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Anatomical peculiarities of reproductive organs of *Lycopersicon esculentum* infected with a severe strain of tobacco mosaic virus

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Summary. The histopathology of tomato sepals, petals, anthers, ovaries and fruit pericarps infected with a highly heat resistant strain of TMV showed many peculiarities. Among these were: undulation and wrinkling of the lower epidermis of sepals and petals, presence of isthmus and failure of xylem and phloem to differentiate. The epidermal cells of the anthers were hooked, the number of pollen sacs was reduced and the vascular elements of the lower region of the fruits appeared thicker than normal, ruptured or disintegrated, and the cortical region appeared small with a wavy or ruptured layers.

Key words: tomato, histopathology, TMV.

Introduction

Although much information is available on histopathological changes in various virus-host systems, little is known about such changes caused by TMV in tomato. In addition, histopathological and anatomical studies were mostly carried out on diseased stems, and very few on roots, flowers or fruits. Awasthi and Singh (1974) studied the histopathology of *Capsicum annuum* L. flowers infected with CMV. Murdock *et al*. (1976) studied the histopathology of pelargonium leaves, stems, roots and flowers infected with tomato ring spot virus. Sayed (1985) studied the histopathology of *Nicotiana glauca* L. stem, petiole and flower infected with Egyptian strains of TMV. In 1997 Eskarous *et al*. studied the histopathology of tomato flower and fruit infected with a highly heat-resistant strain of TMV.

In the present study attention was more particularly directed to the reproductive organs of *Lycopersicon esculentum* Mill. infected with a severe strain of tobacco mosaic virus TMV-M, which was observed in Menoufia, Nile Delta, Egypt, in 1984.

Materials and methods

Seeds of *L. esculentum* cv. Marmande were planted in 15-cm pots filled with sterile clay soil and grown in a greenhouse where the temperature was maintained between 20 and 30°C. Tomato plants (40-50 days old) were inoculated with the virus, and 2 months after inoculation infected flowers and fruits were selected. Control pieces from healthy flowers and fruits were also prepared for comparison. The technique used for the preparation of permanent slides from microtome sections was mainly that of Johanson (1940), Sass (1951) and Purvis *et al*. (1964). Pieces of sepals, petals, anthers, ovaries and fruit pericarps of diseased and healthy flowers were fixed, dehydrated, embedded in paraffin wax and sectioned. The sections were double stained with safranin and light green. The lignified elements were stained shiny red against a light-green background.

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Results

Cross sections of sepals of diseased flowers showed that, in general, the epidermis and mesophyll were similar to those from healthy sepals except that there was undulation and wrinkling of the lower epidermis (Fig. 1) and the mesophyll cells appeared loose with large intercellular spaces (Fig. 2). In some parenchyma cells the chloroplasts were more numerous and bigger (Fig. 3). Xylem and phloem were greatly reduced and almost undifferentiated (Fig. 4). Sometimes two sepals were joined together and the cells of the connecting region or isthmus were thick (Figs 5, 7) with undifferentiated xylem and phloem (Figs 6, 8).

Infected petals exhibited a normal outline in a few cases, but mostly the infected petal was markedly longer, thinner, strongly undulated and wrinkled (Fig 9). The cells of the lower epidermis were bigger than those of the upper epidermis (Fig. 10). Various structural abnormalities in the mesophyll were observed depending upon the particular shape of the infected petals. In normal-sized petals, the parenchyma cells of the mesophyll contained wide spaces between them due to rupture and dissolution of some cells (Fig. 11). In thin blades, the mesophyll was one layer thick and

