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Natural tolerance/resistance of citrus plants to Citrus tristeza disease

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Abstract. Since tristeza is the most serious citrus virus disease worldwide, knowing the natural defense systems of citrus plants is still considered the main control approach. In this paper an account is given of the attempts to use CTV tolerant/resistant cultivars and rootstocks and classical cross-protection programmes to contain tristeza disease.

Keywords. Citrus – Citrus tristeza virus – Resistance – Tolerance.

Tolerance/résistance naturelle des plants d'agrumes à la maladie de la tristeza des agrumes

Résumé. *Vu que la tristeza est la maladie à virus la plus redoutable chez les agrumes à l'échelle mondiale, la connaissance des mécanismes naturels de défense des plants d'agrumes représente aujourd'hui encore la principale méthode de lutte. L'objectif de ce travail est donc de passer en revue les tentatives d'utiliser les cultivars et les porte-greffes tolérants/resistants au CTV et d'appliquer des programmes de protection croisée classiques pour limiter la diffusion de la tristeza.*

Mots-clés. *Agrumes – Virus de la tristeza des agrumes – Résistance – Tolérance.*

I – Introduction

Tristeza is the most devastating virus disease of Citrus worldwide. Millions of trees on sour orange rootstock have been killed or have become unproductive after being infected with *Citrus tristeza virus* (CTV) - induced decline. Tristeza epidemics started in the Western hemisphere in the 1930s whereas in the Mediterranean the first disease outbreaks occurred in the 1950s in Israel and Spain (Roistacher, 1991; Whiteside *et al*., 1988), In the last years, CTV has been reported in the rest of the Mediterranean region (Djelouah and D'Onghia, 2001) and disease epidemics have been found in Italy (Davino *et al*., 2003).

The virus, which is a *Closterovirus*, has been globally distributed through infected citrus propagating material, whereas locally it is transmitted semi-persistently by different aphid species (Gottwald *et al*., 1997), primarily *Aphis gossypi* and *A. spiraecola* in the Mediterranean. *Toxoptera citricidus*, the most efficient CTV vector worldwide, is now present in Northern and Central Portugal and in Northern Spain (Ilharco *et al*., 2005), and represents a serious threat of CTV rapid spread to the other European and Mediterranean citrus-industries.

CTV strains are broadly grouped according to how they affect certain plants or scion/rootstock combinations: decline and death of most cultivars on sour orange rootstock (*Citrus. aurantium* L.) 'quick decline (CTV-QD); seedling yellows symptoms (CTV-SY), stem pitting (CTV-SP) of grapefruit (*C. paradisi* Macf.) and of sweet orange (*C. sinensis* L. Osb.) which usually can induce stunting, poor yield and fruit quality, and rarely the tree death (Lee and Rocha-Pena, 1992).

The virus causes considerable economic losses and the current management approaches (e.g. use of pathogen-free stock, tolerant rootstocks, eradication of infected trees, vector control) are unlikely to provide long-term durable control. However, several attempts have been made for gene manipulation by selection and breeding programmes but this is a very difficult and long practice due to the large genetic distance between resistant and citrus cultivars. The production of CTV resistant biotech citrus plants for commercial purposes is highly attractive, but it is still underway in several laboratories. Hence, the effective management of CTV-induced diseases, based on knowledge of natural plant defence mechanisms, remains an important challenge for the sustainability of the Mediterranean citrus industry.

II – Tristeza tolerance/resistance variability

Alternative rootstocks are chosen instead of the sour orange in order to eliminate the graft-incompatibility induced by CTV-QD in many citrus cultivars on sour orange. In fact, the use of CTV tolerant/resistant rootstocks has contributed to reduce the damages caused by the disease but this has also limited the range of suitable rootstocks as regards other phytosanitary conditions. The trifoliate rootstocks, for instance, are highly susceptible to most citrus viroid diseases (Mestre *et al*., 1997a) and this is a major problem when citrus viroids are present in propagating materials, Therefore the use of 'healthy' propagating material is crucial in any CTV control strategy.

