Inheritance of Resistance to Two-spotted Spider Mite and Glandular Leaf Trichomes in Wild Tomato *Lycopersicon pimpinellifolium* (Jusl.) Mill.

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ABSTRACT. Genetics of resistance to *Tetranychus urticae* Koch and of glandular trichomes of *Lycopersicon pimpinellifolium* accession TO-937 in a cross between susceptible *L. esculentum* Mill. ‘MoneyMaker’ and resistant TO-937 was studied in a greenhouse experiment. Parents, F₁, F₂, and two BC₁ generations, interspersed with susceptible tomato plants to avoid negative interplot interference, were artificially infested. Mite susceptibility was evaluated by a rating based on plant capacity to support mite reproduction. TO-937, BC₁ to TO-937, and F₁ were resistant, ‘MoneyMaker’ susceptible, and the F₂ and the BC₁ to ‘MoneyMaker’ segregated. Resistance was controlled by a single dominant major locus, but modulated by unknown minor loci. TO-937 presented type IV glandular trichomes, their presence governed by two dominant unlinked loci. Type IV trichome density correlated to resistance; however, a causal relationship between type IV trichomes and mite resistance could not be definitively established. The relatively simple inheritance mode will favor successful introgression of resistance into commercial tomatoes from the close relative *L. pimpinellifolium*.

The two-spotted spider mite (*Tetranychus urticae* Koch) causes severe yield loss in tomato crops in temperate regions both in open air and in greenhouse cultivation due to a large capacity for population increase (Berlinger, 1986). Control of mite populations in greenhouse tomatoes needs environmentally undesirable intensive pesticide application because biological methods based on predators (especially *Phytoseiulus persimilis* Athias-Henriot) are only partially successful in tomato (Nihoul and Van Impe, 1992). Predator entrapment in exudate from tomato glandular trichomes (Nihoul, 1994; Van Haren et al., 1987) accounts for a low efficiency of two-spotted spider mite control although a slightly improved control capacity of *P. persimilis* has been reported when reared on tomato plants (Drukker et al., 1997). Breeding for resistant cultivars is considered to be the most attractive alternative strategy for crop protection against pests and diseases (Johnson, 1992) provided that durable genetic resistance is available within the crop. Unfortunately, there is no described tomato cultivar fully resistant to *T. urticae*, although resistance to spider mites has been reported among accessions of the wild, green-fruited tomato species *Lycopersicon pennellii* (Corr.) D’Arcy (Gentile et al., 1969) and *L. hirsutum* Humb. & Bonpl. (Gentile et al., 1969; Rodriguez et al., 1972; Snyder and Carter, 1984). *L. pennellii* and *L. hirsutum* are rather distant relatives of *L. esculentum* Mill., the cultivated tomato

species, but still are cross-compatible with the crop species (Rick, 1979). A novel source of resistance to *T. urticae* was found in the wild tomato *L. pimpinellifolium* accession TO-937 (Fernández-Muñoz et al., 2000). Introgression of the resistance from *L. pimpinellifolium* into *L. esculentum* would be easier and faster than from *L. pennellii* or *L. hirsutum* because *L. pimpinellifolium* is a red-fruited, self-compatible species, closely related to the cultivated tomato (Rick, 1979). Knowledge of the inheritance of resistance in crosses with cultivated tomato could help optimize the breeding of resistance into commercial tomato. In that previous study, resistance was completely dominant in a cross of TO-937 with a susceptible *L. esculentum* cultivar. However, we observed an overestimation of resistant plants (Fernández-Muñoz et al., 2000). This could be explained by negative interplot interference (James et al., 1973) due to the high frequency of resistant genotypes in the experiment.

Stems and leaves of plants of *Lycopersicon* species are densely covered by different types of trichomes (Luckwill, 1943) some of which are glandular. Exudates from glandular trichomes have been related to arthropod pest resistance (Levin, 1973) and to specifically spider mite resistance (Aina et al., 1972). Reported natural insecticides/acaricides of *Lycopersicon* species are: the volatile and toxic methyl ketones 2-tridecanone (Chatzivasilieiadis and Sabelis, 1997; Williams et al., 1980) and 2-undecanone (Farrar and Kennedy, 1987) from *L. hirsutum* var. *glabratum*; the volatile and repellent sesquiterpene family of compounds from *L. hirsutum* var. *hirsutum* (Guo et al., 1993; Weston et al., 1989); and the toxic and possibly sticky acyl sugars from *L. pennellii* (Walters and Steffens, 1990).

This work describes results of experiments with a family of generations from a cross *L. esculentum* (susceptible) × *L. pimpinellifolium* (resistant) to study: 1) the genetics of TO-937

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resistance to *T. urticae* using an experimental design planned to overcome interplot interference; 2) the differences in presence and density of glandular and nonglandular leaf trichomes between the resistant and the susceptible parents and their inheritance; and 3) the possible relationships of leaf trichomes with resistance.