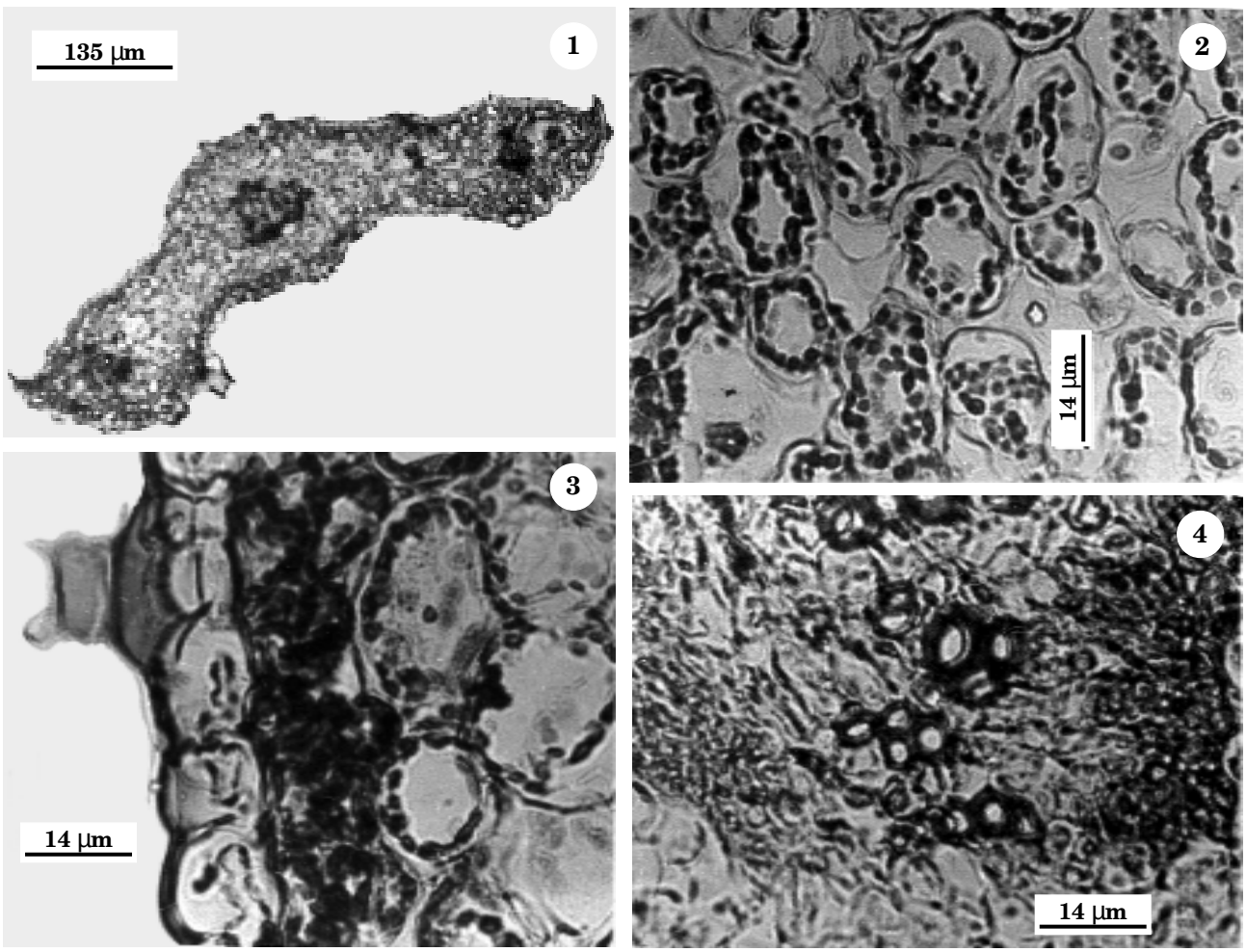


Fig. 1-4. Cross sections of sepal of Marmande tomato flower infected with TMV-M showing the general form of infected sepal (1), magnified part of mesophyll (2), magnified part of epidermis (3), magnified part showing xylem and phloem elements (4).

