Impact of Potato virus Y on anatomical leaflets and tubers of potato plants

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ABSTRACT

A pot experiments were conducted on potato (Solanum tuberosum L.cv. cara) plants during the winter season at 2011 to investigation the abnormalities changes of potato leaflets and tubers infected with Potato virus Y. Anatomical features of terminal leaflets i.e. mean of thickness both, midrib zone, leaflet blade, upper epidermal layer, palisade and spongy tissues, length and width of protoxylem and metaxylem vessels were reduced. However, lower epidermal layer was increased. Stomatal characters i.e. number of stomata and length of stoma pore were increased, while, width of stoma pore, length and width of guard cells were reduced. Anatomical characters of tubers i.e. thickness cork layers was slightly increased, however, its number was sharp reduced compared with healthy tubers. Biochemical changes of potato tubers i.e. number of starch granules and total carbohydrate contents were non-significantly reduced as comparing to the healthy ones. As well as morphological characters i.e. size and tuber weight per plant were significantly reduced, however, its number was significantly increased as comparing to the healthy plant.

Keywords: Potato plants, Leaflet, Anatomy, (SEM), Starch granules, Tubers.

INTRODUCTION

Potato (Solanum tuberosum L.) is a tuber produce whose tubers are carbohydrate-rich source with an im¬portant role in feeding people and is of interest because of its very high yield/ha, so that it produces as much protein and about twice as much carbohydrate as grains. Their tubers are economically valuable and are extensively used in feeding people and live stock in starch production (Mehdi et al., 2011).

Potato plants are susceptible to many viruses that caused heavy losses in the yield of both quality and quantity (Valkonen 2007 and Mansour et al., 2008). Potato virus Y (PVY) the type member of the genus potyvirus, belong to the largest plant virus family Potyviridae (Spetz, et al., 2003). PVY is occurred worldwide wherever potatoes are grown (Crosslin et al., 2006).

Potato virus Y (PVY), considered one of the most damaging potato viruses causing significant yield depression reached to 50% because it spreads easily by sap mechanically, aphids in a non-persistent manner and infected tubers. The mean yield losses from plants with mild, moderate and severe symptoms were 54.5, 92.93 and 95.65% respectively (Hossain and Ali 1992).

Enzyme- linked Immunosorbent assay (ELISA) used to detect the presence of an antigen in a sample (Clark and Adams 1977).

Starch granules in potato are oval-shaped and highly variable in size, with diameter ranging form 5 to 100 um (Noda et al., 2005). Starch is an essential component of pota¬to constituting 17-21% of its fresh weight and about 80% of its dry matter. It is nutritional reserve of most plants and starch granules are in fact compressed packages of glucose polymer (Kaur et al., 2002).

Light microscopy is still important in studying histological abnormalities induced by viral infection. Thus,

histological parameters of both healthy and infected potato plant were studied (Matthews1991). Much information is available on histopathological changes in various virus-host systems, caused by PVY in potato plants were recorded by many authors (Gomaa Hanaa 2003 and Mohamed et al. 2012). The leaf blade is rogues and irregular and the stomata malformation (Eugenia and Walter1980).

In this report it was studied the changes in the histological structure in leaves and tubers infected with PVY, as well as, cork layer, starch granules and total carbohydrate were studied in potato tubers.

MATERIAL AND METHODS

The presented investigation was carried out at fenced farm of the Faculty of Agriculture, Al–Azhar University, Cairo, Egypt, to speculate some light on the effect of potato virus Y on some qualitative, quantitative and anatomical characteristics of Potato plants (Solanum tuberosum L. cv. Cara).

Source of plant materials and virus isolate:-

Potato plants (Solanum tuberosum L. cv. Cara).were inoculated with PVY isolate kindly obtained from Al-Naggar (2015) under green-house condition. Infected potato plants were confirmed using antiserum polyclonal antibody kindly obtained from Al-Naggar (2015) using indirect-ELISA according to (Clark and Adams 1977). The tubers of each plant of PVY infected and healthy ones were harvested.

The potato yield was determined as number, weight and size tubers per plant, as well as number of grains and total carbohydrates tuber per plant.