A first attempt to group symptomless rutaceous plants in relation with citrus tristeza virus strains was made by Bové (1995). For most citrus species considered symptomless, it is not clear yet whether they are tolerant or resistant. When a citrus tree is resistant to a CTV strain, this means that it can not be infected systemically, whereas if it is tolerant the virus can spread systemically without significantly affecting its growth and yield. Even if cultivars and rootstocks are potential sources of resistance, unfortunately their resistance mechanisms are still poorly characterized.

Variability of tolerance/resistance expression is mainly related to the virus isolate and to the environmental conditions. Several examples are reported worldwide. Washington navel and Valencia sweet oranges were tolerant to the Brazilian CTV strains, whereas Pera sweet orange was susceptible to the severe stem pitting isolates. In contrast, CTV isolates in Spain and Israel could induce great damages on Valencia and Washington navels. The K strain of CTV from Corsica (France) is an example of a mixture of strains which, when present, induces no symptom in lime seedlings, the universal CTV indicator, even if the virus multiplies well in the infected limes. However, aphid transmission of this strain separates isolates and these isolates result in strong symptoms in Mexican lime [*C. aurantifolia* (Christm.) Swing.] (Bovè, 1995).

Pummelo [*C. maxima* (Burm.) Merrill or *C. grandis* (L.) Osb.] represents a very good example of the great variability of symptom expression which is strictly related to the CTV isolate type (Fang and Roose, 1999; Garnsey *et al*., 1996). In fact, based on symptom expression (dwarfing and stem pitting), 18 pummelo cvs were classified as tolerant, moderately tolerant and susceptible to CTV-SP (Xueyuan *et al*., 2002). However, resistance in pummelo seems to cover a limited number of CTV isolates. The same condition is observed in other genera within the Rutaceae-Aurantioideae (*Severinia, Atlantia, Fortunella, Glycosmis, Murraya, Triphasia, Feronia, Feroniella, Aegle, Merrillia*) while little is known yet on the resistance mechanisms (Garnsey *et al*., 1987; Mestre *et al*., 1997b; Yoshida, 1996).

Recently, an attempt to select CTV tolerant hosts was made using CTV RNA concentration in the plant tissues (Targon *et al*., 2007); the expression of p23 gene, which has probably a regulatory role in the virus cycle and/or pathogenesis, and of p25 and p27 genes, which are the CTV coat proteins, was higher in a susceptible variety (Pera orange) compared with a tolerant variety (*C. reticulata* Blanco Ponkan mandarin) using Real time PCR.

Other cases are related to the effect of CTV isolates on trees which are grafted onto tolerant rootstocks as in South Africa, where CTV usually occurs as a mixture of stem pitting and seedling yellows strains; the effect of this mixture proved to be more severe on trees grafted onto Troyer citrange (*Poncirus trifoliata* x *C. sinensis*) than on trees grafted onto rough lemon (*C. jambhiri*) and Volkameriana lemon [*C. limon* (L.) Burm.f.] rootstocks. The effect of CTV isolates without the seedling yellows component was less severe on tolerant rootstocks. The symptom severity seemed to be affected by the cultivars (rootstock and scion) and the climate. The reason is still unknown because the citrange parents are trifoliate orange (resistant) x sweet orange (tolerant) (Van Vuuren, 2002). Nevertheless, the occurrence of CTV isolates, which could replicate at a low level in trifoliate orange in New Zealand (Dawson and Mooney, 2000), and of the Indian CTV isolate, which produced the same results (Hilf, 2005), poses a major threat to the effectiveness of CTV resistance derived from trifoliate orange.