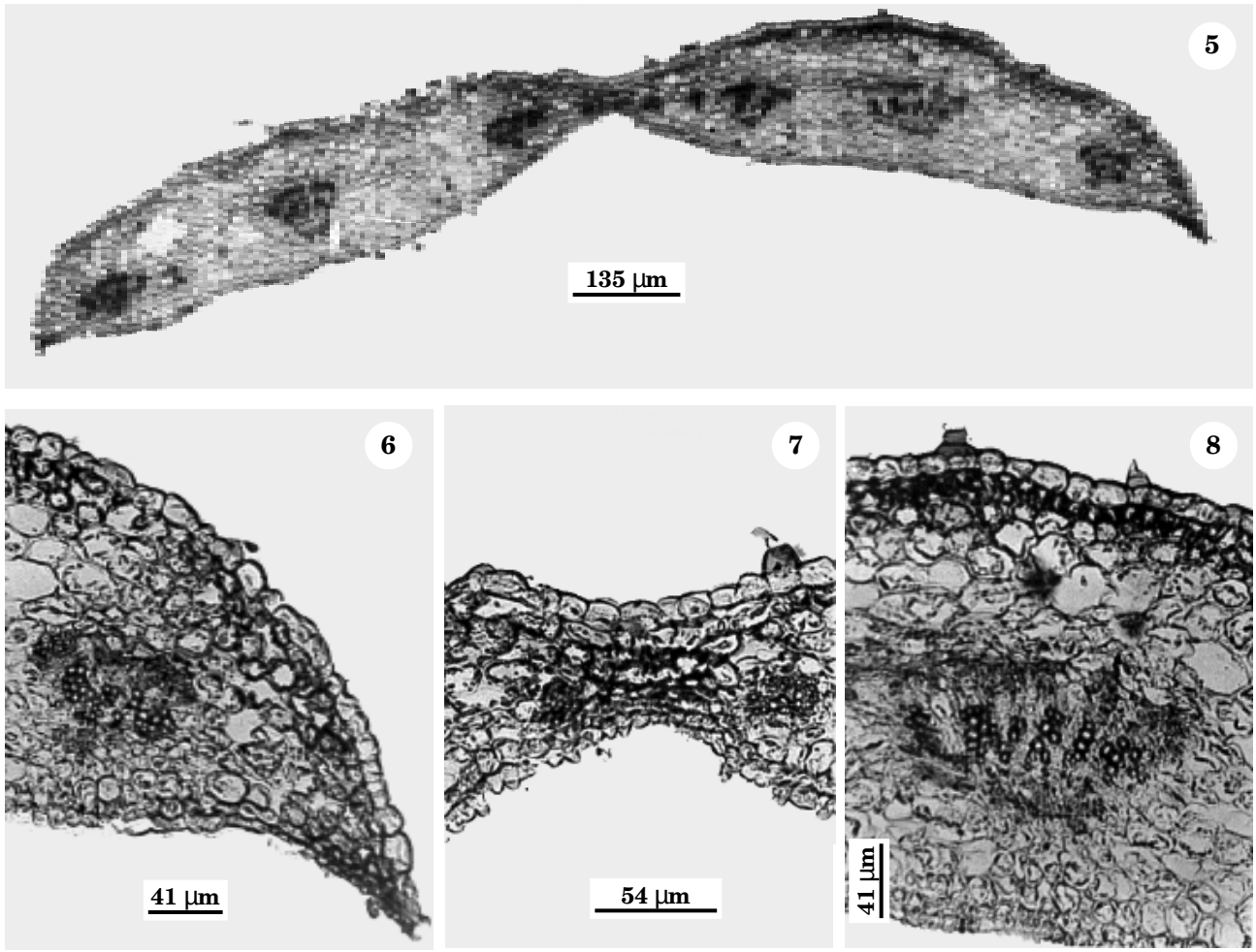


Fig. 5-8. Cross sections of sepal of Marmande tomato flower infected with TMV-M showing the connection of 2 sepals (5), magnified part of lateral vein (6), region of connection or isthmus (7), magnified part of midvein (8).

even this layer was ruptured (Fig. 9). Xylem and phloem were reduced and almost undifferentiated (Fig. 11).

The epidermal cells of infected anthers generally exhibited prominent thickening forming hook-like structures on both sides of the pollen sacs. The anther lobes showed an irregular arrangement, the number of pollen sacs varied from 2 to 4 and the pollen grains were mostly aborted (Fig. 12). Generally, there was no tapetum in sections exhibiting hooked epidermal cells, and the vascular elements were degenerated (Fig. 13).

The epidermal layer of the lower region of infected ovaries was strongly undulated (Fig. 14). In the middle region, there were 3 locules (Fig. 15) or 2 or, rarely, none, instead of the 5 locules in the ovaries of healthy plants. Ovules were generally aborted and vascular elements reduced and undifferentiated. Another common phenomenon was the rupture and sometimes the dissolution of whole cells (Fig.14).

In the fruits, the epidermis of infected pericarp was very thick, covered with a layer of cuticle that was too thick to reveal the epidermal region to be observed (Fig. 16). Sometimes the epidermis was ruptured or disintegrated (Fig. 17). The cortical region was small (Figs 17, 18), composed of a few layers of wavy-walled parenchyma cells with curved cells walls. Sometimes these cells were completely ruptured (Fig.16).