Anatomical studies

PVY infected leaflets samples were taken from the terminal leaflet of fourth leaf developed on stem (after 50 days from sowing). As well as tubers samples were cut from the heel, rose and central core of the tubers at harvesting. Samples were immediately put in F.A.A. solution for 24hrs. 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. Sections were cut at the thickness of 10 um, and then mounted on slides with the aid of egg-albumin as an adhesive. The sections of samples were prepared by the method suggested by (Sass 1958). Sections were stained with safranin and light green schedule then kept in Canada balsam as mounting medium. Sections were microscopically examined by Nikon Camera on a Carl Zeiss Jena microscope. The microscopic scale with the help of the (Olympus–BX40 (model-BX40-3).

Each value means of 10 sections, 10 readings per each film.

Scanning electron microscopy (SEM):

Leaf specimens of both healthy and potato virus Y (PVY) infected plants were taken 35 days post inoculation (d.p.i.). Samples were fixed in 2.5 % glutaraldehyde for 24 h at 4°C, then post fixed in 1 % osmium tetroxide for 1 h at room temperature (Ishak and El-Deeb 2004). The specimens were then dehydrated with acetone, critical point dried and finally sputter-coated with gold. The examination and photographing were done through (Jeol 6300) with TESCAN System for image analysis at the Regional Center for Mycology and Biotechnology, Al-Azhar University. The microscopic scale with

the help of the (Olympus–BX40 (model-BX40-3).

Histological studies of potato tubers and appearance of starch granules:-

Light microscopy was employed to characterize native starches with respect to appearance, shape and size of granules (Schoch and Maywald 1956). The shape of the native starch granules was observed by light microscope that provided camera (Panasonic WV GP 240). It was connected to a computer. The shape was defined by the coloration of starch granules with (0.2 g iodine in 2% KI solution). The size of the native starch granules in suspension was measured on a microscopic scale with the help of the (Olympus – BX40 (model-BX40-3). Photographs were taken with an attached (Panasonic WV GP 240 Camera).

Determination of total carbohydrate contents-:

Total carbohydrate contents were determined as percentage of dry weight of potato tuber plant samples using the colorimetric method described by (Krishnaveni et al., 1984).

The number and size of starch granules were calculated per gram fresh weight tuber. The starch content was 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. two films were prepared for each sample with examination25 field in each film.

RESULTS

Inoculated potato plants (S. tuberosum L. Cara) with PVY isolate showing variable symptoms including leave narrow, veinal necrosis and mosaic (Fig. 1). The PVY infected potato plants and tubers gave positive results with polyclonal antibodies by indirect. Data in (Table 1) showed PVY inoculated plants gave positive values for both leaves and tubers, while healthy potato plants ones gave negative results.

Effect of PVY on anatomical of terminal leaflets:-

A) Light microscopy:

Data in Table (2) showed that, the midrib zone was slightly decreased by (-3.33%) less than the healthy ones. Leaf blade thickness was reduced by (-23.58%) less than the healthy ones (Fig. 2). Such reduction was associated with the decreasing recorded on the thickness of the upper epidermal layer, palisade and spongy tissues by (-38.88%, -27.03% and -20.6%) respectively less than the healthy ones. Although, thickness of lower epidermal layer was increased by (+17.25%) more than the healthy ones. Such response is run parallel with the disorder in upper and lower epidermal layers and lost it is both normal shape and arrangement. The effects were also resulted lost both the normal arrangement of mesophyllic tissue (palisade and spongy tissues), lost their normal shape with greatly disintegration and intercellular spaces between parenchymatous cells in mesophyllic tissue (Fig. 2).

Also, the area occupied by collenchymatous cells behind the main vascular bundle was occupied by larger size and more layers of collenchymatous cells in midrib zone as comparing to the healthy plants (control). Such effects were mainly due to the disarrangement of xylem vessels elements (Fig. 2).

Table (1): Serological confirmation of PVY in leaves and tubers using indirect ELISA.

