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Historical review of *Citrus tristeza virus* and its vectors in Iran

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Abstract. This review outlines the importance of citrus industry in Iran where the different species have been grown for many centuries. Emphasis is laid on the introduction of CTV following the import of large amounts of Satsuma varieties from Japan in the 1970s. A number of surveys were carried out from 1978 to 2007 in the main citrus growing areas across the country and a low level of natural CTV transmission was determined. *Aphis gossypii*, *A. citricola*, *A. craccivora*, *Toxoptera aurantii* and *Myzus persicae* proved to be the most common vectors involved in CTV spread. In contrast, *T. citricidus* has never been reported to date.

Key words. Aphids – Citrus – Citrus tristeza virus – ELISA – Iran.

Rétrospective historique du virus de la tristeza des agrumes et de ses vecteurs en Iran

Résumé. *Dans cette rétrospective historique, l'accent est mis sur l'importance de l'agrumiculture en Iran, où de nombreuses espèces sont cultivées depuis des siècles. Dans ce pays, le CTV a été introduit à la suite des importations massives de satsuma du Japon au cours des années 70. Plusieurs enquêtes ont été réalisées entre 1978 et 2007 dans les principales régions agrumicoles iraniennes et elles ont permis de mettre en évidence le faible niveau de transmission naturelle du virus. En plus, l'Aphis gossypii, l'A. citricola, l'A. craccivora, le Toxoptera aurantii et le Myzus persicae se sont avérés être le vecteurs les plus communs du CTV. En revanche, la présence du T. citricidus n'a jamais été signalée jusqu'à présent.*

Mots-clés. *Pucerons – Agrumes – Virus de la tristeza des agrumes – ELISA – Iran.*

I – Introduction

Citrus has a long history in Iran. Citron was the first citrus fruit to be cultivated in the country more than 2500 years ago, in the times of Median Empire. Sour orange, lemon and small fruited lime were spread throughout Persia and Near East around AD 1000. Sweet orange, called "Portugal" in many Arab countries as well as in Iran, reached the Persian Gulf ports with Portuguese trade vessels during the middle centuries (Ebrahimi, 1996).

At present, citrus orchards cover an area of 268,000 ha with a production of 4,300,000 tons in three major citrus-growing regions in Iran. The Caspian Sea belt (Golestan, Mazandaran and Gilan provinces 106 000 ha) extends in the north over 400 km from east to west, and lies between the shore of the Caspian Sea (29 m below sea level) and the first slopes of the Alborz mountain range. Various citrus varieties are cultivated, seedy local sweet oranges (*Citrus sinensis* L.) (55%); seedy local orange trees grafted on sour orange rootstock (*C. aurantium* L.) (20%); Washington navel, Thompson navel and blood oranges, all on sour orange, (5%); Satsuma (*C. unshiu* Marc.), Clementine (*C. reticulata* Blanco) and local and imported tangerine (*C. reticulata* x *C. sinensis*), on sour and trifoliate orange (*Poncirus trifoliata* Raf.) rootstock, (15%); and others (5%).

Citrus orchards of the Southern Inland Belt (116 000 ha) are scattered through the low valleys of the southern Zagros mountain range, essentially in the provinces of Khuzestan, Fars and Kerman. The main characteristics of these areas are low annual rainfall (100-300 mm) and excessive heat in summer. The main citrus species and varieties are seedy local sweet orange trees on sour orange, Bakravi or lemon (*C. limon* Burn. f.) rootstocks, (51.8%); small-fruited acid seedling trees

(28.1%); seedy local tangerine trees on Bakravi or lemon rootstock (9.6%); Palestine sweet lime (*C. limettoides* Tan.) cutting trees (8.4%); and others (2.1%).

Bakravi is said to be a local natural hybrid between mandarin (*Citrus reticulata* Blanco) and small-fruited acid lime (*Citrus aurantifolia* Chrism).

About 46 000 ha of citrus are spread along the coasts of the Persian Gulf and the Gulf of Oman named Southern Coastal Belt. The climate is tropical with no frost, and well suited to acid lime, sweet lime and lemon. In this area acid lime represents 90 percent of production. Salinity of water and high pH are the limiting factors.

Until 1918 there was no real "citrus industry" and all citrus varieties were propagated by seed. The heavy losses due to foot rot prompted growers to use the phytophthora-resistant sour orange rootstock, but sweet orange seedling still constitute 60% of all orange trees in the Caspian Sea area. Acid lime tolerates a high pH (8.5) and is more resistant to drought than sour orange; therefore, it is often used as rootstock in the southern citrus-growing area.