However, emphasis has been laid on the broad spectrum CTV resistance expressed by trifoliate orange, [*Poncirus trifoliata* (L.) Raf.] compared to the CTV strain-specific resistance found in pummelos. This resistance, which is associated with a single dominant gene at the *Ctr* locus, proved to be effective against all the tested CTV isolates. This was a potentially very useful character since this species is also sexually compatible with *Citrus* members (Barrett, 1990; Mestre *et al*., 1997b). Moreover, *Ctr* was not the only locus responsible for CTV resistance in *P. trifoliata*, but at least one other gene seems to be involved. Given that citrus is a perennial crop, breeding for durable disease resistance should involve selection at both the *Ctr* and *Ctm* loci (Mestre *et al*., 1997a). Although the dominant gene has already been characterized and mapped, much research effort is still focussed on the possibility of transferring this resistance to some important citrus varieties by molecular transformation (Deng *et al*., 2001; Yang *et al*., 2003). Some studies undertaken to determine the type of resistance involved, demonstrated that CTV could replicate in mesophyll protoplasts from trifoliate orange and from other citrus relatives which are resistant to CTV *(Swinglea glutinosa* (Blanco) Merr., and *Severinia buxifolia* (Poir) Ten.). In these cases resistance is probably due to the block of either cell-to-cell movement or long-distance movement, or to an induced resistance response (Albiach-Marti *et al.*, 2004).

III – Cross protection

One of the most effective strategies to face citrus crop losses, where severe CTV strains are endemic and vector populations are abundant, is based on cross protection. Cross protection against CTV is induced by challenging severe virus strains with mild strains in order to prevent disease expression (Lee *et al*., 1987). Strain sources are usually infected plants showing mild infection symptoms or no effect at all in areas where severe CTV strains cause serious problems. In California, the severe strains were eliminated by transferring the isolate to *Passiflora* spp.; moreover, aphid vectors, thermotherapy or shoot-tip-grafting can make a positive contribution to separate mild strains from severe strains. After a preliminary plant screening in the greenhouse of a number of CTV isolates for symptom expression on susceptible varieties, the promising protecting isolates are than tested in the field against natural infections with severe strains. This type of disease control has proved to be the most successful one in many countries worldwide (i.e. Brazil, Australia and South Africa) where susceptible citrus varieties have been planted in the nursery after being infected with a mild CTV strain. Cross protection has prevented low yields and small-sized fruits of Pera sweet orange in Brazil (Costa and Müller, 1980) and Marsh grapefruit in South Africa (Van Vuuren *et al*., 1993). The cross protection mechanism is not well known; however, protection breaks down over time due to many factors such as the variety, the virus strain and the environmental conditions. The continuous challenge of an existing or of a newly introduced different severe strain can also overcome the mild strain effect in cross protection as reported in Florida for sweet orange on sour orange rootstock after eight years (Powell *et al*., 1992). The synergistic effect of another virus with a mild CTV strain can also break the protection effect, as reported for a citrus viroid, on the growth and production of Delta Valencia orange on Yuma citrange rootstock (Van Vuuren and Da Graça, 1996). For this reason a certification scheme was developed in South Africa, using healthy plants which were CTV-preimmunized.

Another successful cross protection programme was also developed in Peru, where economic disasters were caused by a CTV stem pitting strain, which induced a scion disease affecting citrus trees regardless of the rootstock. This situation led Bederski *et al*. (2007) to search for highly productive CTV symptomless carriers within the same citrus cultivars. They found quite a high level of cross protection of grapefruit cvs 'Star Ruby' and 'Flame', when UCLA rough lemon was used as a rootstock. Moreover, a single nine-year old Star Ruby tree on *Citrus shekwasha* remained symptomless for stem pitting and highly productive with uniform large sized fruits, despite the heavy inoculum and vector pressure. In this case, the character involved was not rootstock dependent because 'Star Ruby' was successfully propagated also onto a rootstock other than *C. shekwasha*.

In conclusion, resistant trees to be used for commercial purposes are still many years away. While research into the application of the resistance gene found in *P. trifoliata* is making progress, the use of tolerant/resistant rootstocks and varieties or the development of cross protection programmes are nowadays the only available and efficient alternatives to control tristeza disease and make citrus cultivation possible.

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