Plant	ELISA value at 405 nm			
	Leaves		Tubers	
	ELISA value	Result	ELISA value	Result
Healthy	0.223	-	0.185	-
1*	0.735	+	0.345	-
2	0.649	+	0.536	+
3	0.815	+	0.418	+
4	0.576	+	0.366	-
5	0.680	+	0.475	+
6	0.527	+	0.521	+
7	0.754	+	0.358	-
8	0.596	+	0.452	+
9	0.698	+	0.591	+
10	0.534	+	0.367	-

1* = to 10 inoculated plants (leaves and tubers). += Positive. - = Negative.
+ve control = 0.825 -ve control = 0.208

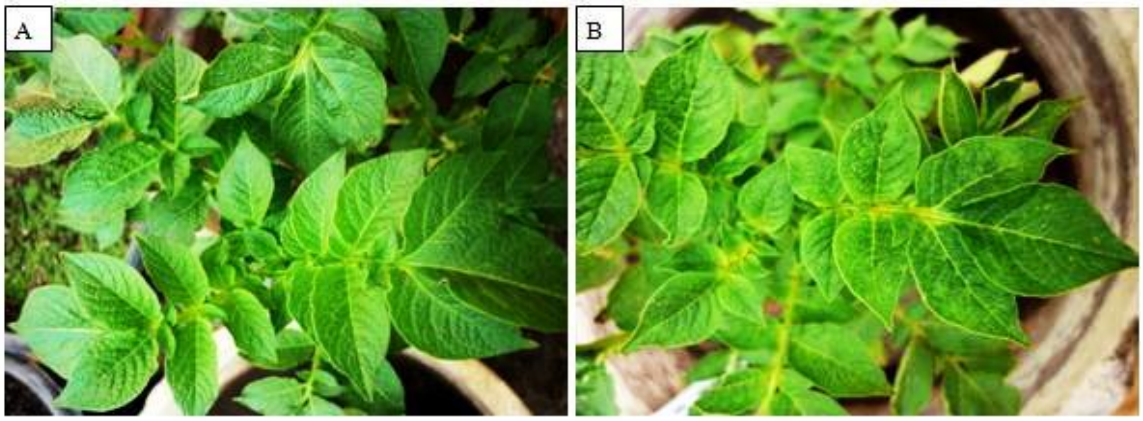

Fig. (1): Potato plants inoculated with PVY showing PVY distinct symptoms; (A): Healthy leaves; (B): infected plant (Inoculation with PVY).

Table (2): Effect of PVY on anatomical characteristics of potato terminal-leaflets.

Characteristic	Healthy leaf	Infected leaf	% Relative Change ±.
	Average of absolute value (um.)	Average of absolute value (um.)	
Thickness of midrib	1334.08	1289.54	-3.33
Thickness upper epidermal layer	26.95	16.47	-38.88
Thickness lower epidermal layer	9.04	10.6	+17.25
Thickness of palisade tissue.	64.39	46.98	-27.03
Thickness Spongy tissue.	89.39	70.97	-20.6
Thickness of leaflet blade.	189.77	145.02	-23.58
Length of protoxylem vessel.	17.04	11.46	-32.74
Width of protoxylem vessel.	16.2	14.06	-13.21
Length of metaxylem vessel.	26.37	21.11	-19.94
Width of metaxylem vessel.	22.00	21.15	-3.86

