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Serological studies on the accumulation and localisation of three tomato leaf curl geminiviruses in resistant and susceptible *Lycopersicon* species and tomato cultivars

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Summary

Accessions of wild *Lycopersicon* spp. and selected F1 hybrid tomato cultivars were compared for their resistance to three whitefly-transmissible geminiviruses: Indian tomato leaf curl virus (ITmLCV) and tomato yellow leaf curl viruses from Sardinia (TYLCV-Sar) and Senegal (TYLCV-Sen). The resistance of different plant lines was expressed in different ways but in most instances a given line reacted similarly to graft inoculation with the three viruses. *L. pimpinellifolium* LA1478 produced as much virus antigen, assessed by triple antibody sandwich-ELISA, as the susceptible cv. Moneymaker but developed only very mild symptoms and is therefore tolerant of infection. In *L. hirsutum* LA1777 and *L. peruvianum* CMV-INRA, very mild or no symptoms developed but antigen concentrations were substantially less than in Moneymaker. *L. chilense* LA1969 remained symptomless and its antigen concentration was <1% of that in Moneymaker. Symptoms were mild or barely evident in the F1 hybrid cultivars. Cultivars Tyking and Fiona had antigen concentrations about 5–10% of those of Moneymaker, whereas TY20, Top 21 and Tyger had intermediate antigen concentrations. In a few instances, the extent to which virus accumulation was restricted depended on the challenge virus. Accumulation of TYLCV-Sen in TY20, Top 21 and Tyger was less affected than that of the other two viruses, and accumulation of TYLCV-Sar in accessions LA1777 and CMV-INRA was less affected than that of TYLCV-Sen or ITmLCV.

Tissue-printing tests showed that ITmLCV and TYLCV-Sen antigens were confined to phloem tissue. In Tyking, the number of virus antigen-containing phloem traces and the antigen content of individual traces were less than in Moneymaker but the partitioning of antigen between internal and external phloem was unaffected.

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Introduction

Leaf curl and yellow leaf curl diseases cause serious losses in tomato crops in many countries in tropical, sub-tropical and warm temperate regions (Green & Kalloo, 1994). The diseases are caused by geminiviruses that are transmitted by the whitefly *Bemisia tabaci sensu lato* (Cohen & Nitzany, 1966; Czosnek *et al.*, 1988). A variety of methods have been used to minimise disease incidence in crops. In Israel, applications of insecticides to decrease vector populations partially controlled leaf curl in the short-term (Nitzany, 1975) but proved less effective in the longer term because insecticide-resistant whitefly populations built up (Cohen & Melamed-Madjar, 1974). In India, putting muslin covers over tomato seed-beds and spraying the enclosed seedlings with insecticide delayed infection and decreased crop loss (Saikia & Muniyappa, 1989). In other areas, delaying the planting date until the *B. tabaci* population had passed its peak, or deterring *B. tabaci* from landing on tomato plants by covering the soil with silver plastic (Sumwan, Akkawi, Al-Musa & Mansour, 1988) had some beneficial effects. However, none of these treatments was effective in all locations or in all conditions.

Breeding programmes to produce leaf curl-resistant tomato cultivars are therefore of prime importance. Little resistance was found among existing tomato cultivars but tests on wild species of *Lycopersicon* detected heritable resistance in some accessions of *L. chilense* (Zakay *et al.*, 1991), *L. hirsutum* (Ioannou, 1985), *L. peruvianum* (Pilowsky & Cohen, 1990) and *L. pimpinellifolium* (Pilowsky & Cohen, 1974). Moreover, Pilowsky & Cohen (1990) were able to transfer the resistance from *L. peruvianum* to a cultivated tomato, the F1 hybrid TY20. In most studies, resistance was assessed on the basis of decreased incidence and/or decreased severity of symptoms in field-grown plants exposed to natural infection. However, in a few studies other criteria, such as decreased frequency of detection or decreased accumulation of viral DNA, were used (Zakay *et al.*, 1991; Rom *et al.*, 1993). Another point to be considered in assessing resistance is the occurrence of different tomato leaf curl-inducing geminiviruses in different countries. For example, six groups of tomato leaf curl virus isolates with different geographical distributions were distinguished by comparing the patterns of reactions of individual isolates with panels of monoclonal antibodies (MAbs) raised against African cassava mosaic virus (ACMV) or Indian cassava mosaic virus (ICMV) (Harrison, Swanson, McGrath & Fargette, 1991; Muniyappa, Swanson, Duncan & Harrison, 1991; Konate *et al.*, 1995). Moreover, comparison of the genomic DNA sequences of representatives of several of these groups of virus isolates has indicated that each is a separate virus species (Hong & Harrison, 1995; Padidam, Beachy & Fauquet, 1995). Hence it is important to ascertain whether the resistances found in wild *Lycopersicon* spp. are effective against all whitefly-transmitted geminiviruses infecting tomato or are specific to one, or a sub-set, of the viruses.

