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Editorial

Starting a Plant Virus Department and its Development a Personal Experience – From Test Plants to Molecular Biology

Loebenstein G*
Department of Plant Pathology, Agricultural Research Organization, Israel

***Corresponding author:** Loebenstein G, Department of Plant Pathology, Agricultural Research Organization, Bet Dagan, Israel; Email: Gad-talma@barak.net.il

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Editorial

Israel (Palestine) before 1918 was part of the Ottoman (Turkish) empire. Most the land was neglected, with some primitive dry farming agriculture (Figure 1). Under the British mandate (1920-1948) Jewish settlements – private, cooperative (Moshav) and collective (Kibbutz), were established and modern agricultural technologies were introduced (Figure 2).

In the mid 1950- ties, Israel's agriculture underwent a transition from dry farming to irrigated crops. Major irrigation projects were developed, carrying water from the Jordan and Yarkon rivers in the North and Center to the Southern parts of the country. Many small holders' farms were established, and plasticulture allowed growing vegetables all the year around. The continuous vegetation enabled the diseases and their vectors to over-summer, resulting in vicious cycles of virus diseases, especially in tomatoes, cucumbers, sweet peppers and sweet potatoes The director of the Agricultural Research (ARO) therefore decided to establish in 1961 a separate department of Plant Virology. The philosophy of the new department was that it is not sufficient to identify the pathogen, but that the research has to lead to a practical solution. This was quite in contrast to ideas prevalent at that time in some departments of plant pathology, where the scientists considered that their research was only to identify the causal agent.

The first step was to raise test plants for virus identification, which required insect protected greenhouses. In addition, we constructed within one-greenhouse glass cabinets with air conditioners to obtain temperatures around 25^{0}C for keeping the inoculated plants.

Crop Related Research Projects

- Viruses in Carnations and other flower crops. Spray carnation culture is well adapted to the Israeli winter for export to Europe. However, yields and flower quality were low, because viruses infected all stocks. The viruses identified were Carnation mottle virus, Carnation etched ring virus, Carnation necrotic (yellow) fleck virus and Carnation vein mottle virus. In cooperation with the Floriculture department, virus free mother stocks were prepared, resulting in yearly exports of 240 million flowers from 200 hectares in 1977-1978.

- Additional studies were done on viruses in Pelagnium. Viruses as Pelargonium flower break virus were identified by ELISA and a transcribed RNA probe [1]. This enabled a marked export of cuttings to Europe.

- As cucumbers were severely infected by Cucumber Mosaic Virus (CMV), a project was started by Dr. F. Nitzani together with Prof. E. Galun from the Weizmann Institute of Science to breed resistance originating from the Japanese cultivar Kjoto-3-feet into the local Bet Alpha cultivar. Several new CMV-resistant cultivars were selected and used by growers.

- Citrus Tristeza Virus (CTV). Prior to 1970, all citrus trees in Israel had sour orange rootstocks, rendering the tree highly susceptible to CTV. No marked natural spread was observed, as Toxoptera citricida is not present and transmission by Aphis gossypii became evident in Israel only in the second half of the 20th century.

Figure 1: Aerial photograph of the Southern Part of the Tiberias Lake in 1917; Barren area.

Figure 2: Aerial photograph of the same area in 1970; Prosperous Jewish settlements and their agricultural crops.

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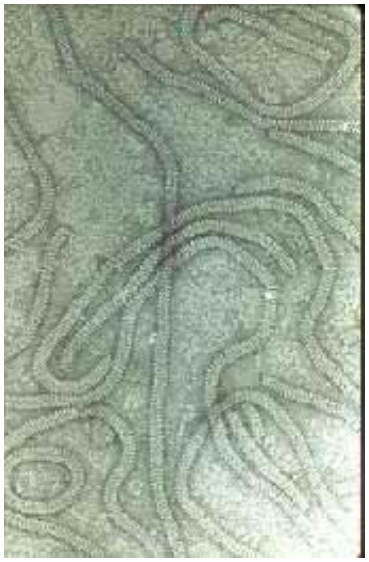

Figure 3: Electron micrograph of Citrus tristeza virus particles, X 150,000.

- Within the Ph.D. thesis of M. Bar Joseph supervised by G. Loebenstein, CTV was purified using electron microscopy as an assay (Figure 3), and partial sequences of the CTV genome were cloned and used to differentiate between CTV isolated. We also showed that a citrus tree could harbor different tristeza variants. This enabled operation of a Tristeza suppression program after several tristeza showing trees were found (Figure 4). About 3.25 million trees were tested during 1979-1985, and about 18 thousand trees in more than 600 groves, were found to be infected. The program delayed the epidemic, but unfortunately was terminated due to "disagreements at the political discussion table".