+ = More than healthy - = Less than healthy

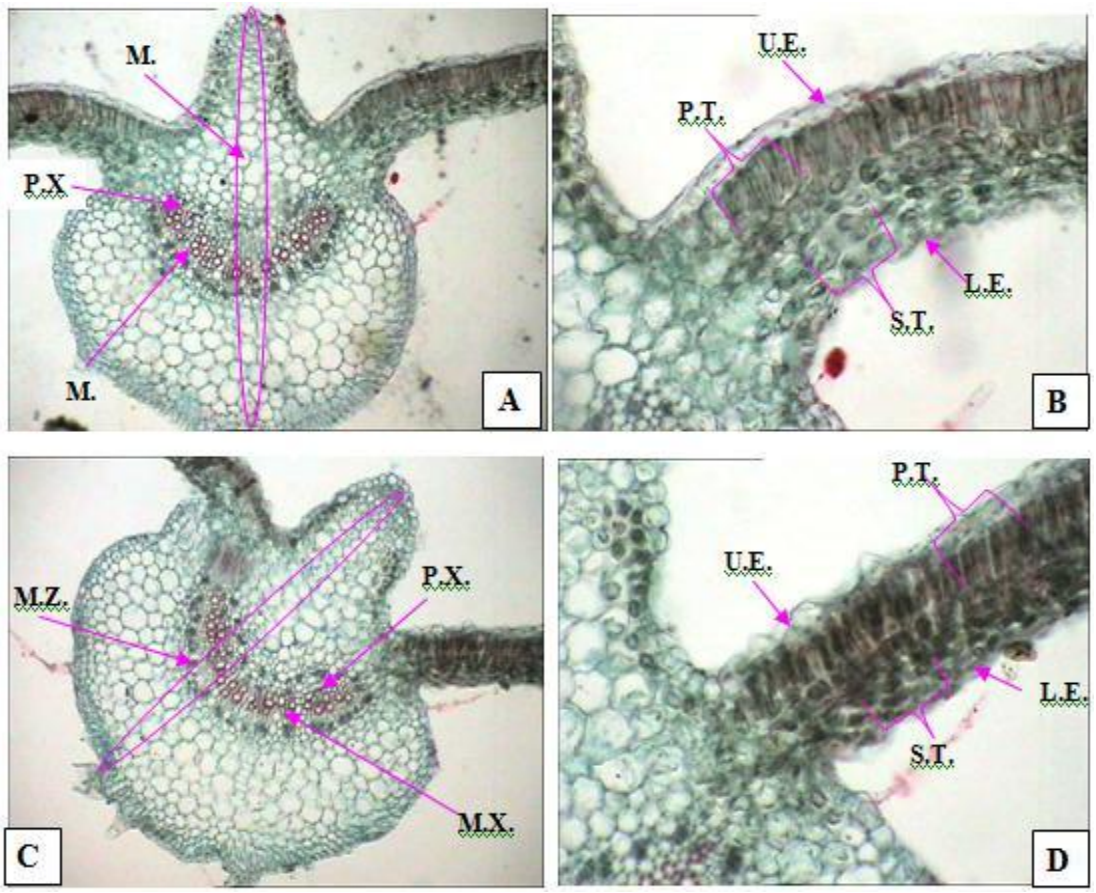

Fig. (2): Light micrographs illustrating leaf-sections of both healthy (A and B) and PVY infected plants (C and D) developed on fourth leaf of potato (Solanum tuberosum L.) plant. (A): Healthy plant (Control) X100. (B): Healthy pant (Control) X200. (C): Infected plant X100. (D): Infected plant X200.
Abbreviation
(L.E.) = Lower epidermal layers, (M.X.) = Metaxylem, (M.Z.) = Midrib zone protoxylem, (P.T.) = Palisade tissue, (P.X.) = Protoxylem, (S.T.) = Spongy tissue and (U.E.) = Upper epidermal layers.

B) Scanning electron microscopy (SEM):

The abaxial surface of healthy plants and virus-infected potato plant were investigated using scanning electron microscopy (SEM). Stomata number was clearly increased by (+151.42%) than in healthy plants due to the viral infection (Table 3) and (Fig. 3).

Measurement of stomatal aperture was increased in length by (+30.09%) more than the healthy plants, moreover width of stomatal aperture was slightly decreased by (-0.75%) as comparing to the healthy plants, length and width stomatal guard cells were decreased by (-8.61% and -24.55%) respectively less than the healthy plants (Fig. 3).

Table (3): Effect of PVY on stomata characteristics of potato terminal leaflets.

Characteristic	Healthy leaf	Infected leaf	% ± of (control) Healthy leaf.
	Average of Absolute value (um.)	Average of Absolute value (um.)	
Length of stoma pore	11.13	14.84	+30.09
Width of stoma pore	2.64	2.62	-0.75
Number of stoma	35	88	+151.42
Length of guard cells	26.47	24.19	-8.61
Width of guard cells	9.61	7.25	-24.55