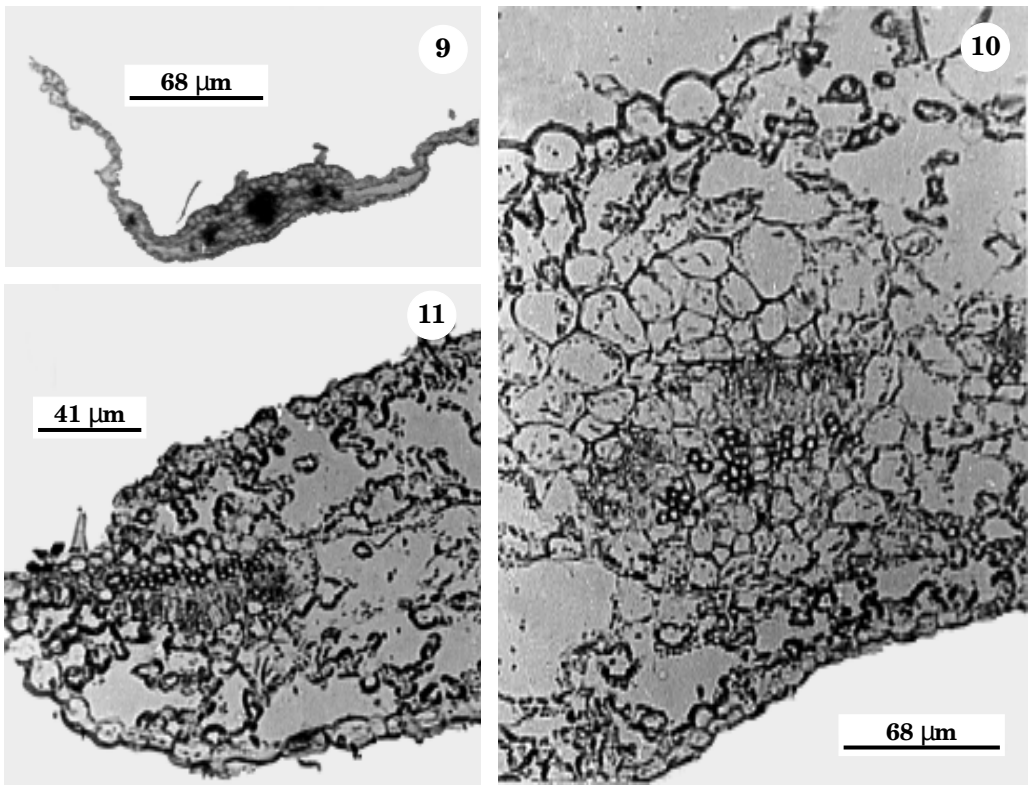


Fig. 9-11. Cross sections of petal of Marmande tomato flower infected with TMV-M showing the general form of infected petal (9), magnified part of midvein (10), magnified part of lateral vein (11).

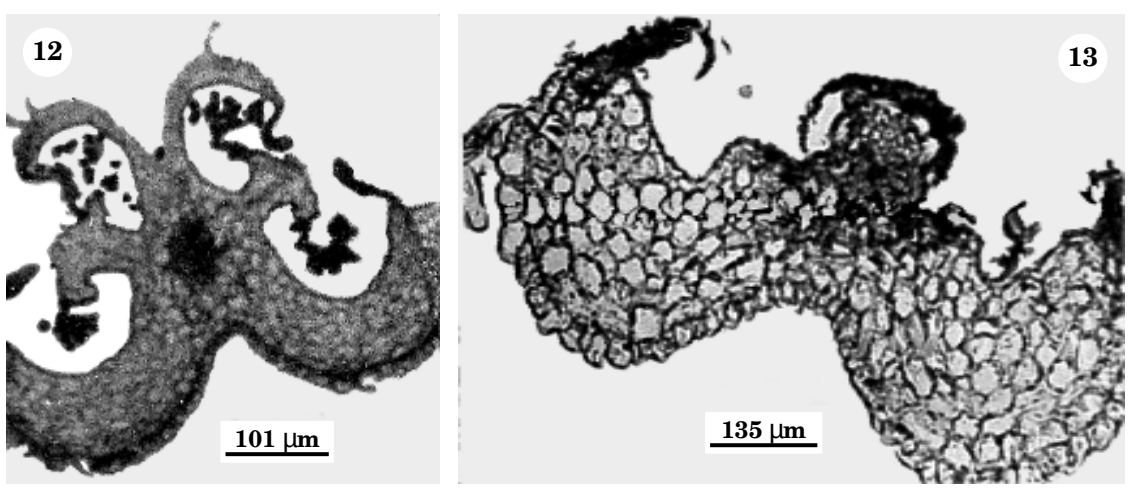


Fig. 12-13. Cross sections of anther of Marmande tomato flower infected with TMV-M showing the general form of infected anther (12), hook-like structure of infected anther (13).

