *Potato Virus* Y. Egyptian Journal of Phyopathology 16:1-2, 53-64.

Johansen, D.A. (1940). Plant microtechnique, MC.Grow Hill Book Co., New York.

Jordan, B.M. and Baker, J.R.(1995). A simple pyronin methyl green technique. Qurai.S.Microscop.Sci. 96:177.

Kim, K.S. (1977). An ultrastructural study of inclusions and disease development in plant cells infected by *Cowpea Chlorotic Mottle Virus*. J.Gen.Virol. 35: 535-543.

Kozar, I.E. & Sheludko, Y.M. (1969). Virology 38-220.

Kishtah, A.A.; Eskarous, J.K.; Habib. H.M. and Ismail M.H. (1983). Effect of *Potato Virus* Y. on the ultrastructure and some physiological processes of *Solanum nigrum*. Egyptian Journal of Phytopathology 15:1-2, 65-76.

Matthews, R.E.F. (1992): Fundamentals of plant virology. Fourth Edition. Academic Press. New York. London. 403 pp.

Montalbini, P.; Buonaurio. R. and Passeri. S. (1991). Effect of *Potato Virus* Y. infection on levels of soluble superoxide dismutases. peroxidase and catalase in tobacco leaves. Phytopathologia-Mediterranea 30(1): 30-40.

Omurg M. and Rosen, G. (1950). Arch.Biochem.Biophys. 25: 262 (c.f. Van Parijs, R. 1976). A procedure for the quantitative determination of DNA in plant tissue. Archives International de Physiologic et de Biochemie. 77.

Palmer, J. k. (1971). The banana. In: Hulme A. C. (ed). The biochemistery of fruits and their products. Academic press. London. New York. pp.65-101.

Pennazio, S. and Roggero, P. (1999). The physiology of crop species systemically infected with viruses. *Solanum tuberosum*. Petria. 9: 1-2, 27-41.

Rodriguez, J.I.; Garcia, M.J.L. and Flores, R. (1978). The relationship between plant growth substances content and infection of *Gynura aurantiaca* by *Citrus exocortis viroid*. Physiol. Plant Path. 13 (3): 355-364.

Salazer, L. F. (1996). The potato and its diseases. In Salazer L. F.(ed.): potato viruses and their content . Peru: Interntional potato center, pp.3.

Salle, A.J. (1967). Laboratory manual on fundamental principles of bacteriology 6th (ed.). McGraw. Hill Book Company. New York., 65-71

Salomon, R. (1989). A possible mechanism for exclusion of plant viruses from embryonic and meristemic tissues. Research in Virology 140 (5): 453-455.

Seevers, P.M.; Daly, J.M. and Caledral, F.F. (1971). The role of peroxidase isozymes in resistance to wheat stem rust disease. Plant Physiol. 48: 353-360.

Stussi-Garaud, C., Tiaeberle, A..M.; Ritzenthaler, C.; Roheritsch. O. and Lebeuvier. G. (1994). Biology of the cell 80 (2-3): 147-153. Rev.of plant path. 74: 165 (1995).

Trevelyan, W.E. and Harrison, J.S. (1956). Studies on yeast metabolism. I. Fractionation and determination of cell carbohydrate. Biochem.J. 160: 23-33.

Tukey, J.W. (1953): Some selected quick and easy methods of statisticalanalysis. Trans.N.Y. Acad. Sci. 16: 88-97.

Van-Parijs, R. (1976). A procedure for the quantitative determination of DNA and RNA in plant tissue. Archives International de Physiologic et de Biochemie 77.

Woolley, D.J. and Woreing, P.F. (1972). The interaction between

growth promoters in apical dominance II. Environmental effects on endogenous cytokinin and gibberellin level in *Solanum andigena*; New Physiol. 71: 1015-1025.

Zein, S.N. (1995). Studies on some aphids bron viruses infecting pepper. M.Sc. Thesis, Fac. Agric., Cairo Univ. and Cairo. Egypt. 80 pp.