- Vector Studies and Control of Virus Dissemination. Serological detection of CMV in Aphids [2]; Transmission of CMV by aphids determined by the coat protein of the virus [3]; Control of CMV and PVY in sweet peppers by oil spays [4].

- Viruses in Sweetpotato. Sweetpotato in the Mediterranean basin are so far a minor crop, grown mainly for local consumption and in some countries like Israel also for exports to Europe. This has been a developing venture and exports have been increasing gradually. Yields in Israel are high, averaging about 34 tons/ha. The main viruses identified in Sweetpotato in Israel are Sweet potato feathery mottle virus, Sweet potato sunken vein virus (synonym Sweet potato chlorotic stunt virus) and Sweet potato virus disease [5]. The best way for control is to supply the grower every year with virus tested planting material.

Research Projects to Understand Resistance Phenomena of Plants to Viruses

- The local lesion phenomenon is one of the most notable resistance mechanisms where virus after multiplying in several hundred cells around the point of entry, does not continue to spread and remains in a local infection. Cells inside the lesion generally contain much less (about 10^3 particles) than cells in a systemic infection that contain between 10^5 and 6 x 10^7 particles per cell. Localization seems to be due to reduced multiplication in the cells of these hosts and not due to barrier substances, as implicated in early research, as possible

Figure 4: Orange tree infected with Citrus tristeza virus showing decline symptoms.

factors preventing virus movement. In the peripheral cells of a starch lesion on cucumber cotyledons the number of TMV particles was about 1/10 compared with those in the central part of the starch lesion, with no barriers or ultrastructural changes at the border of the lesion [6]. In early studies, it was observed that when cucumber cotyledons were treated with antimetabolites one day after inoculation with TMV, virus concentration increased markedly, indicating that during localization a substance is produced that reduces virus multiplication. In further studies, it was shown that localization of TMV in tobacco, containing the N gene, is associated with the presence of a protein with antiviral properties named 'inhibitor of virus replication [7,8]. IVR was released into the medium of TMV-infected protoplasts derived from Samsun NN tobacco IVR inhibited virus replication in protoplasts derived from both resistant Samsun NN, exhibiting local lesions and systemically infected susceptible N. tabacum cv. Samsun plants (Samsun nn). IVR inhibited TMV in protoplasts and leaf disks, the effect being dose responsive. IVR also inhibited PVX, PVY and CMV in leaf disks from different hosts, indicating that IVR is neither host nor virus specific. Production of IVR by infected protoplasts and intact Samsun NN plants was suppressed almost completely when exposed to 35^{0}C [9].

- A ca. 23kDa protein band was always associated with samples of crude protoplast IVR, tissue-IVR and IVR purified from induced-resistant tissue; this protein was absent in samples of uninfected plant tissue and protoplasts derived from them. Purification of the 23kDa protein from SDS-polyacrylamide gels yielded a molecule with antiviral properties in biological tests [10]. Antibodies against the IVR protein neutralized its antiviral activity and enabled immunodetection of the 23 kDA protein [9,10].

- A cDNA was isolated from an expression library prepared from induced-resistant leaf tissue of Samsun NN. This clone, NC 330, expressed a 21.6kDa IVR-like protein in E. coli, recognized by IVR antibody, which exhibited antiviral properties in biological tests. The NC330 clone hybridized with RNA from induced-resistant tissue from N. tabacum cv. Samsun NN but not with RNA from non-induced tissue [11].

- Sequence analysis of clone NC330 indicated that the C-terminus of the deduced protein is highly acidic, rich in aspartic acid and glutamic acid, hydrophobic and with a helical structure [11]. NC330 Protein (accession CAA08776) motif analysis in silico showed the presence of six sites typical for protein kinase one site for N-glycosylation, two N-merystylation sites and Leucine Rich

Repeats (LRR), but it is mainly a Tetratricopeptide Repeat (TPR) protein. These motifs are known to be involved in protein-protein interactions. It is worthwhile to note that TPR motifs are present in many proteins including inducible interferons [12,13].

- Transformation of N. tabacum cv. Samsun nn in which TMV spreads systemically, with NC330 encoding an IVR-like protein, resulted in a number of transgenic plants, expressing variable resistance to TMV and the fungal pathogen Botrytis cinerea. Transformation of tomato plants with the IVR gene resulted in partial resistance to Alternaria alternata, Phytium aphanidermatum and Rhizoctonia solani. The finding that an R gene that is associated with virus localization also induces resistance to some fungal diseases is unique and suggests that some R genes have a wider range of activity than may have been previously assumed.

Foot Note

Part of this chapter was presented at the 13th International Plant Virus Epidemiology Symposium (IPVE), Avignion, France.

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