



A novel source of resistance to the two-spotted spider mite in *Lycopersicon pimpinellifolium* (Jusl.) Mill.: its genetics as affected by interplot interference

Rafael Fernández-Muñoz, Eva Domínguez & Jesús Cuartero
Estación Experimental La Mayora – CSIC, 29750 Algarrobo-Costa, Málaga, Spain

Received 1 February 1999; accepted 10 July 1999

Key words: interplot interference, *Lycopersicon esculentum*, *Lycopersicon pennellii*, *Lycopersicon pimpinellifolium*, spider mites, *Tetranychus urticae*, tomato

Summary

We have previously found an accession of *Lycopersicon pimpinellifolium* (Jusl.) Mill. ('TO-937') that appeared to resist attack by the two-spotted spider mite (*Tetranychus urticae* Koch). *L. pimpinellifolium* is a very close relative of the cultivated tomato (*Lycopersicon esculentum* Mill.) and thereby a potential source of desirable traits that could be introgressed to the crop species. The objective of this study was to investigate the genetics of the resistance present in 'TO-937'. Resistance to infestation by the spider mite was quantified in 24-plant plots of *L. pimpinellifolium* accessions 'TO-937' and 'PE-10', *L. pennellii* accession 'PE-45', *L. esculentum* cultivars 'Moneymaker', 'Roma' and 'Kalohi' (reported to be partially resistant: Stoner & Stringfellow, 1967), and the interspecific F₁ cross, *L. esculentum* 'Moneymaker' × *L. pimpinellifolium* 'TO-937'. Only 'TO-937', the F₁, and 'PE-45' were found to be resistant. Resistance of 'TO-937' was complete when evaluated in two small greenhouses completely planted with 'TO-937' so as to simulate the genotypic homogeneity usual in commercial crops. Generations (P₁, P₂, F₁, F₂, BC₁P₁, and BC₁P₂) of a P₁ (susceptible) × P₂ (resistant) cross ('Moneymaker' × 'TO-937') were studied for resistance in a single-plant per plot design. Resistance of 'TO-937' was inherited with complete dominance and appeared to be controlled by either two or four genes according to whether segregation in the F₂ or the BC₁P₁, respectively, were considered. However, calculation of the number of genes involved in the resistance was complicated by negative interplot interference due to the high frequency of resistant genotypes within most of the generations.

Introduction

A serious pest of tomato (*Lycopersicon esculentum* Mill.) in temperate regions is the two-spotted spider mite (*Tetranychus urticae* Koch). Its control under warm and relatively low humidity conditions is a severe problem because spider mites have an enormous capacity for population increase and yield loss is expected with only one third of the leaves damaged (Berlinger, 1986). Genetic resistance to spider mites is known among accessions of the wild tomato species *L. hirsutum* Humb. & Bonpl. and *L. pennellii* (Corr.) D'Arcy (Gentile et al., 1969). Although these two green-fruited species can be hybridized with the cul-

tivated tomato (Rick, 1979), they are distant relatives of *L. esculentum* and no resistant cultivar has yet been developed for commercial use. We recently observed an accession from *L. pimpinellifolium* (Jusl.) Mill. that appeared to resist attack by spider mites. This red-fruited, self-compatible species is a close relative of the cultivated tomato and so could be a better donor of resistance than either *L. hirsutum* or *L. pennellii*. Breeding for resistance to a pathogen must be based on knowledge of the genetics of the resistance. We will describe in this paper a new source of spider mite resistance from *L. pimpinellifolium* and the genetics of this resistance.

Genetical studies and breeding programmes for resistance to mobile organisms such as arthropod pests are difficult tasks because of the difficulty of maintaining controlled conditions of infestation (Stevens & Rick, 1986). The problem must then be solved by using experimental designs in which several small plots of each genotype are distributed in the experimental area. With this kind of design, however, quantification of resistance is often complicated by interplot interference, one of the components of the 'representational error' defined by Van der Plank (1963). This interference arises from the role of continuous reinoculation among adjacent plants in the development of pathogens with aerial dispersal (which is also applicable to mobile arthropods such as spider mites). Interplot interference leads to overestimation (negative interference) or underestimation (positive interference) of the actual resistance level of a cultivar in experimental designs in which small plots of the cultivar are surrounded by small plots of genotypes that are more resistant or more susceptible than that cultivar, respectively, (James et al., 1973). We estimated interplot interference in this genetic study of resistance to spider mite.