Discussion

This study showed that, in general, the epidermis and mesophyll of infected sepals were similar to those of healthy sepals except that the lower epidermis tended to be undulated and wrinkled, the mesophyll cells appeared loose with large intercellular spaces, and the chloroplasts were more numerous and larger. Infected petals showed severe distortion and abnormalities in general shape, with curvature and wrinkling of the blade. Other peculiarities of both infected sepals and petals were the joining of two sepals or petals together,

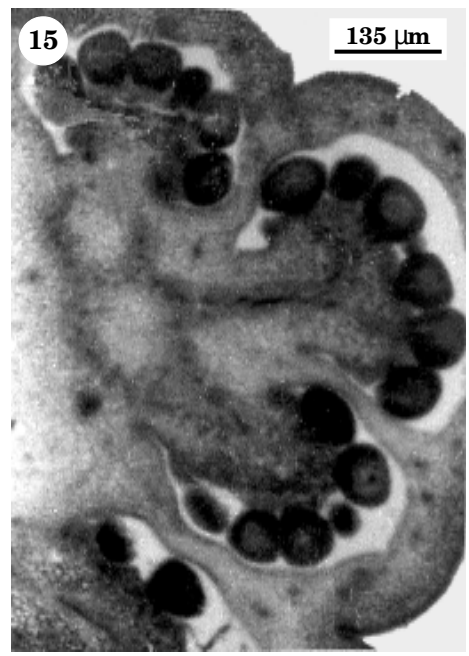
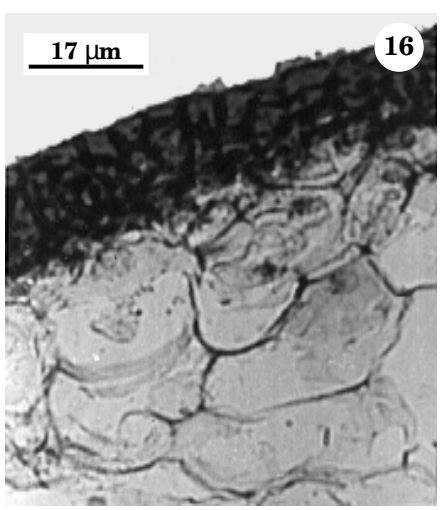
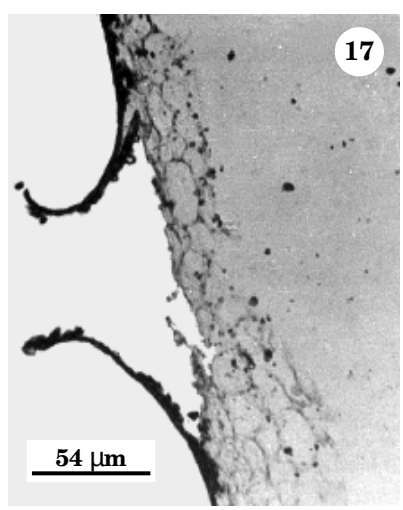


Fig. 14-15. Cross sections of ovary of Marmande tomato flower infected with TMV-M showing the general form of the lower region of the ovary (14), general form of the middle region of the ovary with ovules and carpel traces (15).





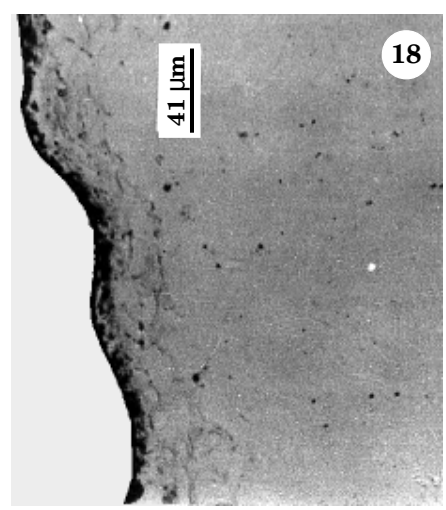


Fig. 16-18. Cross sections of pericarp of Marmande tomato fruit infected with TMV-M showing the epidermal layers very thick and covered also with thick cuticle layer (16), rupture of epidermis (17), undulation of epidermis (18).

the thickening of the cells of the connecting region or isthmus, and a reduction in the number of xylem and phloem vessels, which were undifferentiated.

Esau (1960) and Fahn (1974) stated that the sepals and petals resembled the leaves in their internal structure.