Materials and Methods

PLANT MATERIALS AND GREENHOUSE DESIGN. A six-generation genetic family from a *L. esculentum* × *L. pimpinellifolium* interspecific cross was obtained. The E.E. La Mayora-CSIC germplasm bank accession TO-937, an inbred line derived from four consecutive selfings of *L. pimpinellifolium* material collected by our group at 50 m altitude on the coastal plain of Lambayeque Department, Peru in 1983, was the spider-mite resistant parent. *L. esculentum* 'Moneymaker' was the susceptible parent. Both parents have indeterminate growth habit. Crosses between the two parents produced the interspecific F₁ hybrid. Twenty F₁ plants were then intercrossed to obtain the F₂ generation and they were backcrossed to 20 plants each of the two parents to obtain the backcross 'Moneymaker' × ('Moneymaker' × TO-937) [BC_{1ESC}] and the backcross ('Moneymaker' × TO-937) × TO-937 [BC_{1PIM}]. An experiment to evaluate resistance to *T. urticae* was conducted in a polyethylene greenhouse in 1999. Seeds of 'Moneymaker', TO-937, F₁, F₂, BC_{1ESC}, and BC_{1PIM} were sown in a nursery glasshouse in mid-March. Six weeks later they were transplanted into a polyethylene greenhouse in soil (22% clay, 24% silt, 54% sand). Plants were spaced 0.5 m apart within the rows with 1 m between rows. To prevent the risk of escapes or of overestimation of plant resistance (Fernández-Muñoz et al., 2000), half of the plants in the experiment were test plants and half were susceptible 'Moneymaker' plants (auxiliary plants). Test plants were placed every other position along the rows and auxiliary plants were situated between them. There were 125 F₂, 37 BC_{1PIM}, 38 BC_{1ESC}, 16 TO-937, 17 F₁, and 17 'Moneymaker' test plants and 250 auxiliary plants. They were watered as needed to maintain proper soil moisture and were fertilized every other week with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Plants were trained to one stem and no pesticide treatment was applied. At the end of this experiment, two BC_{1ESC} plants were selected for their mite resistance and served to obtain two BC_{2ESC} families by using their pollen to pollinate 'Moneymaker' plants. Resulting seeds were sown and nine and 14 plants of these BC_{2ESC} families were grown in 16-L pots in a heated greenhouse from October to December 1999 and evaluated for resistance to *T. urticae*.

SPIDER MITE INFESTATIONS AND RESISTANCE EVALUATIONS. Initial controlled infestations were made to all the plants in the greenhouse six weeks after transplanting, when plants were at 10 to 15 leaf stage. A leaf of French bean (*Phaseolus vulgaris* L.) highly webbed and infested with more than 20 living *T. urticae* adult individuals and many nymphs, larvae, and eggs, was placed between the stem and the plastic guide used to train the plant at ca. 30 cm from the apex. When the French bean leaf wilted, spider mites moved onto the tomato plants. A second infestation was done two weeks later. Evaluations of the degree of spider mite attack on test and auxiliary plants started 6 weeks after the first infestations. Mean maximum/minimum daily temperatures and relative humidities from infestation to evaluation dates were 31.5 ± 3.1 °C/19.0 ± 1.8 °C and 79% ± 7%/35% ± 11%, respectively, in the spring-summer experiment and 26.0 ± 2.9 °C/18.5 ± 2.2 °C and 80% ± 6%/61% ± 8% in the fall experiment, respectively. These conditions are not limiting for *T. urticae* (Berlinger, 1986), and mites could complete at least three

consecutive life cycles from infestation to evaluation dates. Inspection with a magnifying glass allowed evaluation of damage symptoms, the number of mites, and the presence of webbing. These evaluations were made independently on all the leaves at four canopy heights of the tomato plants: the basal, the medium-basal, the medium-apical, and the apical quarters. Resistance of each plant was scored as a mite susceptibility rating (MSR) ranging from 1 to 6 (Table 1).

LEAF TRICHOME OBSERVATIONS. Counts of leaf trichomes were initiated three weeks after the first infestations. Only abundant, short trichomes [type IV, V, and VI after Luckwill's (1943) classification] were taken into account because sparse and/or long trichomes are not likely to interfere with spider mites. Density of trichomes is known to be affected by leaf age (because of leaf expansion) and environmental conditions (Nihoul, 1993; Wilkens et al., 1996). To help reduce interference from these effects, all trichome counts were made within one week on leaflets at the same position and developmental stage. Because we were primarily interested in the resistance response of younger canopies, trichome counts were made on young, still expanding leaves. The first pair (starting from terminal position) of opposite leaflets of the third leaf below the apex of each test plant was excised and carried to the laboratory in small petri dishes lined with moistened filter paper to prevent desiccation, with one leaflet placed abaxial surface down and, the other, adaxial surface down. Adaxial surface trichomes were counted on the former leaflet and abaxial surface trichomes on the latter. Counts to calculate density of type IV, V, and VI trichomes/mm² were made using a dissection microscope (40× magnification) over four random sample areas of 1 mm². To maintain homogeneity, only leaflet lamina was considered, as veins were seen to be more hairy.