Experiments with segregating generations, in which the plants have different genotypes and thereby different resistance levels, are needed for genetic studies and selection processes. Nevertheless, the genetic heterogeneity in experiments with segregating generations is very different from the commercial situation typified by monoculture. It is therefore difficult to extrapolate from the design of genetic experiments to commercial situations, because of interplot interference. Therefore, we also evaluate the potential utility of the *L. pimpinellifolium* resistance in a design similar to a commercial crop.

Materials and methods

Three experiments were conducted in a polyethylene greenhouse during spring/summer in 1996, 1997, and 1998. Three genotypes (the susceptible cultivar 'MoneyMaker', the resistant *L. pimpinellifolium* accession 'TO-937' and the interspecific F₁ hybrid 'MoneyMaker' × 'TO-937') were evaluated for spider mite resistance in the 1996 experiment. In each plot, 28 plants of the same genotype were arranged in two 14-plant adjacent rows; the first and the last plant in each of the two rows were considered guard plants and thereby the experimental plots consisted of 24

plants. The three plots were situated along the same rows, with the F₁ plot between the parental plots. A similar design was followed in the 1997 experiment to evaluate resistance within 24-plant plots of the cultivars 'Roma' and 'Kalohi' (previously described as susceptible and partially resistant, respectively, to the two-spotted spider mite by Stoner & Stringfellow (1967)), the *L. pimpinellifolium* accession 'PE-10' and the *L. pennellii* accession 'PE-45'. In 1997 we also grew 'MoneyMaker' (P₁), 'TO-937' (P₂), the F₁ 'MoneyMaker' × 'TO-937', the F₂, the BC₁P₁ 'MoneyMaker' × ('MoneyMaker' × 'TO-937'), and the BC₁P₂ ('MoneyMaker' × 'TO-937') × 'TO-937'. They were evaluated for resistance using a design in 60 small complete blocks distributed along 12 double rows. Each block consisted of five F₂ plants in one row and one plant of the BC₁P₂, BC₁P₁, F₁, 'MoneyMaker', and 'TO-937' in the adjacent row. Because each plant was surrounded by plants with different genotypes, a single plant was considered to represent a plot. This design could overcome the potential effect of microclimate differences in the greenhouse on the final level of infestation. The effect of interplot interference over the advance of the spider mite could be estimated by the comparison of responses of the two parents and the F₁ with their results in the 24-plant per plot experiment in 1996. In order to evaluate the performance of 'TO-937' in commercial crops in which plants with the same level of resistance are grown together, two small polyethylene greenhouses were each planted with 600 'TO-937' plants in 1998.

We based our evaluation of resistance on the size of the mite population on each plant rather than other faster methods (such as the thumbtack bioassay; Weston & Snyder, 1990) that are based on evaluation of the repellence of excised leaves. Our measure reflected the capacity of the plant to arrest proliferation of the spider mite under commercial cultivation conditions. In the experiments, the controlled infestations were begun in late May, when most plants were at the 10–15 leaf stage, by putting on each plant a piece of French bean leaf highly netted and infested with, typically, above 20 living *T. urticae* adult individuals and many nymphs, larvae, and eggs. The leaf piece was situated at ca. 30 cm from the apex, between the main stem and the plastic guide used to train the plant. Spider mites moved onto the tomato plants as the leaf pieces of French bean wilted. A second infestation was made in late June using leaf pieces of eggplant with levels of mite infestation similar to those of the bean leaves used before. Evaluations of spider mite

Table 1. Numbers of plants of several tomato cultivars and accessions of wild relatives classified by their spider mite attack index in 24-plant per plot experiments

Genotypes	Species ^z	Index of <i>T. urticae</i> attack				
		0	1	2	3	4
1996 experiment						
Money maker	esc	0	0	0	0	24
Money maker × TO-937 F ₁	esc × pim	24	0	0	0	0
TO-937	pim	24	0	0	0	0
1997 experiment						
Roma	esc	0	0	0	0	24
Kalohi	esc	0	0	0	0	24
PE-10	pim	0	0	0	17	7
PE-45	pen	24	0	0	0	0