In the country local cultivars are still predominant, in spite of many introductions that have been made since 1933. As part of the economic development of Iran, new citrus species and varieties were introduced for the first time into the Caspian Sea area (Ramsar) in 1933, from Turkey, Italy, Lebanon and Palestine. At that time no attention was paid to the presence or absence of virus diseases. In February 1962, a countrywide frost killed thousands of citrus trees. To rehabilitate the Iranian citrus industry, 700,000 certified buds were imported in 1963-64 from the Willits and Newcomb nursery in California. Additional varieties were introduced into Mazandaran province 1969 from Australia and Japan and in 1971 from California and Morocco. After 1967, a period of heavy frost caused severe damage to citrus in the Caspian Sea region, 40,000 in 1968 and 15,000 satsuma trees in 1970 were imported from Japan. Tristeza virus being endemic in Japan, it could be predicted that each single plant of the 55,000 Japanese satsuma trees was infected with *Citrus tristeza virus* (CTV). These trees were grafted on *P. trifoliata*, a rootstock tolerant to the virus and commonly used in Japan. Hence, the 55,000 Japanese trees have grown well since, but it seems that they were responsible for the introduction of CTV into Iran (Bové, 1995).

The 1985 survey showed that only very limited spread of Tristeza had occurred. The low level of natural transmission is probably due to the fact that *T. citricidus* is absent in the country. *Aphis gossypii, A. citricola, T. aurantii, A. craccivora* and *Myzus persicae* are known as CTV vectors in Iran.

II – Historical review

Ebrahim-Nesbat *et al.* (1978) reported CTV for the first time from Iran by indexing *Citrus unshiumaro*, imported from Japan, on Keylime; this result was confirmed using ISEM method.

Minassian (**1983a)** used ELISA for detection of CTV in aphids from the north of Iran. He detected the virus in the sap of *T. aurantii* and *A. citricola* and showed that out of 22 aphid colonies from both healthy and diseased plants, 15% carried CTV, while 45% of colonies from diseased plants carried CTV.

Minassian (**1983b)** determined the spread of CTV in the north of Iran using ELISA. He tested over 400 citrus trees from different regions in the province of Mazandaran. He showed that in Mahdasht orchards, 90 to 100% of Satsumas on Poncirus stock, introduced from Japan, were infected. He found that 10% of old line of Satsumas on sour orange and less than 10% of Clementines on Poncirus or sour orange were also infected.

Minassian *et al.* (1983) reconfirmed the occurrence of CTV on Satsuma using indexing on key lime which showed vein clearing symptoms, cupping and chlorosis. They confirmed the presence of CTV using ELISA and ISEM.

Ghorbany (1983). detected CTV in its aphid vectors including *T. aurantii, A. spiraecola* and *A. gossypii* in northern Iran by ISEM technique.

Ghorbany (1986) showed that fruit pericarp has the highest CTV content followed by tree bark and leaves using ISEM method. He didn't t see any virus particles in fruit flavedo and seed.

Rahimian (1994), detected strains of CTV in Mazandaran Province in two Mahdasht orchards, considered to be the main infected foci on the basis of biological properties. He identified three strains of CTV: seedling yellows, tristeza and stem pitting. He deduced that a high proportion of Satsuma trees in this area are infected with CTV and the majority of infected trees carry tristeza or stem pitting strains. Infection with the seedling yellows strain was rare.

Aghajanzadeh *et al.* (1996) studied the transmission of CTV by major citrus aphids (*A. citricola, A. gossypii and T. aurantii*) using *C.unshiu and C. aurantifolia* as donor and recipient host species in Mazandaran. None of aphid species examined could transmit CTV from any donor species.

Zarei *et al.* (1997) eliminated CTV from two cultivars of Satsuma (cvs. Miyagawa and Sugiyama) through shoot-tip grafting.

Shayeghan *et al.* (1997) detected CTV in the suburb of Babol (Mazandaran Province) on *C.unshiu* as a new site of infection.

Shafiee and Izadpanah (1998a) purified CTV from the bark of local mandarins in Fars Province and produced an antiserum which reacted with virus isolates both from northern and southern Iran.

Shafiee and Izadpanah (**1998b)** detected distribution of CTV in southern Iran. Kharf, Kazeroon, Fasarood, Janatshahr, Dalaki, Roodfaryab, Khaeez and Jam regions were infected.

Rahimian *et al.* (2000a) showed that the decline of sweet orange on sour orange rootstock is an evidence for possible onset of natural transmission of CTV in Mazandaran.

Alavi *et al.* (**2000a)** differentiated CTV isolates by electrophoretic analysis of cDNA prepared to CTV-RNA. Most severe strains produced a cDNA band with an estimated size of 4.2 kb but shorter than 1 kb about mild strains.

Alavi *et al.* (2000b) evaluated various ELISA and DTBIA methods in order to detect CTV. They found that DAS and PTA-ELISA are more sensitive than indirect ELISA methods and the direct and indirect DIBA was as sensitive as DAS-ELISA. They also showed that a 3% gelatin or a 5% triton X-100 solution gave a clear background; the best results were obtained when used together.

Alavi *et al.* (2000c) purified CTV by sucrose density gradient electrophoresis which could provide preparations of sufficient purity and devoid of contaminating host proteins suitable as immunogen for preparations of CTV specific antisera.