The work described in this paper was done to compare the accumulation in standard conditions of three distinct whitefly-transmitted, leaf curl-inducing geminiviruses in a range of wild *Lycopersicon* spp. and a selection of leaf curl-resistant F1 hybrid tomato cultivars. To exclude the effects of whitefly-related factors, the plants were inoculated by grafting in most of the experiments and, to allow quantitative comparisons to be made, relative virus concentrations were estimated serologically. In addition, information on the distribution of viral antigen within plants was obtained by tissue printing.

Materials and Methods

Plant lines

Accessions of four wild species of *Lycopersicon*, reported to express various degrees of

resistance to leaf curl, were tested. *L. pimpinellifolium* LA1478 has one incompletely dominant resistance gene (Pilowsky & Cohen, 1974); *L. peruvianum* CMV-INRA has five recessive resistance genes (Pilowsky & Cohen, 1990); *L. hirsutum* LA1777 has multigenic dominant resistance (Ioannou, 1985); and *L. chilense* LA1969 has a single dominant resistance gene (Zakay *et al.*, 1991). Seeds of these lines were provided by H Laterrot.

F1 hybrid tomato varieties reported to possess resistance/tolerance were also tested. TY20 has resistance derived from *L. peruvianum* PI126935. In Israel, symptoms develop later and are milder than in control varieties (Pilowsky & Cohen, 1990). Tyger (Royal Sluis) and Top 21 (Clause) are reported to show moderate resistance to leaf curl, and Tyking (Royal Sluis) and Fiona (Sandoz-Sluis en Grot) to have stronger resistance. The sources of the resistance genes in these four cultivars are not stated. The susceptible variety Moneymaker was used as a control in all tests.

Virus isolates

Three virus isolates from widely separated sources were used. Indian tomato leaf curl virus (ITmLCV) was an isolate from Bangalore, India (Muniyappa *et al.*, 1991). Tomato yellow leaf curl virus, Sardinian isolate (TYLCV-Sar; Kheyr-Pour *et al.*, 1991; Konate *et al.*, 1995) was provided by G P Accotto, Torino, Italy, and the Senegal isolate (TYLCV-Sen; Konate *et al.*, 1995) was collected at the Centre pour le Developpement de l'Horticulture (CDH/ISRA), Bambey, near Dakar. The viruses were cultured in graft-inoculated tomato cv. Moneymaker. All infected plants were kept at 15–25°C in an insect-proof containment glasshouse at Dundee under licence from the Scottish Office Agriculture and Fisheries Department (SOAFD).

Inoculation and sampling of plant lines

In most experiments, 4- to 6-week-old plants were inoculated by top-grafting with a young shoot or leaf of infected Moneymaker tomato. The plant lines were tested in two batches, the first consisting primarily of the wild *Lycopersicon* spp. and the second including the F1 hybrid varieties. In each experiment, one of the viruses was inoculated to all the plant lines being tested (at least six plants/line). Two weeks after inoculation, symptoms were recorded, young leaf tissue (*c.* 1 g/plant) was collected and leaf extracts were tested individually by triple antibody sandwich-ELISA (TAS-ELISA) as described below. Additional sets of samples were tested at intervals up to 14 wk after inoculation.

In a few experiments, seedlings with 1–2 true leaves were inoculated by virus-carrying *B. tabaci* (5–10 insects/plant) as described by McGrath & Harrison (1995). The culture of *B. tabaci* was held under licence from SOAFD.

