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Changes in the Bark Proteins of Sour Orange Rootstock Induced by Citrus Tristeza Virus

P. Moreno, J. Ortiz, J. Guerri

ABSTRACT. The effect of citrus tristeza virus (CTV) infection on bark proteins of several citrus rootstocks was studied by polyacrylamide gel electrophoresis (SDS-PAGE). A protein band (P6) from bark of sour orange grafted with sweet orange, grapefruit or clementine scions had lower intensity in CTV-infected than in CTV-free trees. Decrease of P6 intensity was more conspicuous in trees infected with a severe CTV isolate than in those infected with a mild isolate. No difference was observed between bark proteins of CTV-infected and CTV-free sour orange sprouts. Protein profiles of healthy and tristeza-infected Troyer citrange or Cleopatra mandarin, grafted with navel orange, were also indistinguishable. Finally, protein patterns of sour orange grafted with navel orange, severely declined from *Phytophthora* sp. or psorosis, were similar to those of non-declined controls. Thus, decreased intensity of P6 seems to be specifically associated with tristeza in citrus trees grafted on sour orange.
Index words. plant proteins, slab gel electrophoresis, tristeza decline.

Tristeza is probably the most important disease of citrus and it has caused important economic losses in most citrus growing countries. Symptoms induced by citrus tristeza virus (CTV) on different citrus species depend on the virus strain, but most commercial species and cultivars grafted on sour orange decline after virus infection (4).

Recently, progress has been made in strain characterization (1) and methods for quick and reliable detection of the virus (2, 3, 7, 11, 16), but less effort has been directed to study the mechanism of the disease. Schneider (15) pointed out that the first detectable symptom on tristeza-inoculated plants of several citrus species was the formation of a distinctive type of abnormal cells (chromatic cells). In trees of sweet orange grafted on sour orange, necrosis of sieve tubes immediately below the budunion was the primary reaction of infected plants. Beltrán *et al.* (5, 6) detected alterations in the activity of peroxidase, ribonuclease and some of the enzymes involved in callose biosynthesis pathway in bark phloem of orange trees affected by tristeza. They also found a lower protein content in bark phloem of sweet/sour orange trees infected by tristeza than in CTV-free trees (5).

Modifications in the protein profile of different plant species after infection with citrus exocortis viroid (CEV) were detected by slab gel electrophoresis (8, 9, 10).

In this work, we have studied the effect of tristeza on the bark protein profile of the rootstock on several citrus stionic combinations.

MATERIALS AND METHODS

Protein analyses were carried out on bark samples taken from field trees grown at different locations, except in one experiment in which bark from sour orange seedlings grown in the greenhouse were used. Samples consisted of bark pieces, 5 cm long and 2 cm wide, taken 3 mm below the budunion in grafted plants and about 25 cm above ground in ungrafted plants.

Phloem necrosis induced by tristeza at the budunion of trees grafted on sour orange (13) was simulated by girdling 2-year-old sour orange seedlings grown in the greenhouse (14). A bark ring 1 cm wide was removed 25 cm above ground level. Bark samples taken below the girdle were compared

with samples taken 25 cm above ground level from ungirdled plants.

Bark samples were washed, freeze dried and finely ground in a Janke & Kunkel refrigerated mill. Proteins were extracted by homogenizing 1.0 g of the dried powder in 10 ml 0.26M Tris-phosphoric acid buffer, pH 6.9, with the aid of a Polytron homogenizer and clarifying by centrifugation at 15000 *xg* for 20 minutes. An aliquot of the supernatant was boiled 4 minutes in a medium with 1.77% sodium dodecyl sulphate (SDS), 77 mM Tris-HCl, pH 8, 3% glycerol, and 4.4% mercaptoethanol. Protein separation was accomplished by 3-layer slab SDS-PAGE following the method described by Conejero and Semancik (9). Intensity of protein bands was measured at 550 nm with a Beckmann CDS-100 F densitometer. Molecular weight estimations were accomplished by using calibration kits ranging from 14,400 to 669,000 daltons.

RESULTS

Preliminary trials showed that protein patterns of sour orange bark from trees grown at different locations or from the same tree at different seasons were similar. Also sour orange trees grafted with different varieties had analogous protein patterns.

