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A Survey for Tristeza and Greening in Punjab (Pakistan)

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ABSTRACT. A survey for citrus tristeza virus (CTV) and greening was carried out in Punjab province. More than fifty orchards and ten nurseries were sampled in different areas of the province. ELISA tests and electron microscopic observations showed that CTV was present in different districts in the varieties Mosambi, Bloodred and Pineapple sweet orange. Mosambi variety was the most affected (7 positive out of 35). Many orchards showed symptoms of greening on different species (lemon, grapefruit, sweet orange and mandarin). Electron microscopic observations revealed the presence of the greening organism in phloem cells. *Diaphorina citri* was found widespread in the province.

Index words. ELISA, electron microscopy, bud union crease.

The citrus cultivated surface in Punjab is 122,000 ha, with 94.8% of the total in Pakistan. Kinnow mandarin represents 70% of the entire citrus production. The other major commercial citrus varieties are: Feutrell's early mandarin, Mosambi and Bloodred sweet oranges. Almost all the trees in Punjab are grafted on rough lemon rootstock.

Tristeza and greening occurrence in Punjab has been suspected for a long time (5, 10). Rough lemon rootstock was introduced in 1947 to prevent tristeza decline. Symptoms of greening were suspected in the north of the Pakistan, since 1976 (8). The presence of the vectors in Punjab and the occurrence of these diseases in neighbouring India corroborated suspicions (3, 4, 7, 9, 13).

The present paper reports some of the results of a survey carried out in Punjab during 3 yr (1987-1989) within the framework of the "Project for Research and Development in Cultivation of Fruits Vegetables and Olives," as a joint program between Italy and Pakistan.

Preliminary results of a survey for citrus virus and virus-like diseases and of an electron microscopy investigation have been presented elsewhere (6, 11).

MATERIALS AND METHODS

Field inspections were carried out during January-February and November 1987, November 1988 and April

1989 in the most important citrus areas in the Punjab.

Approximately fifty commercial citrus orchards, ten nurseries and different variety collection plots in Sahiwal, Faisalabad, Sargodha, Lahore, Toba tek Singh, Okara and Rawalpindi districts were surveyed.

Tristeza. Samples of budsticks with green bark and/or young leaves were collected in plastic bags at 5 C and transported to Catania for enzyme-linked immunosorbent assay (ELISA) test to detect CTV, using the standard procedures (1) obtained from the T4 isolate of CTV (11). The citrus extracts were prepared from cortex or leaves. One gram of tissue was ground by mortar and pestle in the presence of liquid nitrogen and extracted in buffer (PBS-T+2%PVP). Mexican lime seedlings inoculated with an isolate of CTV from Japan (TJ) and sour orange seedlings inoculated with isolate T4 were used as positive controls. The results were determined by measurement of absorbance at 405 nm with a Titertek Multiskan MCC. We considered all the samples positive which had an absorbance value 2.5 times the extracts from the uninoculated control.

Greening. Two to 5 mm pieces of columella and 2 mm long pieces of leaf veins coming respectively from fruits showing small size, poor colour, malformation or leaves showing yellow veins or mottling have been immediately fixed in 3% glutaraldehyde in



Fig. 1. Greening organism in the sieve tube of a rough lemon tree, Faisalabad (24,000X magnification).

0.1M phosphate buffer at pH 6.8 and processed in Catania for electron microscopy observations. Four grams of N-pyrrolidynomethyl tetracycline (Revering-Hoechst) dissolved in 60 ml of distilled water have been injected in April 89 in two trees of Kinnow mandarin and one of Pineapple sweet orange suspected to be infected by greening.

Samples of nymphs and adults of citrus psylla were collected (March-April 1989) in different areas and transferred into vials containing denatured alcohol or simply closed in hatching boxes. Those in alcohol have been used for taxonomic identification purpose whereas the others have been kept under observation to check the casual presence of any predator or parasite.