TAS-ELISA

Relative concentrations of virus particle antigen were determined essentially as in the procedure of Thomas, Massalski & Harrison (1986), using cross-reacting antibodies raised against ACMV or ICMV. MAbs SCR20 and SCR23 (raised against ACMV; Thomas *et al.*, 1986) were used to assay TYLCV-Sar and TYLCV-Sen, whereas SCR60 (raised against ICMV; Aiton & Harrison, 1989) was used to assay ITmLCV. Leaf extracts were made in 0.05 M Tris-HCl, 0.06 M sodium sulphite, pH 8.5 (10 or 20 ml g^{-1} leaf tissue). Wells in microtitre plates were coated with polyclonal antiserum to ACMV (Sequeira & Harrison, 1982) diluted 1:10000, bound antigen was exposed to MAbs in tissue culture supernatant fluids diluted 1:3 and the MAbs were detected with rabbit or goat anti-mouse globulin/ alkaline phosphatase conjugate (Sigma). Absorbances ($A_{405\,nm}$) were recorded after incubation with p-nitrophenyl phosphate for 1–2 h at room temperature followed by 16–18 h at

4°C. Unless otherwise stated, values for virus-free extracts were deducted from those for virus-containing extracts. The data were analysed by the least significant difference test (Wilkinson, 1992).

Tissue printing

The immunoblotting procedure used to determine the distribution of virus particle antigen in tissue sections is based on that of Cassab & Varner (1987). Freehand sections (*c.* 1 mm thick) were cut from the tissue to be tested, rinsed in distilled water for 3 s, then dried on paper towels. They were placed on prepared nitrocellulose sheet and covered with paper tissue, which was overlaid with a glass plate that was pressed down by hand for 30 s. The plant tissue was then removed, and the sheet air-dried for 20 min before shaking in blocking buffer (50 g litre^{-1} defatted milk powder in TBS [10 mM Tris-HCl, 9 g litre^{-1} NaCl, pH 7.4]) for 1 h at 37°C. The blot was shaken for 1 h at 37°C in virus antibody diluted in blocking buffer that contained sap from virus-free tomato leaves (1 g tissue/5 ml buffer). After three 5-min rinses in TBS, the blot was exposed for 1 h at 37°C to anti-rabbit or anti-mouse globulin/alkaline phosphatase conjugate (Sigma) at 1:2000 in blocking buffer, rinsed in TBS and treated with substrate (5-bromo-4-chloroindolyl phosphate plus nitroblue tetrazolium [Sigma] made up as directed by the supplier) in darkness at 37°C without shaking. Colour development was stopped by rinsing three times in 10 mM Tris, 10 mM EDTA, adjusted to pH 8.0. The blots were air-dried and examined with a binocular microscope.

Tests for ITmLCV used rabbit polyclonal antiserum or mouse MAbs raised against ICMV, whereas tests for TYLCV-Sen used rabbit polyclonal antiserum or mouse MAbs raised against ACMV. The antisera were diluted 1:1000 and the MAbs were used at 1:3 dilution of tissue culture supernatant fluid.

Results

Virus accumulation in Lycopersicon accessions

In the tests on accessions of wild *Lycopersicon* spp., none of the three virus isolates produced symptoms in LA1777 or LA1969. Slight vein-yellowing and leaf curling developed in one test in which CMV-INRA plants were inoculated with TYLCV-Sen but no symptoms were observed in other experiments with any of the viruses. Only faint or dubious symptoms developed in plants of LA1478 inoculated with ITmLCV or TYLCV-Sen (TYLCV-Sar was not inoculated). In contrast, all three viruses induced severe symptoms in Moneymaker. In TY20, obvious but less severe symptoms appeared; these decreased in intensity with increasing time of infection.

Table 1 shows the results of serological assays at intervals after inoculation. The figures are directly comparable within columns but are only roughly comparable within rows because of uncontrolled differences in the sensitivity of TAS-ELISA conducted on different occasions. Inspection of the figures for individual plants revealed considerable plant to plant variation. In addition, virus was not detected in successive tests on a few plants of TY20 and LA1478, and on several of CMV-INRA, probably because the grafts had failed to unite properly and the plants were not infected. These plants were excluded from the calculation of the mean values given in Table 1.