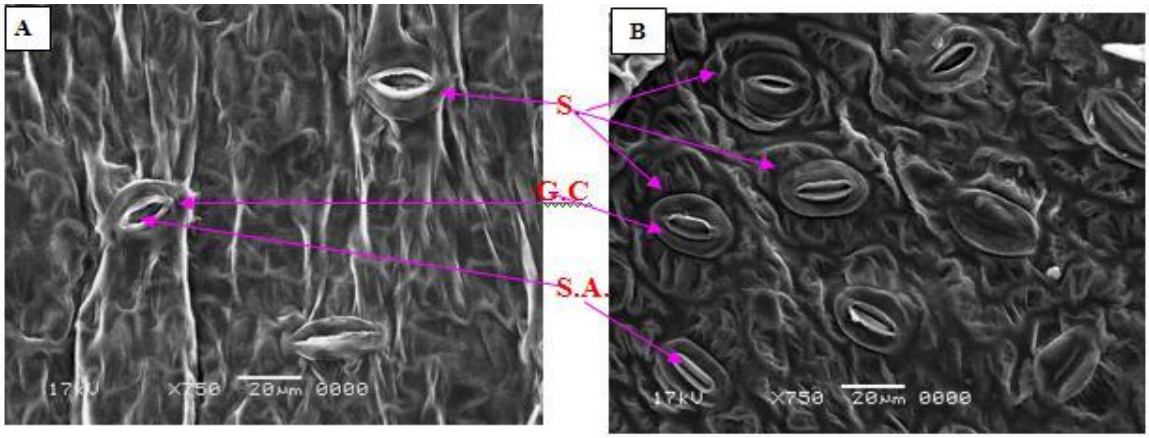

Fig. (3): Scanning electron micrographs: Showing the abaxial surface of both health (A), and PVY infected (B) potato leaf.
Abbreviations: (G.C.) = Guard cells, (S) = Stomata and (S.A.) = Stoma aperture.

Effect of PVY on potato tubers:-

The PVY infection due to rupture and increasing the cork layers of tubers and compared with healthy tubers. Data in Table (4) and (Fig. 4) showed that, thickness of the cork layers was slightly increased due to PVY infection over that of healthy tubers. On the contrary, it was found that the number of cork layers was sharp reduced as compared to that of control. This type of cells becomes more elongated and thickness in shape under influence of infected with PVY. The mostly apparent anatomical response of these effects is observed on the rolled shape and shrinkage witch is more pronounced in some cork regions as comparing to the control. It also reduced the number of vascular bundles in potato tubers infected than in healthy plants as well as the observed both an increase was seen diameter xylem vessels and brown walls in potato cells infected only. (Fig. 4).

Table (4): Effect of PVY on anatomical characteristics of potato tubers.

Characters	Thickness of cork layers (um.)		Number of cork layers	
	Absolute value	% ± of (control)	Absolute value	% ± of (control)
Healthy plants*	171.19	00.00	12.50	00.00
Infected plants*	173.36	1.27	6.25	-50.00

*=Ten plants replicate.

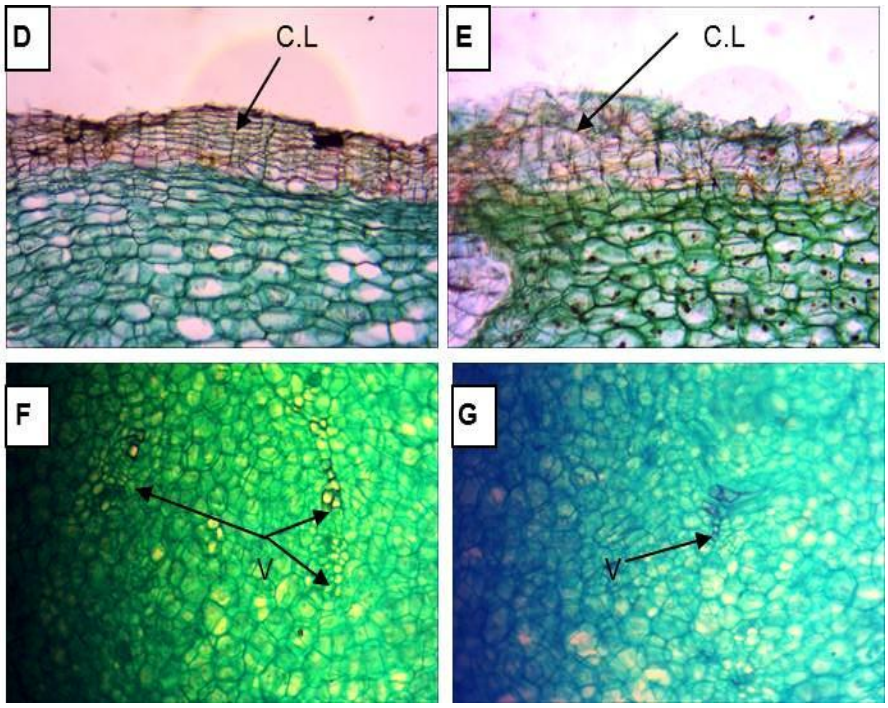

Fig. (4): Cross- sections of potato tuber, (C.L.): Cork Layers; (V): Vascular; (A and B): Healthy; (C and D): Infected. (all, X100).