RESULTS

Tristeza. Since rough lemon is virtually the only rootstock used in commercial orchards of the province, CTV symptoms were observed in variety

collection plots where sweet orange trees were grafted on sour orange rootstock. These showed a mild inverse pitting below the budunion line. Trees on rough lemon appeared symptomless. No stem pitting symptoms were observed in several trees examined. ELISA tests and electron microscopy observations on samples collected for greening studies confirmed field observations and revealed CTV infections on asymptomatic trees too. A list of tested varieties, and results of the test for CTV are reported in Table 1. All the CTV infected plants were sweet oranges (Mosambi, Bloodred and Pineapple varieties). None of the trees adjacent to the infected ones were found to be infected by CTV. Some infections were found in a nursery in Mosambi and were probably due to the use of infected budwood.

Toxoptera citricidus Kirk was not found during the surveys, but *Aphis citricola* Van der Goot, *A. gossypii*

TABLE 1
NUMBER OF CITRUS TRISTEZA VIRUS
INFECTED PLANTS/TOTAL OF TESTED
PLANTS FOR DIFFERENT VARIETIES IN
PUNJAB

| | ELISA for CTV ^a |
|-----------------|----------------------------|
| Sweet orange | |
| Mosambi | 7/35 |
| Bloodred | 1/8 |
| Pineapple | 1/3 |
| Mandarin | |
| Orlando | 0/1 |
| Kinnow | 0/18 |
| Feutrell'searly | 0/4 |
| Lemon | |
| Eureka | 0/8 |
| Prior Lisbon | 0/1 |
| Others | 0/9 |
| Total | 9/87 |

^aResults were determined by measurement of absorbance at 405 nm with a Titertek Multiskan MCC. We considered positive the samples which had an absorbance value 2.5 times extracts from the uninoculated control. For all test we used polyclonal antibody obtained from the T4 isolate of CTV.

Glover, *T. aurantii* (B.d.F.) were present over the province.

Most trees of Mosambi showed a larger growth of rootstock associated with a poor performance of scion, which recalls of bud-union crease of sweet oranges on rough lemon described by McClean (14).

Greening. The characteristic symptoms of greening (tree dieback, malformed and poor coloured fruits, seed abortion, leaf midvein yellowing and leaf mottling) were observed in different areas of the province on almost all cultivated varieties. The symptoms were more evident during cool seasons. Two of 23 samples examined with the electron microscope revealed the presence of greening organism (GO) in phloem cells in ultrathin sections of columella and leaf midvein tissues taken from rough lemon rootstock and Mosambi sweet orange, in two different districts (Faisalabad and Okara). Surprisingly, it was not