DATA ANALYSIS. MSR frequency distributions of the segregating generations were tested for normality by the Kolmogorov-Smirnov Z statistic (SPSS, 1999) and considerations about the shape of the distributions were made based upon the coefficient of bimodality $b = (m_3^2 + 1) / [m_4 + (3(n-1)^2) / ((n-2)(n-3))]$, where m_3 = skewness, m_4 = kurtosis, and n = number of observations (SAS Institute Inc., 1989). The mean values of density of each trichome type of the six generations and their standard errors were used to estimate gene effects by the methods described by Mather and Jinks (1982). In the cases in which one of the parents completely lacked a particular type of trichome, an arbitrary standard error of 0.1 was assigned to the mean of that parent to allow calculation of gene effects. The midparent value m together with additive [d] and dominance [h] gene effects were estimated. The adequacy of such mdh additive-dominance model was tested by ABC joint scaling test. For those cases in which any of the scaling tests was significant, the following models of gene effects including additive × additive [i], additive × dominance [j], and dominance × dominance [l] terms of digenic epistatic interactions were then calculated: $mdhi$, $mdhj$, $mdhl$, $mdhij$, and $mdhil$. No degree of freedom is left in the six-parameter $mdhijl$ model and therefore it was not calculated. Finally, the best-fit model was chosen as that in which expected means deviated least from the observed means of the generations as indicated by a higher probability associated with the corresponding χ^2 test. Correlations between the densities of the various trichome types, and between MSR and trichome densities, were calculated by use of Pearson's r product-moment coefficient; relationships between MSR and trichome densities were studied by multiple regression analysis, method STEPWISE (SPSS, 1999) with the following criteria: F test with $\alpha \leq 0.05$ for a variable to enter and $\alpha \geq 0.10$ to exit the model; and the contrast of differences in trichome densities of resistant vs. susceptible F₂ and BC_{1ESC} plants were tested by one-way ANOVA (SPSS, 1999).

Table 1. Assignment of mite susceptibility ratings (MSRs) to tomato plants depending on their levels of *T. urticae* infestation on leaves at four canopy heights.

MSR	Basal leaves	Medium-basal leaves	Medium-apical leaves	Apical leaves
1	None or a few individuals		No spider mites present	
2	Isolated individuals or small groups of mites, light leaf damage but with no visible webbing		One or a few individuals	
3	Grouped individuals, clearly visible leaf damage, light webbing		One or a few individuals	
4	Grouped individuals, clearly visible leaf damage, light webbing		Small groups of mites, light webbing	
5	Large populations, severe leaf damage, intense webbing but still restricted to each leaf			
6	Large populations, severe leaf damage, intense webbing joining several leaves together			

Results and discussion

RESISTANCE TO *T. urticae*. Mites developed well in the greenhouse, allowing us to start the evaluation of resistance six weeks after initial infestations. All ‘Moneymaker’ test plants had severe mite damage, with MSR of 5 to 6 (Fig. 1). ‘Moneymaker’ auxiliary plants exhibited a similar damage (data not shown), indicating a sufficiently high mite population throughout the greenhouse. Plants of the resistant accession TO-937 showed MSR of 1 to 3, indicating that apical and medium-apical leaves were free of mite reproduction, reflected by complete absence of webbing at these two canopy heights. Dominant inheritance of the resistance was evident because all F_1 and BC_{1PIM} plants behaved like those of TO-937. The BC_{1ESC} and the F_2 segregated widely for the character, with MSR ranging from 1 to 6. If a multiple gene model controlling MSR were assumed, frequencies of MSR in the BC_{1ESC} (Fig. 1) should follow normal distribution. However, significant deviations from normality were observed ($P \leq 0.01$). Deviations from normality were also observed in the F_2 but this was expected because of dominance for the character. Interestingly, both distributions proved to be bimodal ($b = 0.773$ for the F_2 and $b = 0.583$ for the BC_{1ESC} frequency distributions, $P \leq 0.05$). This type of distribution provided evidence that the resistance to *T. urticae* of TO-937 could be a trait controlled

by one major locus. Then, assuming resistance as a qualitative trait, F_2 and BC_{1ESC} plants that had behaved like the TO-937 plants (MSR of 1 to 3) were scored resistant and those plants with a response similar to ‘Moneymaker’ (MSR of 5 to 6) were scored susceptible. Plants with a MSR of 4 were considered intermediate. So, there were 92 resistant, 3 intermediate, and 28 susceptible F_2 plants; and 16 resistant, 2 intermediate, and 17 susceptible BC_{1ESC} plants. Those ratios fit the 3 : 1 and 1 : 1 ratios expected if resistance were controlled by a single dominant gene, regardless of how the intermediate plants were classified (Table 2). The data for the two BC_{2ESC} families from two BC_{1ESC} resistant plants also supported a single-locus model. About two thirds of F_2 resistant plants had a MSR ≤ 3 , with most of these having a MSR of 1 (highly resistant). Most of BC_{1ESC} plants having a MSR ≤ 3 suffered light spider mite attacks on older leaves (MSR of 2 to 3) (Fig. 1). Of the 12 resistant plants in the two BC_{2ESC} families, none had a MSR of 1, only one had a MSR of 2, and 11 had a MSR of 3.