^z esc: *Lycopersicon esculentum*; pim: *L. pimpinellifolium*; pen: *L. pennellii*.

infestation were made at late July by visual observation of numbers of mites (with a magnifying glass) and of the characteristic symptoms of leaf damage. In the 1998 experiment, an additional evaluation was made two weeks after the second controlled infestation. The mean daily maximum/minimum temperatures from infestation to evaluation dates were $35.0 \pm 2.5/22.0 \pm 2.0^\circ\text{C}$ for the 1996 experiment, $34.0 \pm 3.0/19.5 \pm 2.0^\circ\text{C}$ for the 1997 experiment and $35.0 \pm 3.0/21.5 \pm 2.0^\circ\text{C}$ for the 1998 experiment. These temperatures were high enough to allow the spider mite to complete its life cycle and to reproduce (Berlinger, 1986). A Spider Mite Attack Index (SMAI) ranging from 0 to 4 was given to each plant: 0, no spider mite was seen; 1, one or few individuals; 2, several isolated individuals or small groups of mites, light leaf damage but without visible webbing; 3, mites present in groups together with leaf damage, light webbing; 4, large mite population, severe leaf damage and intense webbing. Those plants displaying SMAI of 0–1 were considered resistant (a few individuals on a plant could come from a highly infested neighbour plant), with SMAI of 2 intermediate, and with SMAI of 3–4 susceptible (mites not only surviving, but reproducing).

Results

All the plants of 'Money maker', 'Roma', the reportedly partially-resistant 'Kalohi' and, to a lesser degree, the *L. pimpinellifolium* accession 'PE-10' were severely attacked by the spider mites and consequently

considered susceptible in the 24-plant per plot experiments in 1996 and 1997 (Table 1). No spider mites were seen on the 24 plants of the *L. pimpinellifolium* accession 'TO-937', the F₁ 'Money maker' × 'TO-937', and the *L. pennellii* accession 'PE-45' at the time of evaluation (Table 1) and these were then considered resistant.

In the 'Money maker' × 'TO-937' family experiment, all the plants of 'TO-937', the BC₁P₂, and the F₁, 96% of the F₂ plants, and 93% of the BC₁P₁ plants performed as resistant (Table 2), confirming the completely dominant inheritance indicated by the 1996 experiment. Although 'Money maker' showed spider mite susceptibility, only 15% of the 'Money maker' plants had an SMAI of 4 and no sign of mite reproduction was seen on 34% of the plants (intermediate plants, SMAI of 2) (Table 2). With the single-plant per plot experimental design, and because of the dominance in the 'TO-937' × 'Money maker' family, most 'Money maker' plants were surrounded by resistant plants. Since all the 'Money maker' plants had an SMAI of 4 in the 24-plant per plot 1996 experiment (Table 1), a negative interplot interference leading to an over-estimation of the resistance of 34% could be calculated for this parent in the 1997 experiment. One susceptible 'Money maker' plant was included in each of the 60 blocks and this plant could produce positive interplot interference on the resistant genotypes. 'TO-937' and the F₁ had an SMAI of 0 for all the 24 plants in the 1996 experiment (Table 1) and showed 25 and 42%, respectively, of plants with an SMAI of 1 in the 1997 experiment (Table 2). Nevertheless, this positive interplot interference is almost negligible since all the 'TO-937' and F₁ plants could still be classified as resistant.

No susceptible plant was found in the BC₁P₁ and only one plant with an SMAI of 3 (0.3% from the total) appeared in the F₂ (Table 2). Nevertheless, susceptible genotypes in these segregating generations could be classed as intermediate due to negative interplot interference as occurred with the 'Money maker' plants. Hence, plants showing an SMAI of 2 or above were counted as susceptible for the estimation of the number of genes involved in the spider mite resistance. A ratio of 289:11 resistant:susceptible plants in the F₂ corresponded with an expected 15:1 ratio for a model with two dominant genes ($\chi^2 = 3.417$, $0.90 < p < 0.95$). This model was inadequate for the BC₁P₁ as data did not fit the 3:1 ratio expected. Instead, the 55 resistant:4 susceptible BC₁P₁ plants conformed very

Table 2. Numbers of plants in a family of generations of *L. esculentum* 'Money-maker' × *L. pimpinellifolium* 'TO-937' cross according to their spider mite attack index

Generations	Index of <i>T. urticae</i> attack				
	0	1	2	3	4
Money-maker P ₁	0	0	20	30	9
Money-maker × (Money-maker × TO-937) BC ₁ P ₁	32	23	4	0	0
(Money-maker × TO-937) F ₂	154	135	10	1	0
(Money-maker × TO-937) F ₁	34	25	0	0	0
(Money-maker × TO-937) × TO-937 BC ₁ P ₂	38	21	0	0	0
TO-937 P ₂	44	15	0	0	0

closely to the expected 15:1 ratio for a model with four dominant genes ($\chi^2 = 0.028$, $0.10 < p < 0.20$).