Protein profiles of sour orange bark from tristeza-infected trees had a band, peak 6 in figure 1 (P6), with lower intensity than those from CTV-free trees (figure 1a and c). The estimated molecular weight of this band was about 36,000 daltons. This difference between CTV-infected and CTV-free trees was observed on sour orange grafted with Marsh grapefruit, Washington navel orange, Clementine mandarin and Berna sweet orange topgrafted with Navelina navel orange.

Alteration of the P6 band in tristeza infected trees was similar when sour orange bark was sampled 3 mm or 25 cm below the budunion.

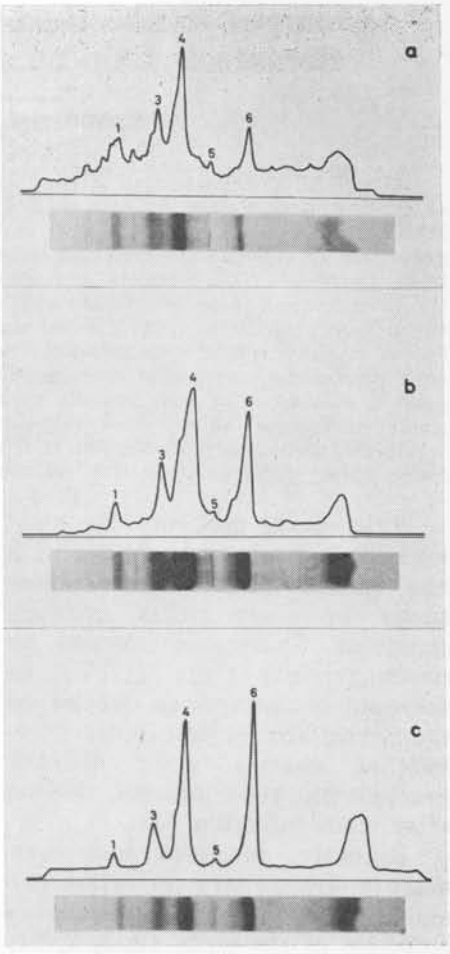

Fig. 1. Electrophoretic profiles and densitometric tracings of bark proteins of sour orange from: a) a tree infected with a severe CTV isolate; b) a tree infected with a mild CTV isolate; c) a CTV-free control.

The effect of CTV on bark proteins of two tristeza-tolerant rootstocks grafted with navel orange was also studied. Protein profiles of Troyer citrange (figure 2) and Cleopatra mandarin (figure 3) from CTV-infected and CTV-free trees were similar.

The alteration of the protein band P6 of sour orange bark from CTV-infected trees was related to the severity of the isolate. Electrophoretic profiles of sour orange

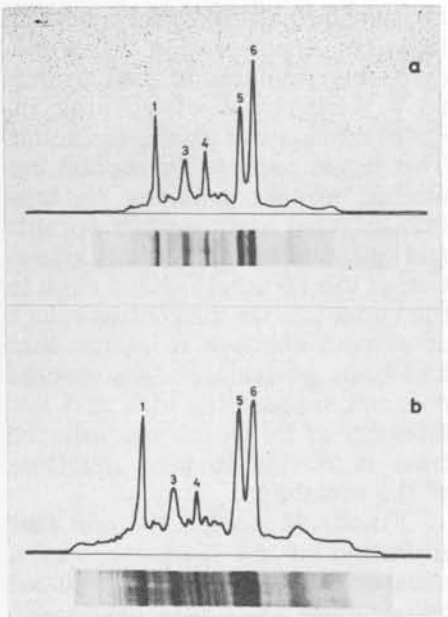

Fig. 2. Electrophoretic profiles and densitometric tracings of bark proteins of Troyer citrange from: a) a CTV-infected tree; b) a CTV-free control.

bark from a CTV-free control, a tree severely declining from tristeza and a tristeza-infected tree with healthy appearance are compared in figure 1. Decrease of P6 intensity was more conspicuous in the tree infected with a severe CTV isolate than in the one infected with a mild isolate.