In the present study, a few plants of *L. pimpinellifolium* TO-937, the F_1 , and the BC_{1PIM} showed low numbers of mites present on older leaves (Fig. 1). At the time of evaluation of resistance, plants

Fig. 1. Frequency distributions of MSR in the six generations of the ‘Moneymaker’ x TO-937 family.

Table 2. Chi-square tests and observed and expected ratios of R (resistant) to S (susceptible) mite susceptibility ratings based on a single dominant gene model for control of resistance to *T. urticae* for F₂, BC₁ESC, and BC₂ESC generations of the cross *L. esculentum* 'MoneyMaker' x *L. pimpinellifolium* TO-937.

Generation (R : S hypothesis)	Observed		Expected		$\chi^2_{(1 \text{ df})}$	P
	R	S	R	S		
F ₂ (3:1)						
Intermediate plants pooled with resistant	95	28	92.25	30.75	0.328	0.567
Intermediate plants pooled with susceptible	92	31	92.25	30.75	0.003	0.958
Intermediate plants removed	92	28	90	30	0.178	0.673
BC ₁ ESC (1:1)						
Intermediate plants pooled with resistant	18	17	17.5	17.5	0.029	0.866
Intermediate plants pooled with susceptible	16	19	17.5	17.5	0.257	0.612
Intermediate plants removed	16	17	16.5	16.5	0.030	0.862
BC ₂ ESC (1:1)						
Family 1	6	3	4.5	4.5	1.000	0.317
Family 2	6	8	7	7	0.286	0.593
Families 1 and 2 pooled	12	11	11.5	11.5	0.043	0.835

typically contained red fruits in the basal canopy, mature green fruits in the medium-basal canopy, open flowers and recently set fruit in the medium-apical canopy, and flowering trusses in the apical canopy. Then, plants scored with a MSR of ≤ 3 were able to maintain canopies that were actively growing completely free of spider mites. Low mite populations on the older canopies could be explained by presence of mites on the adjacent auxiliary susceptible plants; in a previous experiment in which TO-937 plants were grown alone in a greenhouse, in a similar way to that of commercial tomatoes, no spider mites were seen at time of evaluations (Fernández-Muñoz et al., 2000). Hence, *L. pimpinellifolium* TO-937 can probably be considered completely resistant to *T. urticae* in spite of the low levels of mite infestation observed in this experiment.

In a previous study (Fernández-Muñoz et al., 2000), resistance to

T. urticae from TO-937 appeared to be governed by two to four loci. In the present study, negative interplot interference, one of the components of the 'representational error' defined by Van der Plank (1963), was avoided by using an experimental design with susceptible auxiliary plants. The resistance (measured as the plant capacity to arrest mite reproduction on young and mature-young leaves) was controlled by a single major locus. Nevertheless, the low number of plants in the BC₁ESC and BC₂ESC that were completely free of spider mites was evidence that other minor loci, whose effects could be more marked in *L. esculentum*-like genetic backgrounds, may be also involved. These observations pointed to a tendency to reduction in the resistance of older leaves when introducing the resistance gene into *L. esculentum* genetic background. Breeding for commercial tomatoes with resistance level similar to that of TO-937 should

Table 3. Number of plants where type IV, V, and VI trichomes were present or absent, and mean of trichome density (number of trichomes per square millimeter) on leaflets of six generations of the cross *L. esculentum* 'MoneyMaker' x *L. pimpinellifolium* TO-937.

Trichome type and generation	Adaxial surface			Abaxial surface		
	Present	Absent	Mean density (±SE)	Present	Absent	Mean density (±SE)
Type IV trichomes						
TO-937	13	0	8.3 ± 1.5	13	0	16.3 ± 1.4
BC ₁ to TO-937	37	0	7.7 ± 0.6	37	0	17.9 ± 0.8
F ₁	15	0	7.0 ± 1.4	15	0	15.4 ± 2.1
F ₂	100	25	3.5 ± 0.4	121	4	10.7 ± 0.6
BC ₁ to MoneyMaker	13	24	0.5 ± 0.1	24	13	3.5 ± 0.8
MoneyMaker	0	15	0.0 ± 0.0	0	15	0.0 ± 0.0
Type V trichomes						
TO-937	0	13	0.0 ± 0.0	0	13	0.0 ± 0.0
BC ₁ to TO-937	8	29	1.1 ± 0.4	5	32	0.4 ± 0.2
F ₁	2	13	7.2 ± 1.2	10	5	7.6 ± 1.8
F ₂	18	107	6.8 ± 0.4	83	42	6.4 ± 0.6
BC ₁ to MoneyMaker	37	0	10.2 ± 0.4	37	0	16.3 ± 0.8
MoneyMaker	15	0	11.1 ± 1.0	15	0	17.3 ± 1.2
Type VI trichomes						
TO-937	13	0	5.9 ± 0.8	13	0	3.3 ± 0.5
BC ₁ to TO-937	37	0	4.6 ± 0.3	37	0	2.6 ± 0.2
F ₁	15	0	8.1 ± 0.7	15	0	3.4 ± 0.2
F ₂	125	0	5.2 ± 0.2	125	0	2.5 ± 0.1
BC ₁ to MoneyMaker	37	0	6.6 ± 0.5	37	0	3.0 ± 0.2
MoneyMaker	15	0	6.5 ± 0.5	15	0	3.5 ± 0.2