The data in (Table 5) indicated that, the measurements of starch granules in parenchyma cells showed that, significant decrease in size was (66.5) less than healthy ones (120.8). Moreover, the starch granule tended to be circular and irregular on shape and lost it is the normal oval shape in storage parenchyma cells (Fig. 5).

The number of starch granule in infected tubers had non-significant reduction as comparing to the healthy tuber. It was mean number starch granules in suspension (4.77x10-5 and 2.86x10-5) both healthy and infected tubers respectively (Table 5) and (Fig. 5).

On the other hand, the shape of starch granules was abnormal shape (Malformation) in infected tuber compared with healthy starch which showed normal levels of starch (Fig. 5).

The number of tubers both healthy and infected plants are tabulated in (Table 5). It was found that, the infected plants gave significant increasing in tubers number per plant than the healthy ones. While, showed significant decreasing in size tubers compared with healthy plants. It was mean size of tuber (120.6 and 30.8) mm both healthy and infected plants respectively. Mean tubers weight of the infected plants showed significant decreasing of tubers weight compared with healthy plants, it was mean of tubers weight (122.1 and 36.65 g) both healthy and infected plants respectively. Total carbohydrate content in PVY infected tubers were non-significantly increased compared with healthy tuber ones, were as 40.49and 39.80 mg D glucose for gram dray weight respectively.

Table (5): Effect of PVY on quantitative and qualitative characters of yield tubers.

Potato tuber	Quantitative					Qualitative			
	Mean number of tubers per plant**	Size of tubers	mean size of tuber	mean of tubers weight	mean of tuber weight	Number of starch granules in suspension**	Size of Granule (Diameter)	Number of starch granules in cells	Starch soluble (mg)
Healthy^{-1}	10	1206 mm	120.6mm	1221g	122.1 g	$4.77x10^{-5}$	120.80	41	40.49
Infected^{-1}	18	554.5 mm	30.8 mm	655g	36.65 g	$2.86x10^{-5}$	66.50	29	39.80
L.S.D at 5%	**6.95***	**195.34***	**19.43***	**220.43***	**21.01***	**١.23**	**17.67***	**7.15**	**3.72**

* = Significant. ** = average from ten plant replicates. ***= Fresh weight. -1= Ten plants replicate.