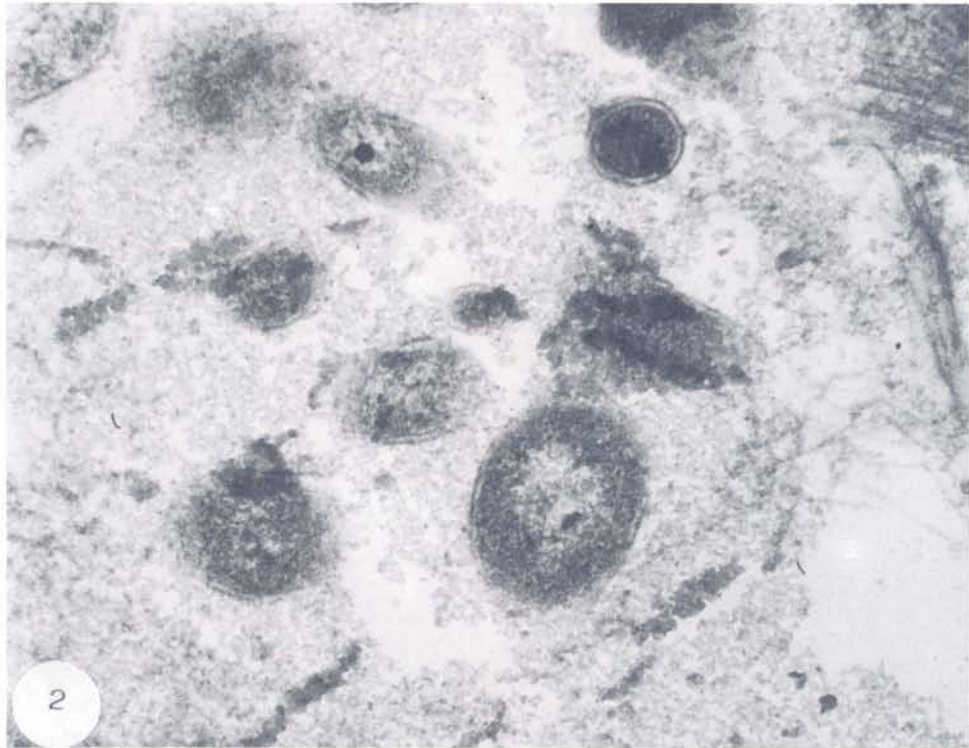


Fig. 2. Ultrathin section from a sweet orange columella showing one sieve tube cell with the greening organism (60,000X magnification).



Fig. 3. Greening diseased leaves of sweet orange showing vein yellowing.

possible to demonstrate the presence of the GO in samples of Kinnow mandarin trees showing all symptoms of the disease.

Tetracycline injections of infected trees resulted in symptom remission with both infected trees showing no fruit symptoms 8 months later, whereas the check remained badly affected.

During the spring survey it was noticed that citrus psylla (*Diaphorina citri* Kuw.) is quite widespread throughout the province on citrus as is



Fig. 4. Malformed and less coloured fruit of Kinnow mandarin.

Murraya paniculata, a citrus relative. However, in Taxila, Islamabad, Okara and Sahiwal, no citrus psylla was found on young citrus leaves because of unseasonably cool temperatures and heavy rainfalls during March 1989.

Identification revealed that the same citrus psylla was present in all the samples. In the hatching boxes no parasites of *D. citri* were found parasitizing the sampled psyllas.

DISCUSSION

Tristeza and greening were both found in Punjab. Mosambi sweet orange was the only cultivar found to be severely infected by CTV. The stunting or decline observed, however, appeared to be more related with a budunion crease on rough lemon which was described by Mc Clean (14) to be caused by a graft-transmissible pathogen. Both tristeza and greening play an important part in the citrus decline. Other problems observed included poor cultural practices, gummosis, and root rot.

CTV infections have been found distributed in the province in variety collections, in commercial orchards, and in a nursery. Because of the lack of infection in trees adjacent to CTV infected trees, the virus was likely spread by grafting. Up to now there is no evidence of CTV transmission by *Aphis citricola*, *A. gossypii* and *Toxoptera aurantii* although these species are present in Punjab (3) and, according to the present results, spread, if any, must be relatively inefficient. Nevertheless since Bové (2) has reported symptoms of the disease in Kagzi lime seedlings, further investigations are needed.

The low percentage of samples found infected by GO does not agree with the distribution of the symptoms in the field. Similar results have been reported by Bové (2). The low infection rate could be attributed to the poor condition of the samples. According to the seasonal variability of symptoms better results could be achieved in samples collected earlier in autumn. In fact, the geographic and climatic condi-

tions and the lack of *Trioza erytreae* Del Guercio suggest that the greening strain present in Punjab is similar to the Asian greening described in India. *D. citri* is an efficient vector of greening and its presence, also in Pakistan, is related with the characteristic symptoms of the disease.

Since *Tetrastichus radiatus*, an efficient parasite of *D. citri* is present in the neighbouring India, its presence in Pakistan must be more deeply investigated. In case of *T. radiatus* is lacking, it could be introduced from abroad.

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