Table 4. Estimates of gene effects ($\pm$ SE), scaling tests ($\pm$ SE), and chi-square test of best-fit model adjustment for the densities of leaf trichomes (number of trichomes per mm²) from a six-generation *L. esculentum* 'Moneymaker' $\times$ *L. pimpinellifolium* TO-937 family.

Mean components and scaling tests ^c	Type IV ^y		Type V ^x		Type VI ^x	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
<i>m</i>	0.53 $\pm$ 1.40	8.17 $\pm$ 0.68	5.53 $\pm$ 0.25	4.00 $\pm$ 2.01	6.21 $\pm$ 0.48	1.75 $\pm$ 0.28
[<i>d</i>]	3.93 $\pm$ 0.70	8.17 $\pm$ 0.68	5.53 $\pm$ 0.25	8.84 $\pm$ 0.58	0.30 $\pm$ 0.48	0.23 $\pm$ 0.19
[<i>h</i>]	5.71 $\pm$ 2.38	3.03 $\pm$ 3.36	1.20 $\pm$ 0.64	5.53 $\pm$ 3.28	-5.68 $\pm$ 1.70	1.56 $\pm$ 0.47
[<i>i</i>]	3.40 $\pm$ 1.46	---	---	4.84 $\pm$ 2.05	---	1.48 $\pm$ 0.35
[<i>j</i>]	7.22 $\pm$ 1.54	12.55 $\pm$ 2.55	7.20 $\pm$ 1.31	12.68 $\pm$ 1.70	3.00 $\pm$ 1.39	---
[<i>l</i>]	---	4.15 $\pm$ 4.73	---	---	7.60 $\pm$ 1.85	---
<i>A</i>	0.15 $\pm$ 2.34	4.17 $\pm$ 2.94	2.17 $\pm$ 1.53	7.76 $\pm$ 2.70	-1.35 $\pm$ 1.27	-0.86 $\pm$ 0.49
<i>B</i>	-6.09 $\pm$ 1.40	-8.38 $\pm$ 2.60	-4.98 $\pm$ 1.45	-6.76 $\pm$ 1.86	-4.90 $\pm$ 1.25	-1.47 $\pm$ 0.66
<i>C</i>	-8.31 $\pm$ 3.42	-4.08 $\pm$ 5.12	1.53 $\pm$ 2.84	-6.94 $\pm$ 4.43	-8.08 $\pm$ 1.88	-3.32 $\pm$ 0.76
χ^2	0.712 (1 df)	0.001 (1 df)	5.181 (2 df)	2.597 (1 df)	2.119 (1 df)	1.327 (2 df)
<i>P</i>	0.399	0.970	0.070	0.107	0.145	0.515

^z*m* = mid-parent value; [*d*] = additive effects; [*h*] = dominance effects; [*i*] = additive $\times$ additive effects; [*j*] = additive $\times$ dominance effects; [*l*] = dominance $\times$ dominance effects; *A*, *B*, *C* = scaling tests.

^y*P*₁ = TO-937, *P*₂ = 'Moneymaker'.

^x*P*₁ = 'Moneymaker', *P*₂ = TO-937.

not rely exclusively on a direct backcrossing-and-selection program because not all of these hypothetical minor loci have to be dominantly inherited to the resistant parent.

From the above comments, it seems clear that the use of auxiliary susceptible plants within the tests helped to reduce the overestimation of resistance derived from negative interplot interference, but led to a certain degree of underestimation of resistance due to positive interplot interference. Nevertheless, we still recommend this design because for selection work, it is more proficient at avoiding escapes.

Resistance to *T. urticae* was evaluated, based on the capacity of plants to constrain reproduction of *T. urticae* during a rather long time period, instead of other less time consuming methods that are related to plant repellency to the mites such as the thumbtack assay (Weston and Snyder, 1990). However, long-term greenhouse experiments where no pesticide is applied risk losing plants to attacks by other pests or viruses transmitted by insects. Therefore, simple methods to evaluate reproduction-based resistance like the one developed by Balkema-Boomstra et al. (1999) are desirable for selection work.