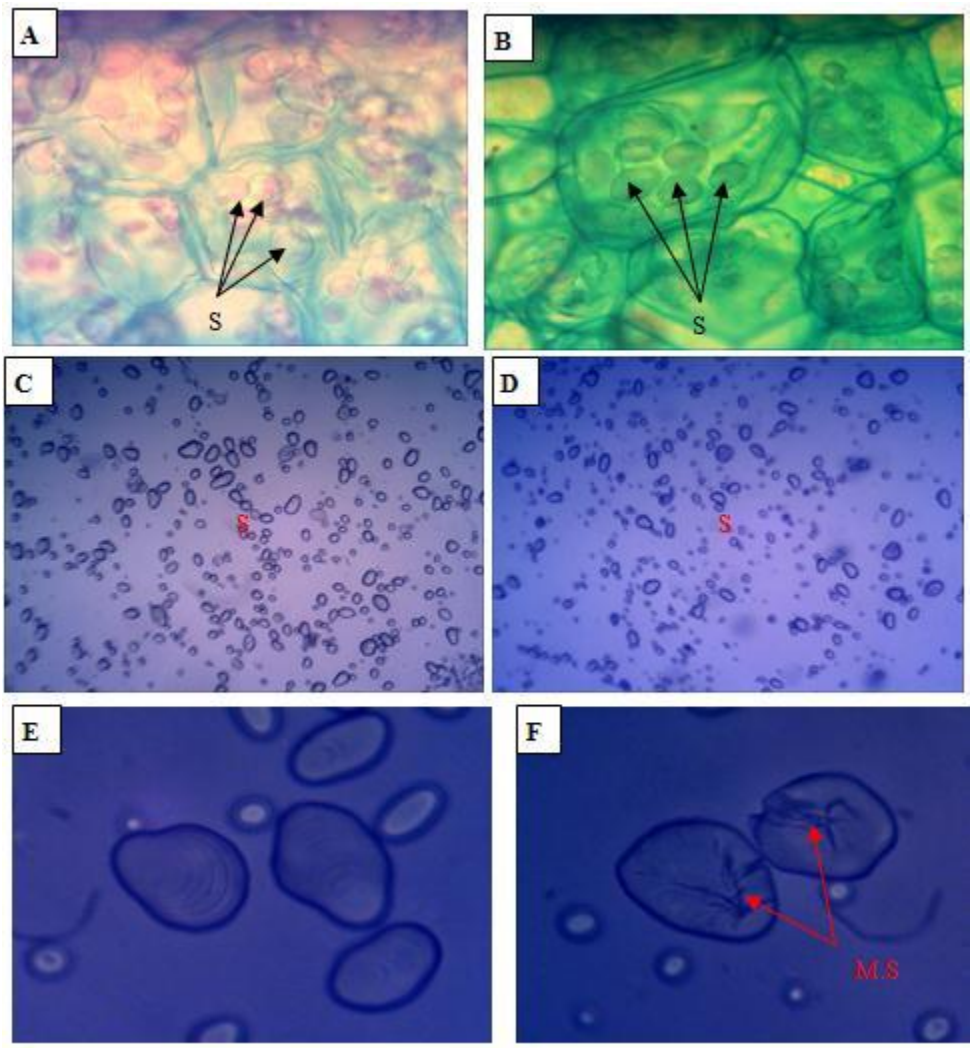

Fig. (5): Micrographs of starch grains of both healthy and PVY infected plants (A and B: X400); (C and D: X100); (E and F: X200).

DISCUSSION

The collected potato plants manifesting viral signs were found to be infected by PVY based on ELISA results. The method for virus detection which is used most extensively consists of enzyme-linked immunosorbent assay (ELISA) on polystyrene microtiterplates (Fegla and Kawanna 2013). Several investigations showed that ELISA is on of the most easily and widely used serological techniques for plant virus diagnosis (Gomaa Hanaa 2003 and Mahfoze et al., 2004).

Light microscopy is still important in studying histological abnormalities induced by viral infection. Several alterations were observed in the potato leaflets infected with PVY in the present study. The study suggested that there is a correlation between virus infection-induced anatomical abnormalities with the shift in metabolic profiles. The results exhibited a reduction of midrib zone, upper epidermal layer, palisade and spongy tissues, leaflet blade thickness, length and width both protoxylem and metaxylem vessles, although, lower epidermal layer was increased only. These results are in harmony with Gomaa Hanaa (2003) and Mohamed et al. (2012) revealed that, plates represent a transverse section in potato leaf infected by potato virus Y. This plate shows that the mesophyll cells are less differentiated with fewer chloroplasts, normal exylem elements and deformation in phloem elements. In addition to enlargement of lower epidermal cells and reduced size of upper epidermal cells

In this regard, Ishak and El-deeb (2004) reported that, the most important changes due to sweet potato chlorotic stunt virus (SPCSV), infection were confined to the vein region. In general, almost all the anatomical characters of the midrib region investigated by light microscopy were increased. However, a reduction was observed in the diameter of xylem vessels and phloem area as well as the thickness of the leaf blades. Similar results were obtained by Mohamed et al. (2012) who reported that, infected phloem tissues showed less active sieve elements, and phloem radial thickness and secondary phloem fibers were reduced also, the thickness of xylem tissue and vessels diameter were also reduced.

The viral infection greatly increased the stomata number compared to the healthy plants. Measurement of stomata aperture was increased in length compared to the healthy plants. Moreover, width of stomata aperture was slightly decreased compared to the healthy plants. Also, length and width stomatal guard cells were decreased less than the healthy plants.