Alavi *et al.* (**2000b)** reported *A. gossypii* as a vector of CTV in the north of Iran.

Pakniat *et al.* (2002a) investigated a decline of Navel orange characterized by yellowing and browning of veins in Darab (Fars Province) since 1998. They detected CTV in affected trees. They could eliminate the disease by shoot tip grafting (STG), while CTV was still present. So, they deduced that although CTV may play a role in the development of Navel orange disorder, other factors, which were excluded by STG, are also involved.

Pakniat *et al.* (2002b) studied the aphids ability in the transmission of CTV. They found that *M. persicae* can be a dangerous vector in the north while *A. gossypii* and *A.craccivora* are vectors in Fars Province.

Pakniat *et al.* (2002c) investigated the distribution of CTV in Fars and Bushehr provinces. Fasaroud was the most infected district with 50% infection in Key lime and Talha the lowest one

with 20%.Their data also showed the role of vector in CTV transmission in the area as Key lime and sour orange trees are grown from seeds.

Rahimian *et al.* (**2002)** found seedling yellows as the predominant CTV strain transmitted by aphids in Mazandaran Province.

Barzegar *et al.* (**2005a)** characterized 22 isolates of CTV collected from the north and the south of Iran based on CP gene sequences. They showed high similarity between Iranian isolates, California SY568 severe stem pitting and Japanese NUagA seedling yellows strain up to 97% based on RFLP profile, nucleotide and deduced amino-acid sequences. Also they couldn't obtain major dissociation between the isolates from northern and southern region of Iran.

Barzegar *et al.* (2005b) investigated the biological and molecular properties of four CTV strains isolated from sweet orange trees in north of Iran. Sequence analysis showed 98-99% sequence homology of Iranian isolates with the Californian CTV severe stem-pitting isolate SY568 and 97-98% homology with the Japanese seedling yellows isolate NUagA.

Alavi *et al.* (**2005)** divided seven isolates of CTV collected from the north of Iran into two groups based on symptoms in different host plants: the first causing stem pitting and severe decline and the second, seedling yellows. Isolates in the first could be transmitted by *A. gossypii*. Based on the phylogenetic analysis of CP gene on the isolates from the eastern part of Mazandaran Province, they suggested at least two independent introductions of CTV into Iran.

Ahmadi *et al.* (**2006)** determined distribution and analysis of genetic diversity of CTV isolates in Kerman Province. They found that Jiroft and Hosseinabad were the most infected regions in this province. They divided the isolates in two distinct clusters. They grouped all isolates from Kerman, except Bam isolate, in one cluster and the isolate of Bam with other isolates from Iran in another cluster.

Alebouye *et al.* (**2006)** determined the prevalence of CTV infection in the Mahdasht area in Sari. Their results showed that all Satsumas in one plot in Mahdasht, which were among the ones imported from Japan over 35 years ago, were infected and there was an inverse relationship between the proportion of infected trees in the adjacent and more distantly located groves and their distance from the Mahdasht grove.

Pakniat *et al.* (**2006a)** detected CTV infection in all southern provinces of Iran (Fars, Kohgiluyeh-Boyerahmad, Hormozgan, Sistan-Baluchestan and Kerman). They couldn't detect any severe strain of this virus in these regions. In contrast, northern isolates recently introduced from Japan included severe CTV strain. They found the highest rate of infection in sweet orange followed in decreasing order by local mandarin, sweet lime and lime.

Pakniat *et al.* (**2006b)** demonstrated that an unidentified strain of CTV is associated with vein yellowing and browning in navel sweet orange varieties using biological methods.

Barzegar *et al.* (**2006a)** compared sequence analysis of CP27 gene of aphid *A. gossypii* transmissible and aphid non-transmissible CTV isolates. They found 73 nucleotide changes and 5 amino-acid substitution between them. Three of these amino acids were important for interaction of CP27 and aphid style. In their study comparison of the CP27 deduced amino-acid sequence with their pfam members revealed the conservancy of the Arg and Asp amino-acids needed for formation of salt bridge.

Barzegar *et al.* (**2006b)** revealed sequence variability of CP25 gene of 15 CTV isolates by single strand conformation analysis. They observed 8 distinct patterns. SSCP profile of some isolates was in accordance with CP25 gene sequence. But, totally there was no direct relationship between nucleotide differences and SSCP patterns as, sometimes two isolates with a few nucleotide differences produced distinct patterns.

Maghsoudi *et al.* (**2006)** evaluated 5 natural citrus rootstocks to CTV in north of Iran. They found that natural Kotra hybrid, Shell mahalleh and Khoram abad lemon hybrid are fairly tolerant, off-type sour orange is tolerant and Ramsar lemon hybrid is susceptible to CTV.

Fifaei *et al.* (**2007)** produced Washington navel sweet orange free of tristeza virus using STG technique with four treatments and four replications. All of them were not infected and T-budding method was more successful. They showed that there was no significant difference between used rootstocks.

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