LEAF TRICHOMES INHERITANCE. In addition to very tall trichomes, leaves of *L. esculentum* 'Moneymaker' plants were densely covered with nonglandular, single-cell based, 0.10 to 0.20 mm long, trichomes [type V after Luckwill (1943)]; less abundant, four-cell glandular headed, single-cell based, $\approx$ 0.10 mm long, trichomes [type VI after Luckwill (1943), more precisely type VIa following Channarayappa et al. (1992) classification]; and sparse, multi-cell

glandular headed, very small (<0.05 mm long) type VII trichomes. *L. pimpinellifolium* TO-937 leaves also were pubescent and had similar type VI glandular trichomes; no type VII glandular trichomes were seen on TO-937. An unexpected, outstanding observation was that TO-937 plants showed no type V leaf trichomes. Instead, single-cell based, 0.08 to 0.15 mm long, trichomes had a small glandular vesicle at the tip. They were very similar in appearance and size to the type IV glandular trichomes described and drawn by Luckwill (1943) for *L. hirsutum* and also named type IV by Channarayappa et al. (1992) for *L. hirsutum* and *L. pennellii*. Therefore, in this paper we have classified these trichomes as type IV. Among the *Lycopersicon* species, only *L. hirsutum* and *L. pennellii* are known to possess type IV glandular trichomes. In this study we make the first report of the presence of type IV trichomes in another *Lycopersicon* species, *L. pimpinellifolium*. This is not an exception in this species, because in later observations made on *L. pimpinellifolium* accessions from La Mayora Experimental Station germplasm bank, three out of seven additional accessions presented such trichomes (data not shown). Survey of extent of this trait within *L. pimpinellifolium* natural populations by examining wider germplasm resources would be most interesting not only for breeding purposes but also for biosystematic studies to elucidate if the presence of type IV trichomes in *L. pimpinellifolium* originates from occasional natural interspecific hybridizations with *L. hirsutum* or *L. pennellii*.

Table 3 shows data for type IV, V, and VI leaf trichome densities on adaxial and abaxial leaf surfaces of parents and progenies. Type

Table 5. Coefficients of correlation between MSR and the adaxial and abaxial density of type IV, V, and VI trichomes for the F₂ (below diagonal) and the BC₁ to 'Moneymaker' (above diagonal) generations of a *L. esculentum* 'Moneymaker' $\times$ *L. pimpinellifolium* TO-937 family.

Coefficient	MSR	Type IV adaxial	Type V adaxial	Type VI adaxial	Type IV abaxial	Type V abaxial	Type VI abaxial
MSR		-0.443**	-0.199	-0.075	-0.554**	0.538**	-0.214
Type IV adaxial	-0.402**		-0.306	0.045	0.775**	-0.679**	0.065
Type V adaxial	0.298**	-0.738**		0.444**	-0.006	0.350*	0.338*
Type VI adaxial	-0.016	0.015	0.071		0.295	0.149	0.594**
Type IV abaxial	-0.479**	0.651**	-0.441**	0.190*		-0.705**	0.239
Type V abaxial	0.471**	-0.663**	0.634**	-0.053	-0.847**		0.129
Type VI abaxial	0.011	0.169	-0.070	0.412**	0.153	-0.077	

***Significant at $P \leq 0.05$ or 0.01, respectively.

Fig. 2. Frequency distributions of the density of type IV trichomes in susceptible and resistant plants within the two generations that segregated for mite resistance in the *L. esculentum* 'MoneyMaker' $\times$ *L. pimpinellifolium* TO-937 family. When a class of trichome density is represented by an interval, the interval does not include the value of its lower limit.

VI glandular trichomes were seen on all plants in this study. Regarding the generations of the family, the most noteworthy observation was that all the plants within those generations that were completely resistant to *T. urticae* (the parent TO-937, the BC₁PIM, and the F₁) also possessed type IV trichomes. Calculation of the number of genes that control qualitative traits such as presence of type IV and V trichomes was jeopardized because for certain generations the ratios of presence : absence were different for adaxial and abaxial surfaces (Table 3). For type IV trichomes, the ratio of presence : absence for adaxial and abaxial surfaces were the same in the parents, the F₁, and the BC₁PIM. However, in the F₂ a 100 : 25 ratio of presence : absence for adaxial surface and a 121 : 4 ratio for abaxial surface and in the BC₁ESC a 13 : 24 ratio for the adaxial surface and a 24 : 13 ratio for the abaxial surface were observed. A few TO-937 had less than one adaxial type IV trichome/mm². This could lead to mistaken classification of plants of the generations into the two categories of presence : absence and then qualitative trait genetic analyses could not be properly applied to this character. For abaxial type IV trichomes this problem was less evident and, with the observed presence : absence ratios, a model with two independent loci with dominance for presence of type IV trichomes fitted the expected 15 : 1 for the F₂ ($\chi^2_{(1\text{ df})} = 1.985$, $P \leq 0.159$) and 3 : 1 for the BC₁ESC ($\chi^2_{(1\text{ df})} = 2.027$, $P \leq 0.155$). The model with three loci was equally valid for F₂ as data deviated little from the expected 63 : 1 ratio ($\chi^2_{(1\text{ df})} = 2.179$, $P \leq 0.140$) but it was very inadequate to fit the expected 7 : 1 BC₁ ratio ($\chi^2_{(1\text{ df})} = 17.330$, $P \leq 0.001$). In two wild *Solanum* species (Gibson, 1979) and in

another Solanaceae species, *Datura wrightii* Regel (Van Dam et al., 1999), a single gene is responsible for the inheritance of presence or absence of type IV trichomes. In this study, two dominant unlinked loci were necessary to explain BC₁ and F₂ segregation for type IV trichome presence, which has been also found for a *L. esculentum* $\times$ *L. pennellii* family (Lemke and Mutschler, 1984). Inheritance of presence of type IV trichomes in crosses *L. esculentum* $\times$ *L. hirsutum* follows a different pattern because dominance for nonpresence has been found (Snyder and Carter, 1985). Reliable calculation of number of loci involved in expression of the other trichomes that differed between parents, type V trichomes, was not possible as the response of F₁ plants was not clear; five out of the 15 F₁ plants showed no type V trichome.