Compared with Moneymaker, the plant lines fell into four categories on the basis of virus antigen content. Despite having only faint or no symptoms, LA1478 contained as much antigen as Moneymaker, regardless of virus isolate, and is therefore tolerant of infection. TY20 contained somewhat less ITmLCV and, in some tests, TYLCV-Sar than Moneymaker,

Table 1. *Virus content of* Lycopersicon *accessions at intervals after graft–inoculation with ITmLCV, TYLCV-Sar or TYLCV-Sen*

Plant line	ITmLCV			TYLCV-Sar			TYLCV-Sen		
	4 wk*	6 wk	9 wk	5 wk	7 wk	10 wk	2 wk	11 wk	14 wk
L. esculentum Moneymaker	0.55†	0.61	0.23	1.85	2.11	2.31	1.01	1.47	0.42
L. esculentum F1 TY20	0.52	0.28	0.13	1.38§	2.21	1.80	1.10	1.77	0.26
L. pimpinellifolium LA1478	0.75	0.78	0.34	—	—	—	1.06	1.27	0.58
L. hirsutum LA1777	0.11§	0.11§	0.02	0§	2.12	1.52	0.04§	0.65§	0.11§
L. peruvianum CMV-INRA	0.11§	0.15§	0.05	0.12§	1.71	2.12	0.17§	0.62§	0.12§
L. chilense LA1969	0.03§	0.01§	0.02	0.03§	0.06§	—	0.05§	0.13§	0.02§

* Period after inoculation.
† Mean $A_{405\,nm}$ for 4–12 plants.
§ Difference from Moneymaker significant at $P = 0.05$.

Table 2. *Virus content of tomato varieties at intervals after graft–inoculation with ITmLCV, TYLCV-Sar or TYLCV-Sen*

Plant line*	ITmLCV		TYLCV-Sar			TYLCV-Sen		
	6 wk†	8 wk	2 wk	6 wk	10 wk	2 wk	6 wk	8 wk
Moneymaker	1.41‡	2.24	2.35	2.33	1.69	1.89	1.75	2.30
F1 Fiona	0.52§	0.65§	0.70§	1.44§	0.27§	1.76	0.75§	1.55§
F1 Top 21	0.75§	1.87	0.94§	2.29	0.71§	1.27	2.44	2.28
F1 TY20	0.51§	0.99§	1.39§	1.85	0.77§	1.98	1.94	2.31
F1 Tyger	0.63§	1.78	1.01§	1.76	0.86§	1.84	1.42	2.01
F1 Tyking	0.41§	0.68§	0.73§	0.98§	0.20§	1.44	0.85§	1.04§
CMV-INRA	0.17§	0.37§	0.32§	1.05§	0.09§	1.58	1.22	2.02

* CMV-INRA is *L. peruvianum*; all other lines are *L. esculentum*.
† Period after inoculation.
‡ Mean $A_{405\,nm}$ for 4–6 plants.
§ Difference from Moneymaker significant at $P = 0.05$.

but there was no difference in concentration of TYLCV-Sen. It has a degree of infection tolerance together with somewhat decreased accumulation of two of the three viruses tested. LA1777 and CMV-INRA are more resistant than LA1478 or TY20. They developed few or no symptoms and had relatively low concentrations of ITmLCV and TYLCV-Sen. The decrease in concentration was much smaller for TYLCV-Sar, although virus accumulation was delayed compared to that in Moneymaker. LA1969 had very low concentrations of all three viruses. Indeed, most of the ELISA figures for individual plants fell within the range of experimental error for virus-free Moneymaker. To ascertain whether LA1969 was infected, scions from several plants inoculated with ITmLCV or TYLCV-Sen were grafted to Moneymaker plants, which were tested by ELISA a few weeks later. The viruses were detected in almost all the plants tested. LA1969 is therefore highly resistant to all three virus isolates. No symptoms developed and virus accumulation remained undetectable or barely detectable by ELISA. Comparisons with values for dilution curves of extracts from Moneymaker (not shown) indicated that LA1969 contained <1% of the virus found in the susceptible cultivar.

Virus accumulation in improved tomato varieties

In general, symptoms in the improved tomato cultivars were mild or dubious. Table 2 summarises the results of the serological tests. The F1 hybrids fell into two groups. Tyking and Fiona had the lowest concentrations of each of the viruses, probably about 5–10% of the concentrations reached in Moneymaker. TY20, Top 21 and Tyger had intermediate concentrations of ITmLCV and TYLCV-Sar. However, TYLCV-Sen reached as great concentrations in TY20 and Top 21 as in Moneymaker. ITmLCV and TYLCV-Sar accumulated at lower levels in CMV-INRA than in Tyking or Fiona but there was a small opposite effect at 6 wk and 8 wk after inoculation with TYLCV-Sen.