Two weeks after the second infestation in the 1998 experiment, 8% of the 'TO-937' plants showed levels of mite attack equivalent to an SMAI of 2, 13% equivalent to an SMAI of 1, and 79% plants were free of mites. However, no sign of spider mites living on the plants was evident at the time of evaluation.

Discussion

The conditions of artificial infestation in the experiment described ensured that the susceptible cultivars 'Money-maker' and 'Roma', together with the *L. pimpinellifolium* accession 'PE-10', were severely attacked by the two-spotted spider mite in the 24-plant plots. The cultivar 'Kalohi', described by Stoner & Stringfellow (1967) to be partially resistant to spider mite oviposition, feeding damage and defoliation, performed as susceptible in our experiments, however. *L. pennellii* 'PE-45' and *L. pimpinellifolium* 'TO-937' were completely resistant to *T. urticae* because no spider mite population was present on the plants after two months from the initial artificial infestation. Accessions from *L. pennellii* had been reported to be highly resistant to spider mites (Gentile et al., 1969) and thereby the resistance response seen in 'PE-45' was expected. To our knowledge, this is the first report of an *L. pimpinellifolium* accession resistant to mites. Plant defense against phytophagous arthropods is usually attributed to glandular trichomes (Levin, 1973). Abundant glandular trichomes were observed on the stems and leaves of resistant *L. pennellii* 'PE-45' and *L. pimpinellifolium* 'TO-937' whilst non-glandular trichomes predominated on the *L. pimpinellifolium*

accession 'PE-10' susceptible to the spider mite. However, the density of glandular trichomes on 'TO-937' plants did not seem to be greater than on plants of the susceptible cultivar 'Money-maker' (data not shown). Further studies are required to elucidate whether or not the 'TO-937' resistance comes from trichome secretion of repellent compounds such as methyl ketones and sesquiterpenes, known to play an important role in mite resistance of *L. hirsutum* accessions (Weston et al., 1989; Guo et al., 1993), or of acyl sugars, the natural insecticides present in *L. pennellii* (Walters & Steffens, 1990).

Negative interplot interference was observed in the 'Money-maker' × 'TO-937' family experiment resulting in an apparent enhancement of the spider mite resistance level of the susceptible genotypes. The more individuals with susceptible genotypes a generation contains, the stronger this interference is expected to be in experiments with single-plant per plot designs. This could explain why results from the F₂, whose average resistance is expected to be higher than that of the backcross to 'Money-maker', pointed to inheritance controlled by two genes, whilst the backcross to 'Money-maker' pointed to inheritance controlled by four genes. We may expect that not more than two resistance genes would be calculated if experimental designs to overcome interplot interference were to be used in future genetic studies. Nevertheless, it would be practically impossible to design comparisons of resistance to mobile pests among many cultivars or genotypes within segregating generations under conditions free of interplot interference. Several strategies to reduce the incidence of interference could be applied. Parlevliet & Van Ommereen (1984) proposed the inclusion in the experiments of a range of check genotypes with well-characterised levels of partial resistance.

However, such check genotypes are not always available nor always perform as reputed (as was the case with 'Kalohi' in this present study). In studies with segregating generations, it would be possible to obtain clones of each individual plant via *in vitro* culture, make one small plot for each clone, and evaluate only those few plants being completely surrounded by clones of the same genotype. Obtaining clones of each genotype would not be difficult but would be expensive and require a large amount of space. Consequently, this strategy would not be applicable to long-term breeding programmes but could be used in studies on the genetics of the resistance or in the precise evaluation of resistance level that would be required for locating molecular markers linked to resistance gene(s). Indirect selection with molecular markers, in which infestation is no longer necessary, would overcome interplot interference completely. Gonçalves et al. (1998) suggested that indirect selection could also be achieved using easily measured traits such as the content of methyl ketone 2-tridecanone (that is repellent to spider mites and is abundant in *L. hirsutum* f. *glabratum* accessions). When no indirect selection method is available, the segregating population must be evaluated for resistance and this could be achieved using an experimental design in which each plant of the segregating population was surrounded by plants of a susceptible cultivar. No negative interplot interference would then be expected and the potential appearance of positive interplot interference would be even of assistance to the selection process by reducing escapes.