In most cases, wide ranges of trichome densities were observed in the generations (Table 3). Abaxial type IV and V densities tended to be higher than adaxial densities, and the opposite was seen for type VI trichomes. Adaxial and abaxial type IV mean trichome densities showed a similar trend, with the BC₁PIM and the F₁ very close to the *L. pimpinellifolium* parent, the F₂ intermediate, and the BC₁ESC closer to the *L. esculentum* parent. For both adaxial and abaxial type V

trichomes, the BC₁ESC was similar to the *L. esculentum* parent, both the F₁ and the F₂ were intermediate, and the BC₁PIM was very close to the *L. pimpinellifolium* parent. No significant difference was seen for type VI trichome densities, except a slight positive heterosis in the F₁ for adaxial density.

Adjustment of generation mean data to quantitative trait genetic models in which deviations from expected were not significant was possible for densities of the three types of trichomes on both leaflet surfaces (Table 4). In no case did ABC scaling tests reveal that trichome densities followed an additive–dominance model. Therefore, epistatic components were necessary to explain the observed generation means for the three types of trichomes on both leaflet surfaces. The best-fit models including these epistatic components are given in Table 4. Mean components of the generations for density of adaxial and abaxial type IV trichomes differed in that dominance effects were more important than additive effects for the adaxial surface while the opposite was observed for the abaxial surface. Regarding epistatic effects, the additive $\times$ dominance interaction component was highly significant, which explained the great similarity in trichome density of both BC₁ generations to their respective recurrent parents. For type V trichomes on both leaflet surfaces, additive effects were more important than dominance effects (which were not significant) and, like type IV trichomes, the close similarity between backcrosses and their recurrent parents was also explained by high additive $\times$ dominance effects. Differences in means of F₁ hybrid generations when the parents do not differ can be only explained by epistatic gene action and, in the case of adaxial type VI trichomes, strong dominance $\times$ dominance effects can account for the observed positive heterosis in the F₁. In the genetics of type IV trichome density in a *L. esculentum* $\times$ *L. pennellii* family, strong dominance $\times$ dominance epistatic effects were detected (Lemke and Mutschler, 1984). Similarly, for the *L. esculentum* $\times$

L. pimpinellifolium family in our study, the inheritance of type IV trichome density could be explained only if an epistatic interaction was considered, in this case, additive $\times$ dominance effects that made backcrosses closer to their recurrent parents. This implies that successful breeding for high densities of type IV trichomes by a backcrossing program would be unlikely unless a more time-consuming scheme of sib-mating backcross progenies followed by selection were applied. Genetics of presence of type V trichomes could not be studied, but the negative correlation between type IV and type V trichomes densities (see below) is evidence that these two trichome types could share genetical control such as observed for *D. wrightii* (Van Dam et al., 1999).

RELATIONSHIP BETWEEN MITE RESISTANCE AND LEAF TRICHOMES. Susceptibility to *T. urticae* significantly correlated to density of type IV and V trichomes (except for adaxial type V density in the BC₁ESC) but not to type VI density (Table 5). Abaxial densities in general correlated to MSR better than adaxial. Correlations of type IV trichomes with MSR were negative, indicating positive correlation with resistance. Adaxial density of a trichome type significantly correlated to abaxial density of the same trichome type. Densities of type V trichomes were negatively correlated to densities of type IV trichomes, and the highest coefficient of correlation ($r = -0.847$) was that between abaxial densities of these two trichome types in the F₂. Densities of type VI trichomes only correlated to densities of type VI on the opposite surface.