Helena et al. (2006) founded that, Transpiration rates (E) were lower in healthy C/C, T, and T/C, but decreased less after the PVY infection compared with C. Stomatal conductance (gs) was the highest in healthy C and decreased more significantly after PVY infection than in other plant types. Relative water content (RWC) decreased significantly in both control types infected by PVY, while the infection had no effect in T and T/C.

The viruses infecting potato have been an object of intense and careful research. Alternations of physiology in virus-infected potato plants have raised poor interest. Increased respiration and decreased photosynthesis have been generally described, but the molecular mechanisms relative to both the function are still unknown. Alternation of plant

hormone level present hitherto neglected subject, with the exception of cytokinins which were investigated in potato virus Y (PVY) infected potato (Pennazio and Roggero 1991a).

Ishak and El-deeb (2004) founded that, examination of the leaf epidermis obtained through scanning electron microscopy (SEM) revealed that the viral infection increased the pearl glands in number as well as its diameter. Diameter of the stomata guard cells was also increased.

In this regard, infection with PVY may increase the transpiration rates and cytokinin production, which plays an important role in cross divisions, especially in the epidermal layer cells, resulting in an increase in the number of stomata per unit area.

The number of cork layers was sharply reduced as compared to that of control. These types of cells become more elongated and thick in shape under influence of infected with PVY.

Hormonal disorders may occur as a result of infection with PVY, which leads to an abnormal increase in the size of the infected plant cells compared to healthy plants.

Potato virus Y infection reduced the number of vascular bundles in potato tubers than in healthy ones as well as it was observed the increasing in diameter xylem vessels and brown cell walls in xylem vessels infected only, this result agreement with (Naser-El Din 2007) and (Gomaa Hanaa 2003). On the other hand, tuber constituents with cultivar and growing conditions. Estimated amounts of constituents may also reflect differences in methods of chemical analyses (Hooker 1982).

Mohamed et al. (2012) who reported that, infected phloem tissues showed less active sieve elements, and phloem radial thickness and secondary phloem fibers were reduced also, the thickness of xylem tissue and vessels diameter were also reduced.

The tuber surface permits or excludes entrance of pathogens regulates rate gas exchanges or water loss, and protects against mechanical damage. The surface is not fixed and static but will maintain and regenerate itself through wound healing reactions which influence disease incidence and severity, preservation in storage and seed germ inability and performance (Wigginton 1974).

PVY infection induced the infected cells to produce the compounds phenols in infected cells compared with healthy ones, which results in an increase due to in the thickness of the xylem vessels.

The shape of starch granules were abnormal (Malformation) in infected tuber compared with healthy starch which showed normal levels of starch.

The starch granules were significantly decreased in diameter compared to the control. Also, deformation of morphological tuber was observed Noda et al. (2005) found that, starch granules in potato are oval-shaped and highly variable in size, with diameter ranging from 5 to 100 um.

Due to the virus infection, the oxygen free radicals affect the starch granules membranes and destruct it so lead to destruction in starch shapes and changes in starch layers and decreasing in starch content.

In the present study we found that the infected potato plants were significantly higher in the number of tubers and showed significant decreasing in the size of tubers. Also, it showed significant decreasing of tubers weight. These results are in agreement with those obtained by (Badarau et al. 2011).

Under greenhouse conditions, the infected plants produced a higher number of tubers than the uninfected controls, relative to uninfected control. Increased number and reduced weight of tubers is a characteristic response to stress of PVY on potato plants. At the same trend Mansour et al. (2008) and Valkonen (2007) founded that the number of tuber in infected plants gave increasing significantly higher of number tubers than the healthy ones. While, showed significant decreasing of size tubers compared with healthy ones.

Total carbohydrate content in PVY infected tubers were not significantly increased compared to healthy ones. The reduction of total carbohydrate content in PVY infected potato tubers with viruses related to the affect indirect virus infection on chlorophyll content. Several investigations found reduction in tuber chloroplast and degradation (Pompe-Novak et al., 2001 and Guo Xing Qi, et al., 2002).

Summarizing, we may state, as Eugenia and Walter (1980) pointed out, that hypertrophy (epidermis and mesophyll), hyperplasia (mesophyll), hypoplasia (stomata) and eventual necrosis, are usually associated to a viral infection. The character of the pathological changes differs according to the maturity of the tissue infected, the localization and the intensity of the processes.

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