To ascertain whether the differences in concentration of virus antigen depended on the method of virus inoculation, a small-scale test was done in which plants of Moneymaker, Tyking (the most resistant F1 hybrid) and CMV-INRA were inoculated as small seedlings by *B. tabaci*. As with graft-inoculated plants, the concentration reached by TYLCV-Sen in Tyking was only 2-5% of that in Moneymaker, and lower than the concentration in CMV-INRA (Table 3). These effects therefore seem to be independent of mode of inoculation and of plant age at inoculation.

Virus distribution in tissues

Initial experiments established that ITmLCV could be detected with MAbs SCR52 or SCR60 but the best results were obtained with ICMV polyclonal antibody. TYLCV-Sen

Table 3. *TAS-ELISA of TYLCV-Sen in whitefly-inoculated tomato seedlings*

	Infected		Not infected	
Plant line*	1/50	1/500	1/50	1/500
L. esculentum Moneymaker (5)	2.10†	2.09	0.01	0.04
L. esculentum Tyking (5)	1.41	0.52	—	—
L. peruvianum CMV-INRA (11)	1.94	0.97	0.02	0.06

* Plants were tested 4 wk after inoculation. Figures in parentheses are the number of plants tested.

† Mean $A_{405\,nm}$ after deducting mean value for buffer control.

was detected with ACMV polyclonal antibody or MAb SCR20, but SCR23 gave the strongest staining. ICMV polyclonal antibody and SCR23 were therefore used routinely in further tests, which showed that purple spots associated with phloem tissue could be readily seen in imprints of sections of stems infected with ITmLCV (Fig. 1) or TYLCV-Sen but not in imprints of virus-free stems. Up to 100 spots/stem imprint could be seen, distributed somewhat erratically among vascular bundles and occurring in both internal and external phloem. Other tissues were not stained. The viruses could be detected throughout infected plants. For example, in graft-inoculated plants, TYLCV-Sen was detected in main stems (29.7 spots/imprint), upper stems (6.0), young stems (3.0), old petioles (3.0) and petiolules
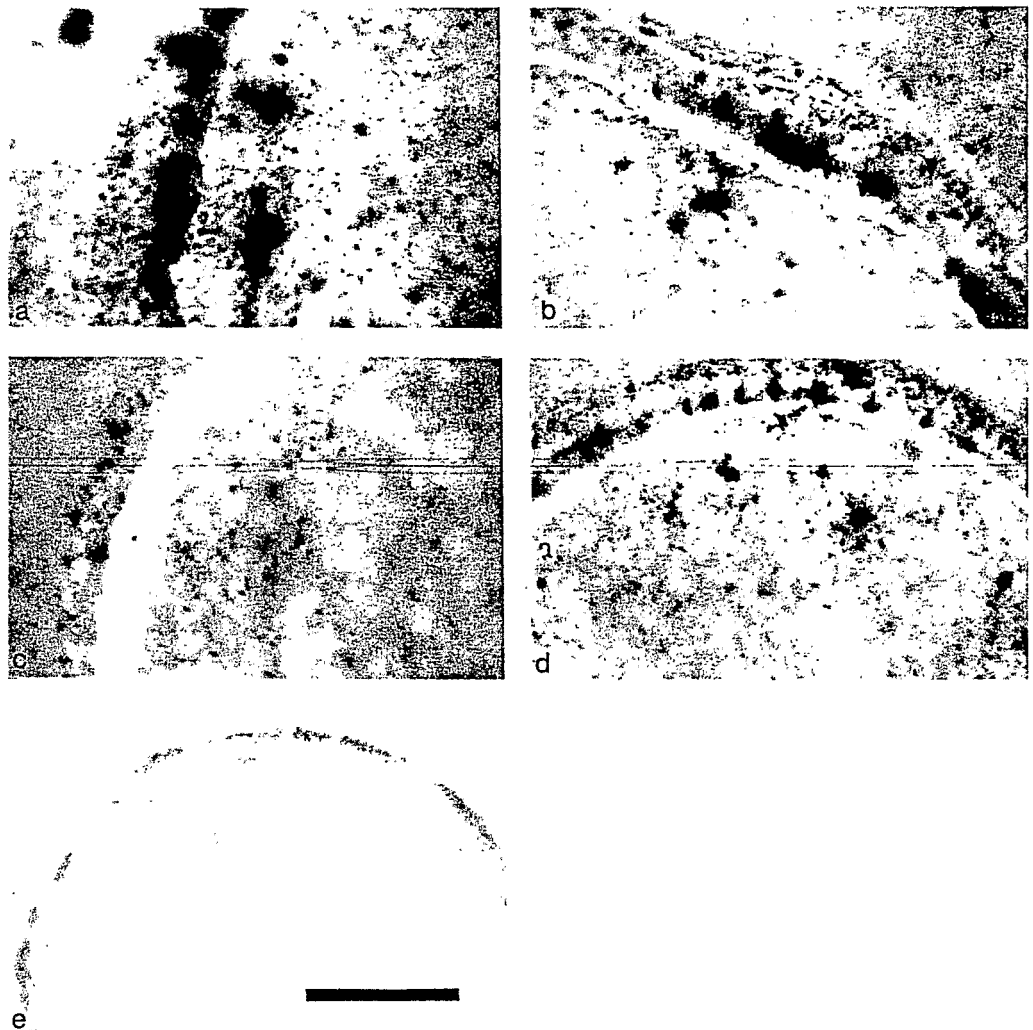