In conclusion, *L. pimpinellifolium* 'TO-937' appears to be a very promising source of resistance to the two-spotted spider mite for breeding commercial resistant tomato cultivars because: (1) the level of resistance shown by 'TO-937' was high enough to arrest spider mite proliferation in monoculture greenhouse crops, typical of commercial practice; (2) *L. pimpinellifolium* is a red-fruited, self-compatible species which has better agronomic traits than other potential donors of resistance genes; (3) useful genes from this species may be quickly and easily introgressed because it is very closely related to the cultivated tomato species and no linkage drag would be expected; and (4) the inheritance of the resistance was completely dominant and this would facilitate introgression by backcrossing.

Acknowledgements

This work was partially funded by the Spanish CICYT Project No. AGF95-0023-CO2-01. We are indebted to Dr Anthony R. Yeo for critically reviewing of the manuscript.

References

- Berlinger, M.J., 1986. Pests. In: J.G. Atherton & J. Rudich (Eds), *The Tomato Crop: A Scientific Basis for Improvement*, pp. 391–441. Chapman & Hall, New York.
- Gentile, A.G., R.E. Webb & A.K. Stoner, 1969. *Lycopersicon* and *Solanum* spp. resistant to the carmine and the two-spotted spider mite. *J Econ Entomol* 68: 834–836.
- Gonçalves, M.I.F., W.R. Maluf, L.A.A. Gomes & L.V. Barbosa, 1998. Variation of 2-Tridecanone level in tomato plant leaflets and resistance to two mite species (*Tetranychus* sp.). *Euphytica* 104: 33–38.
- Guo, Z., P.A. Weston & J.C. Snyder, 1993. Repellency to two-spotted spider mite, *Tetranychus urticae* Koch, as related to leaf surface chemistry of *Lycopersicon hirsutum* accessions. *J Chem Ecol* 19: 2965–2979.
- James W.C., C.S. Shih, L.C. Callbeck & W.A. Hodgson, 1973. Interplot interference in field experiments with late blight of potato (*Phytophthora infestans*). *Phytopathology* 63: 1269–1275.
- Levin, D.A., 1973. The role of trichomes in plant defense. *Quart Rev Biol* 48: 3–15.
- Parlevliet, J.E. & A. Van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. *Euphytica* 33: 685–697.
- Rick, C.M., 1979. Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. In: J.G. Hawkes, R.N. Lester & A.D. Skelding (Eds), *The Biology and Taxonomy of the Solanaceae*, pp. 667–678. Academic Press, New York.
- Stevens, M.J. & C.M. Rick, 1986. Genetics and breeding. In: J.G. Atherton & J. Rudich (Eds), *The Tomato Crop: A Scientific Basis for Improvement*, pp. 35–109. Chapman & Hall, New York.
- Van der Plank, J.E., 1963. *Plant diseases: epidemics and control*. Academic Press, New York.
- Stoner, A.K. & T. Stringfellow, 1967. Resistance of tomato varieties to spider mites. *Proc Amer Soc Hort Sci* 90: 324–329.
- Walters, D.S. & J.C. Steffens, 1990. Branched chain amino acid metabolism in the biosynthesis of *Lycopersicon pennellii* glucose esters. *Plant Physiol* 93: 1544–1551.
- Weston, P.A., A.D. Johnson, H.T. Burton & J.C. Snyder, 1989. Trichome secretion composition, trichome densities, and spider mite resistance of ten accessions of *Lycopersicon hirsutum*. *J Amer Soc Horticult Sci* 114: 492–498.
- Weston, P.A. & J.C. Snyder, 1990. Thumbtack bioassay: a quick method for measuring plant resistance to two-spotted spider mites (Acari: Tetranychidae). *J Econ Entomol* 83: 501–504.