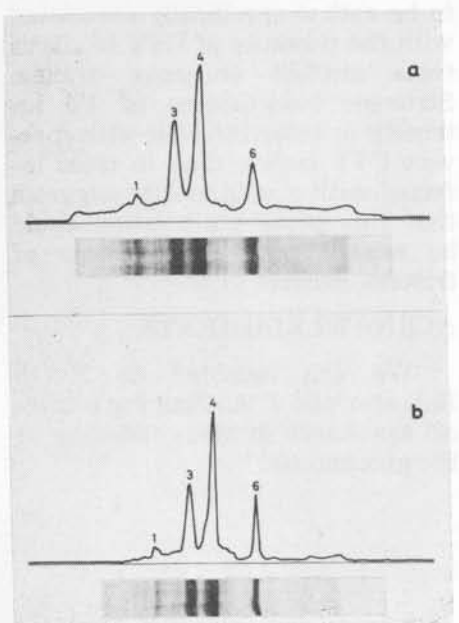

Fig. 3. Electrophoretic profiles and densitometric tracings of bark proteins of Cleopatra mandarin from: a) a CTV-infected tree; b) a CTV-free control.

Modification of P6 intensity associated with tristeza on stionic combinations on sour orange was not observed on ungrafted sour orange. Electrophoretic profiles of about 3 cm diameter sour orange sprouts from CTV-infected and CTV-free trees, showed similar intensity of the P6 band. Protein profiles of sour orange sprouts were very close to those of CTV-free sour orange rootstocks (figure 1c).

Bark proteins of trees declining from causes other than tristeza were also studied. Protein profiles of sour orange rootstock from navel orange trees declining from tristeza, psorosis or *Phytophthora* were compared. Only CTV-declining trees showed decreased intensity of the P6 band. Psorosis and Phytophthora-declining trees had protein profiles similar to control trees free of scaly bark and foot rot symptoms (figure 1c).

Phloem necrosis induced by girdling had no effect on the protein pattern of sour orange seedlings. Plants girdled for two months and ungirdled plants gave profiles with similar intensity in the P6 band. They were also very close to protein profiles of CTV-free sour orange rootstocks (figure 1c).

DISCUSSION AND CONCLUSIONS

A decreased intensity of a protein band (P6) has been observed in protein profiles of sour orange bark from CTV-infected trees compared with CTV-free controls. The same alteration of proteins has been found on diseased trees from different locations, ages and stionic combinations in sour orange. In addition, decrease of P6 intensity is more conspicuous in trees in-

fected with a CTV isolate inducing severe decline than in trees infected with a mild isolate. Thus, the modification found in the P6 protein band of sour orange bark seems to be related in some way with tristeza infection, independently of the scion variety or growing conditions.

CTV-infected sour orange sprouts did not show any change in protein profile compared with CTV-free trees. This is an indication that low intensity of P6 band found below budunions is not a direct effect of the virus but rather the result of an interaction between CTV and tristeza-susceptible combinations. Unaltered protein patterns found in CTV-infected trees on tolerant rootstocks also support this hypothesis.

Most citrus trees in Spain are infected by one or several viruses (12) and some of the trees sampled in this work indexed positive for exocortis, psorosis or both; but, since CTV-free controls were also infected by those viruses and CTV-infected trees from different origins gave the same protein alteration we can associate this alteration with the presence of CTV.

The possibility existed that decreased intensity of the P6 band was a result of altered proteins in necrotic cells produced by tristeza on sour orange. Nevertheless, phloem necrosis on infected trees is more intense just below the budunion (13) whereas alteration of P6 was similar 3 mm or 25 cm below the budunion.

Alternatively, modification of the protein pattern could reflect an impaired translocation of photosynthesis products to sour orange as a consequence of girdling induced by tristeza at the budunion. This hypothesis would explain unaltered protein patterns on tristeza-infected sour orange sprouts and the finding of similar alteration of the P6 band far and close to the budunion. On the contrary, lack of protein changes in plants that had been girdled for two months does not support the idea that low intensity of P6 on tristeza infected trees is related to poor nutrition of the rootstock.

Finally, it could be thought that alteration of P6 intensity was a nonspecific effect of decline induced by tristeza on infected trees. Nevertheless, trees declining from *Phytophthora* or psorosis did not show any modification on protein profiles of sour orange bark sampled below the affected area. This is an indication that low intensity of P6 is not a nonspecific effect of tree decline but it seems to be rather specifically associated with the presence of CTV in citrus trees grafted on sour orange. Stronger modification of P6 intensity in trees infected with a severe CTV isolate than in trees infected with a mild isolate suggests that this protein alteration could be related with pathogenesis of tristeza decline.