Fig. 1. Serological detection of ITmLCV in sections of tomato stems by tissue printing. The first antibody was rabbit polyclonal antiserum to ICMV and the second antibody was goat anti-rabbit globulin/alkaline phosphatase conjugate. Bar represents 1 mm in all prints. Prints are from sections of (*a*) and (*b*) infected stems of cv. Moneymaker, (*c*) and (*d*) infected stems of cv. Tyking, and (*e*) virus-free stem of cv. Moneymaker.

Table 4. *Distribution of ITmLCV and TYLCV-Sen in stem sections of tomato varieties Moneymaker and Tyking as determined by tissue printing*

Experiment no. (wk after inoculation)	Virus	cv. Tyking				cv. Moneymaker			
		No. imprints examined	External phloem	Internal phloem	Spots/ imprint	No. imprints examined	External phloem	Internal phloem	Spots/ imprint
1 (3)	ITmLCV	12	174*	124	24.5	15	310	291	40.0
	Control	5	0	0	0	5	0	0	0
2 (5)	ITmLCV	9	110	29	15.4	9	352	294	71.8
	Control	4	0	0	0	4	0	0	0
3 (3)	ITmLCV	15	40	57	6.5	11	245	188	39.4
	Control	5	0	0	0	5	0	0	0
4 (3)	ITmLCV	10	24	17	4.1	15	170	234	26.9
	Control	5	0	0	0	5	0	0	0
Total	ITmLCV	46	348	227	12.5	50	1077	1007	41.7
5	TYLCV-Sen	8	5	9	1.8	8	13	82	11.9
	Control	4	0	0	0	4	0	0	0
6 (7)	TYLCV-Sen†	10	57	139	19.6	10	233	290	52.3
	Control	5	0	0	0	5	0	0	0
Total	TYLCV-Sen	18	62	148	11.7	18	246	372	34.3

* Total number of stained spots.
† Plants were inoculated by whiteflies; all other inoculations were by grafting.

(4.6). In another test on the stem of a 14 cm-tall plant inoculated with ITmLCV by *B. tabaci* when a young seedling, imprints taken at 2 cm intervals from the top to bottom had 3, 11, 17, 35, 43, 20 and 30 spots, respectively. In subsequent tests, the middle part of the stem was sampled, using stems of similar diameter.

A series of experiments was made to compare the number and distribution of stained spots in imprints of stems of Moneymaker and Tyking infected with ITmLCV or TYLCV-Sen. Imprints of Moneymaker contained more numerous spots than those of Tyking, with the spots obtained with Tyking tending to be smaller than those obtained with Moneymaker (Fig. 1). Little difference was found in the relative numbers of spots in the internal and external phloem of the two varieties (Table 4). The difference between Moneymaker and Tyking was independent of the virus isolate, time after inoculation (3–7 wk) and mode of inoculation (grafting or whiteflies).

Concurrent tests by TAS-ELISA on extracts of stem sections infected with TYLCV-Sen indicated that the concentration of virus antigen in Moneymaker was 20- to 50-fold greater than that in Tyking. However, the numbers of stained spots in stem imprints differed only about 3-fold. This suggests that the somewhat larger spots in imprints of Moneymaker stems must each represent substantially more virus antigen than the smaller spots in those of Tyking.