Sayed (1985) showed that the blades of sepals of *N. glauca* and *Petunia hybrida* infected with an Egyptian strain of TMV were either singly curved upwards or did not develop a midvein. The epidermal cells were small and their cuticle was thin. The epidermal cells appeared either loose with large intercellular spaces or were elongated and long-lobed in a palisade-like manner. The xylem and phloem were not well developed. Diseased petals did not show alterations in internal structure except for a marked undulation of the abaxial epidermal surface. The blade appeared thinner. The mesophyll cells were somewhat collapsed and distorted. The epidermal cells of infected anthers exhibited prominent thickening, forming hook-like structures on both sides of the pollen sacs. The anther lobes showed an irregular arrangement, the number of pollen sacs varied from 2 to 4, and the pollen grains were mostly aborted. Generally there was no tapetum in sections exhibiting hooked epidermal cells and the vascular elements were degenerated.

The epidermal layer of the lower region of infected ovaries was strongly undulated. In the middle region of the ovaries there were 3 or 2 locules or, rarely, none at all, instead of 5 in ovaries of healthy plants. Ovules were generally aborted and the xylem and phloem were reduced and undifferentiated. Another common phenomenon was the rupture and sometimes the dissolution of whole cells in the blade.

Awasthi and Singh (1974) and Murdock *et al.* (1976) reported the following histological symptoms for infected anthers and ovaries: anther lobes showed an irregular arrangement with aborted pollen grains, and the ovaries were smaller with abnormal, aborted and faintly stained ovules.

The main histopathological symptoms observed in infected pericarp were either the rupture and disintegration or alternatively a notable thickening of epidermal cells and cuticle. The fleshy layers below the epidermal layers were composed of undulated parenchyma cells which were sometimes completely ruptured.

It has been suggested that anatomical abnormalities of flowers and fruits in infected plants may be due either to altered growth habit (Goodman *et al.*,1986) or to an altered metabolism of growth hormones (Hamacher and Quadt, 1990).

Literature cited

Awasthi, D.N., B.P. Singh, 1974. Histopathological studies of reproductive organs in *Capsicum annuum* infected with cucumber mosaic virus. *Indian Phytopathology*, 27, 218-223.

Esau, K., 1960. The flower. *In:* Anatomy of Seed Plants, John Wiley and Sons, Inc., New York, NY, USA, 296-297.

Eskarous, J.K., S. Sarkar, M. El-Shamy, 1997. Histopathological changes in tomato flower and fruit infected with a highly heat resistant strain of tobacco mosaic virus in Egypt. *In*: 17th International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops, June 23-27, 1997, Bethesda, MD, USA.

Eskarous, J.K., E.T. Sayed, and M. El Shamy, 1999. Further studies on a severe Egyptian strain of TMV on tomato. EPPO/ MPU Conference on Cucurbitaceae and Solanaceae Vegetable Diseases in the Mediterranean Area, October 11-14, 1999, Kerkyra, Greece.

Fahn, A., ed. 1974. Histology of sepals and petals. *In*: Plant Anatomy. Pergamon Press, Oxford, UK, 2nd ed., 445-446.

Goodman, R.N., Z. Kiraly, and K.R. Wood, 1986. Biochemistry and Physiology of Plant Disease. Univ. of Missouri Press, Columbia, MO, USA.

Hamacher, J. and A. Quadt, 1991. Light and electron microscopic studies of cherry leaf roll virus (CLRV) on European ash (*Fraxinus excelsior* L.). *Journal of Phytopathology*, 131, 215-226.

Johansen, D.A., 1940. General methods. *In*: Plant Microtechnique, (Sinnot, E.W., ed.), Mc Graw-Hill Book Company, Inc., New York, NY, USA, 27-170.

Murdock, D.J., P.E. Nelson, S.H. Smith, 1976. Histopathological examination of Pelargonium infected with tomato ringspot virus, *Phytopathology*, 66, 844-850.

Purvis, M.J., D.C. Collier, D. Walls, 1964. Preserving, fixing and embedding; section cutting and mounting; stains and staining techniques. *In:* Laboratory Techniques in Botany. Butterworth and Co., Ltd. London, UK, 56-137.

Sass, J.E., 1951. Botanical Microtechnique. The Iowa State College Press, Ames, IA, USA.

Sayed, E.T., 1985. Further studies on a mosaic virus affecting *Nicotiana glauca*. PhD Thesis, Department of Botany, Faculty of Science, University of Cairo, Giza, Egypt.

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