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LITERATURE CITED

1. BALARAMAN, K. and K. RAMAKRISHNAN
   1978. Studies on strains and strain interaction in citrus tristeza virus. Univ. Agric. Sci., Bangalore. Tec. Ser. No. 19: 1-62.
2. BAR-JOSEPH, M., S. M. GARNSEY, D. GONSALVES, M. MOSCOVITZ, D. E. PURCIFULL, M. F. CLARK, and G. LOEBENSTEIN
   1979. The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69: 190-94.
3. BAR-JOSEPH, M., S. M. GARNSEY, D. GONSALVES, and D. E. PURCIFULL
   1980. Detection of citrus tristeza virus. I. Enzyme-linked immunosorbent assay (ELISA) and SDS-immunodiffusion methods, p. 1-8. *In* Proc. 8th Conf. IOCV, 1979. IOCV, Riverside.
4. BAR-JOSEPH, M., C. N. ROISTACHER, S. M. GARNSEY and D. J. GUMPF
   1981. A review on tristeza, an ongoing threat to citriculture. 1981. Proc. Int. Soc. Citriculture 1: 419-423.
5. BELTRAN, J. P., J. CARBONELL, and V. CONEJERO
   1976. Actividades e isoenzimas de peroxidasa y ribonucleasa en combinaciones resistentes y sensibles de naranjos afectados de tristeza. Rev. Agroquím. Tecnol. Alim. 16: 195-208. L
6. BELTRAN, J. P., J. CARBONELL, and V. CONEJERO
   1976. Actividades de los enzimas de la vía de bosíntesis de callosa en floema de corteza de naranjos afectados de tristeza. Rev. Agroquím. Tecnol. Alim. 16: 367-79.
7. CAMBRA, M., P. MORENO, and L. NAVARRO
   1979. Detección rápida del virus de la "tristeza" de los cítricos (CTV) mediante la técnia inmunoenzimática ELISA-sandwich. An. INIA/Ser. Prot. Veg./No. 12: 115-25.
8. CONEJERO, V., and J. S. SEMANCIK
   1977. Exocortis viroid: alteration in the proteins of *Gynura aurantiaca* accompanying viroid infection. Virology 77: 221-32.
9. CONEJERO, V., and J. S. SEMANCIK
   1977. Analysis of the proteins in crude plant extracts by polyacrylamide slab gel electrophoresis. Phytopathology 67: 1424-26.
10. CONEJERO, V., I. PICAZO, and P. SEGADO
    1979. Citrus exocortis viroid (CEV): protein alterations in different hosts following viroid infection. Virology 97: 454-56.
11. GARNSEY, S. M., R. G. CHRISTIE, K. S. DERRICK, and M. BAR-JOSEPH
    1980. Detection of citrus tristeza virus. II. Light and electron microscopy of inclusions and viral particles, p. 9-16. *In* Proc. 8th Conf. IOCV, 1979. IOCV, Riverside.
12. NAVARRO, L., J. F. BALLESTER, J. JUAREZ, J. A. PINA, J. M. ARREGUI, and R. BONO
    1981. Development of a program for disease-free citrus budwood in Spain. 1981 Proc. Int. Soc. Citriculture 1: 70-73.
13. SCHNEIDER, H.
    1954. Anatomy of bark of bud union, trunk, and roots of quick-decline-affected sweet orange trees on sour orange rootstock. Hilgardia 22: 567-81.
14. SCHNEIDER, H.
    1954. Effect of trunk girdling on phloem of trunk of sweet orange trees on sour orange rootstock. Hilgardia 22: 593-601.
15. SCHNEIDER, H.
    1959. The anatomy of tristeza-virus-infected citrus, p. 73-84. *In* J. M. Wallace (ed.) Citrus Virus Diseases, Univ. Calif. Div. Agr. Sci., Berkeley.
16. TSUCHIZAKI, T., A. SASAKI, and Y. SAITO
    1978. Purification of citrus tristeza virus from diseased citrus fruits and the detection of the virus in citrus tissues by fluorescent antibody techniques. Phytopathology 68: 139-42.