Discussion

Previous work has shown that the leaf-curl resistance of different wild *Lycopersicon* accessions is under a variety of types of genetic control, ranging from five recessive genes to one dominant major gene. Our results show that the resistances of these accessions are expressed in different ways: tolerance of infection with little (LA1478) or substantial (LA1777, CMV-INRA) diminution in virus accumulation, or symptomless infection associated with the presence of vanishingly small amounts of virus (LA1969). In contrast, the resistance expressed by all the F1 hybrids was of the same type but differed in degree, ranging from a decrease in symptom severity and virus content only slightly inferior to that seen in LA1777 and CMV-INRA (Tyking, Fiona) to the noticeably weaker resistance found in TY20, Top 21 and Tyger.

Our resistance rankings based on serological assays appear to reflect the relative field performance of the same plant lines. For example, the ranking of the wild *Lycopersicon* accessions based on symptoms and presence of viral DNA in plants in a field trial in Israel (Zakay *et al.*, 1991) was similar to our ranking. Also, in field trials of F1 hybrids in Cuba (Gomez, 1995) and Martinique (Langlais, 1995), Tyking and Fiona were highly resistant to yellow leaf curl whereas Tyger was less resistant. This parallelism in rankings suggests that the results of serological assays on graft-inoculated plants can provide a short cut in assessing virus resistance, by their apparent usefulness for predicting field performance without doing field trials. Moreover, the tests could be done on plants grown in glasshouses outside the area affected and at any time of year.

Tissue printing gave valuable information, which complemented that provided by ELISA. The detail revealed was remarkably great and allowed individual vascular traces to be discerned. However, although many fewer antigen-containing traces were found in stems of Tyking than in Moneymaker, the difference was smaller than expected from the results of ELISA on extracts of stem tissue. Assuming that the efficiency of extraction of viral antigen from tissues of the two cultivars was the same, the disparity could be explained by a difference in number of infected phloem cells per vascular bundle or in amount of virus antigen per infected cell. The observation that the stained spots in tissue imprints tended

to be smaller for Tyking than for Moneymaker would be consistent with either or both of these possibilities. In Tyking, both the decrease in number of virus-containing phloem traces and a decrease in number of infected cells per trace could result from resistance expressed as an impaired ability of the virus to spread through phloem tissue. The more extreme type of resistance controlled by the *Ty-1* gene from *L. chilense* LA1969 is likewise characterised by impaired spread of TYLCV (Israeli form) through the plant: delayed and decreased accumulation of viral DNA in whitefly-inoculated leaves, and slow, low-level accumulation in systemically infected leaves (Michelson, Zamir & Czosnek, 1994). The type of resistance expressed in Tyking also occurs in lines of potato carrying genetic resistance to another phloem-limited virus, potato leafroll luteovirus (PLRV; Barker & Harrison, 1986). In such lines, PLRV antigen accumulates to only about 5% of the concentration reached in susceptible cultivars, and infected plants are much poorer sources of virus for transmission by aphids in laboratory tests or field conditions than are plants of susceptible cultivars (Barker & Harrison, 1986; Barker & Woodford, 1992). This resistance to PLRV is associated with a much larger decrease in number of infected cells in the external phloem than in the internal phloem, an effect that was not seen in geminivirus-infected Tyking tomato. External phloem is probably the main source of virus for vector aphids and whiteflies and so, although tomato plants with low virus contents are expected to be inferior sources of geminiviruses for vector whiteflies, they may not be as inferior as plants of PLRV-resistant potato lines are for aphids.

Good progress is clearly being made in breeding leaf curl-resistant tomato cultivars, although plant lines with enhanced resistance are badly needed. Also, it is not yet known how durable the currently available forms of resistance will prove to be when the resistant cultivars are exposed to variant forms of virus that may appear in naturally occurring virus populations over a period of years. Some encouragement can be taken from the fact that some forms of resistance were expressed to all three of the geminiviruses we tested, although exceptions were noted with other forms. Experience with a comparable situation in cassava also encourages optimism. In this species, the mosaic disease resistance originally selected in coastal East Africa, where East African cassava mosaic geminivirus occurs, and which is expressed as decreased virus accumulation and impaired virus spread within individual plants, is also effective against ACMV and has proved to be durable (Hahn, Terry & Leuschner, 1980; Jennings, 1994). However, the durability of leaf curl-resistance in tomato can only be established by experience. For the present, the best strategy is probably to combine different types of resistance, such as those found in the best F1 hybrid cultivars and in LA1969.

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