To know what particular trichome type (and on what leaflet surface) or combinations of trichome types were important to predict the levels of mite susceptibility, an analysis by stepwise multiple regression of MSR on the six trichome density variables was conducted for each of the two generations that segregated for the character. In both analyses, the only variable that met the requirements for entry into the model was abaxial type IV trichomes density (for the F₂, $P \leq 0.001$, corrected $R^2 = 0.221$; for the BC₁ESC, $P \leq 0.001$, corrected $R^2 = 0.286$). So, the abaxial type IV trichomes variable alone could statistically explain the ratings of susceptibility to *T. urticae*. However, this could be a statistical artifact in part. In fact, when only resistant plants ($MSR \leq 3$) were included in analyses, correlation between MSR and density of abaxial type IV trichome was no longer significant; this was more evident for the F₂ ($r = -0.089$, $P \leq 0.462$, $n = 71$) than for the BC₁ESC ($r = -0.408$, $P \leq 0.117$, $n = 16$). Therefore, density of type IV trichomes was apparently valid to predict if a plant was resistant or susceptible, but the level of resistance of a resistant plant could not be inferred from its density of type IV trichomes. Hence, frequency distributions for density of these trichomes were plotted separately for resistant and susceptible plants (Fig. 2). The maximum trichome densities always corresponded to resistant plants and, in general, resistant plants were more hairy with regard to type IV trichomes; calculation of one-way ANOVA showed that resistant plants had significantly higher type IV trichome densities than susceptible ones for adaxial ($MS_{R \text{ vs. } S} = 398.6$, $df = 1$; $MS_e = 25.5$, $df = 117$; $P \leq 0.001$) and abaxial ($MS_{R \text{ vs. } S} = 2008.0$, $df = 1$; $MS_e = 73.1$, $df = 117$; $P \leq 0.001$) densities in the F₂, and for adaxial ($MS_{R \text{ vs. } S} = 6.0$, $df = 1$; $MS_e = 0.82$, $df = 31$; $P \leq 0.011$) and abaxial ($MS_{R \text{ vs. } S} = 367.7$, $df = 1$; $MS_e = 26.8$, $df = 31$; $P \leq 0.001$) densities in the BC₁ESC. Nevertheless, distributions of type IV trichome densities of susceptible and resistant plants overlapped between susceptible and resistant plants and there were F₂ and BC₁ESC resistant plants with no or very few type IV trichomes (Fig. 2).

Part of the data analyses in this present study seem to demonstrate that presence and density of type IV trichomes are important for the resistance of plants: significant correlations of trichome densities with MSR, the multiple regression analysis, and the ANOVA

testing of differences in trichome densities between resistant and susceptible plants. Similar evidences have been also observed on *L. hirsutum* accessions and in *L. esculentum* $\times$ *L. hirsutum* hybrids, in which variations in type IV trichome density were responsible for most variation in spider mite resistance (Carter and Snyder, 1985, 1986; Good and Snyder, 1988). However, type IV trichomes cannot be the sole source of resistance, such as can be inferred from Fig. 2, because several plants with a relatively high density of type IV trichomes were susceptible and a number of resistant plants did not present type IV trichomes on their leaves. Similarly, one mite-resistant plant that completely lacked type IV trichomes also has been observed in a segregating generation from a *L. esculentum* $\times$ *L. hirsutum* cross (Snyder and Carter, 1984). Then, the observed relationship between resistance and type IV trichomes may be attributable simply to chance as these two characters come from the same parent and are dominantly inherited and, thereby, are difficult to separate in early segregating generations. A complementary hypothesis would be that type IV trichomes could modulate expression of the resistance gene. For example, if such a major gene were responsible for the production of any substance deleterious for mites that was exuded by glandular trichomes, the observed correlation between resistance and density of these trichomes would be satisfactorily explained. This last hypothesis is fully compatible with the appearance of susceptible F₂ plants with high density of type IV trichomes but it seems not to match with the observation of resistant plants with no or very low densities of type IV trichomes. Nevertheless, all plants in this study presented type VI trichomes and there is no reason to assume that different types of glandular trichomes in the same individual plant exudate completely different chemical compounds. So, plants could manifest resistance in spite of lack of type IV trichomes. Carter and Snyder (1985) observed that in *L. hirsutum* and in *L. esculentum* $\times$ *L. hirsutum* hybrids, density of type VI trichomes correlated to mite resistance when density of type IV trichomes was low. Future development of advanced inbred lines derived from the 'Moneymaker' $\times$ TO-937 cross will make clear whether or not mite resistance from this accession is a consequence of type IV glandular trichomes.

Conclusions and Future Prospects

L. pimpinellifolium TO-937 shows complete resistance to *T. urticae* in the greenhouse. Resistance is dominant and governed by a single major locus whose effect seems to be modulated by minor genes. Evidence that there is action of the newly reported type IV glandular trichomes of *L. pimpinellifolium* on *T. urticae* resistance has been provided. Whether this action has a chemical or a mechanical (or both) basis will be clarified in further investigations, especially when advanced inbred lines are obtained. Hypothetical minor genes modulating resistance and epistatic gene effects on type IV trichome density can reduce efficiency of introgression of strong resistance into commercial cultivars. Moreover, greenhouse resistance is difficult to evaluate and reproducibility along the breeding process may be poor. Hence, long-term, difficult recurrent or sib-mating backcrossing schedules seem to be necessary. Resistance to spider mites based on glandular trichomes may diminish the possibility of biological control of the pest by using mite predators because these are also very negatively affected by trichome exudates (Nihoul, 1994; Van Haren et al., 1987). Therefore, the obtained breeding lines may need to be completely resistant to be useful. Nevertheless, the taxonomic proximity of red-fruited *L. pimpinellifolium* and *L. esculentum* tomato species and the nonexcessively complex inheritance mode of resistance found

makes TO-937 a very promising resistance source for genetic resistance to an arthropod pest available in cultivated tomato.

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