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Resistance of 14 accessions/cultivars of *Lycopersicon* spp. to two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), in laboratory and greenhouse

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Abstract

Fourteen accessions and cultivars of *Lycopersicon* spp. were studied to evaluate their resistance to two-spotted spider mite, *Tetranychus urticae* Koch, under laboratory and greenhouse conditions. *In vitro* studies were carried out using thumbtack and leaf disk bioassays. *Lycopersicon hirsutum* and *L. pennellii* accessions supported more mites on the tack. The highest number of eggs (5.15 ± 0.48 eggs / ♀ / d) was recorded on *L. pimpinellifolium* LA2533 and the lowest number (0 egg / ♀ / d) was recorded on *L. hirsutum* and *L. pennellii* accessions. The highest mite mortality and lowest damage score occurred on the leaf disks of *L. pennellii* and *L. hirsutum* accessions that were strikingly in contrast to our observations on *L. esculentum* varieties (Nandi and Sankranthi). The developmental time of the mite was longest (8.61 ± 0.18 days) on leaf disks of *L. esculentum* NDTVR-73 and shortest (7.18 ± 0.51 days) on *L. pimpinellifolium* LA2533. In the greenhouse, the mite was unable to establish on *L. hirsutum* and *L. pennellii* accessions. Amongst the rest, Nandi and Sankranthi accessions/cultivars supported the highest, while NDTVR-73 supported the lowest mite population. The highest and lowest density of type VI glandular trichomes were recorded on *L. esculentum* NDTVR-73 (67.33 ± 6.34 trichomes/mm²) and *L. pennellii* LA2963 (0.79 ± 0.15 trichomes/mm²), respectively. No relation was found between density of type VI trichomes and resistance to the mite. Type IV glandular trichomes were observed only on the foliages of *L. hirsutum* and *L. pennellii*. A positive relation exists between the density of type IV glandular trichomes and resistance to the mite.

Key words: *Tetranychus urticae*, survival, oviposition, avoidance, tomato, glandular trichomes

چکیده

مقاومت چهارده رقم و لاین از گونه‌های مختلف جنس *Lycopersicon* نسبت به کنه‌ی تارتن دولکه‌ای، *Tetranychus urticae* Koch، در آزمایشگاه و گلخانه بررسی شد. مطالعات آزمایشگاهی بر اساس دو روش زیست‌سنجی پونز (thumbtack) و دیسک برگی (leaf disk) صورت گرفت. گونه‌های *Lycopersicon hirsutum* و *L. pennelii* بیش‌ترین تعداد کنه روی پونز را داشتند. بیش‌ترین تعداد تخم (۰/۴۸ ± ۵/۱۵ تخم بر ماده در روز) روی *L. pimpinellifolium* LA2533 و کم‌ترین تعداد تخم (صفر) روی گونه‌های *L. pennellii* و *L. hirsutum* مشاهده شد. مرگ و میر بالایی از کنه و خسارت پایینی روی دیسک‌های برگی *L. hirsutum* و *L. pennellii* دیده شد، درحالی‌که کم‌ترین مرگ و میر کنه و بالاترین خسارت روی دیسک‌های برگی گونه‌ی *L. esculentum* (ارقام Nandi و Sankranthi) مشاهده گردید. طولانی‌ترین دوره‌ی تکاملی (۰/۱۸ ± ۸/۶۱ روز) روی دیسک‌های برگی *L. esculentum* NDTVR-73 و کوتاه‌ترین دوره‌ی تکاملی (۰/۵۱ ± ۷/۱۸ روز) روی *L. pimpinellifolium* LA2533 به‌دست آمد. در شرایط گلخانه، آفت قادر به استقرار روی گونه‌های *L. pennellii* و *L. hirsutum* نبود. در بین دیگر ارقام و لاین‌های مورد مطالعه، ارقام Sankranthi و Nandi بیش‌ترین جمعیت کنه را داشتند، درحالی‌که لاین NDTVR-73 کم‌ترین میزان جمعیت و کم‌ترین میزان خسارت را داشت. بیش‌ترین و کم‌ترین تراکم کرک‌های غده‌ای نوع VI به‌ترتیب روی *L. esculentum* NDTVR-73 و *L. pennellii* LA2963 (۶/۳۴ ± ۶۷/۳۳ و ۰/۱۵ ± ۰/۷۹ کرک بر میلی‌متر مربع) شمارش گردید. ارتباطی بین تراکم کرک‌های غده‌ای نوع

VI و مقاومت به کنه‌ی تارتن وجود نداشت. کرک‌های غده‌ای نوع IV فقط روی گونه‌های *L. pennellii* و *L. hirsutum* مشاهده شدند. همبستگی معنی‌دار و مثبتی بین تراکم کرک‌های غده‌ای نوع IV و مقاومت به کنه‌ی تارتن وجود داشت.

واژگان کلیدی: *Tetranychus urticae*، بقا، تخم‌ریزی، اجتناب، گوجه‌فرنگی، کرک‌های غده‌ای

Introduction

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is an important pest of tomato plants, especially during hot and dry weather. Feeding of *T. urticae* occurs primarily on the leaf surface that affects the rate of leaf transpiration and photosynthesis. Intensity and duration of the mite feedings are negatively correlated with yield (Sances *et al.*, 1979). The short life span, high fecundity and development of resistance to many acaricides make chemical control of the pest particularly difficult. Therefore, a program of integrated pest management is essential for the control of TSSM (Luczynski *et al.*, 1990). A potential alternative method of control is host plant resistance, which may be mediated by glandular trichomes.

Wild tomato species, *Lycopersicon hirsutum* Humb & Bonpl and *L. pennellii* (Corr) D'Arcy, have been reported as the sources of resistance to many tomato pests (Carter *et al.*, 1989; Weston *et al.*, 1989; Eigenbrode & Thrumble, 1993; Simmons *et al.*, 2003). Resistance in these species has been attributed to both quantitative and qualitative aspects of glandular trichomes (Snyder & Carter, 1984). Sticky and toxic exudates of type IV and VI glandular trichomes of *Lycopersicon* spp. may entrap, irritate or kill the invader pest (Stoner & Gentile, 1968; Ania *et al.*, 1972; Simmons *et al.*, 2003). Many studies implicated trichome secretions as important resistance factors of wild tomatoes to arthropods. *Lycopersicon hirsutum* accessions were reported as resistant to *T. urticae* (Weston *et al.*, 1989) and insect pests such as *Spodoptera exigua* (Hübner) (Eigenbrode & Thrumble, 1993), *Leptinotarsa decemlineata* (Say) (Kennedy & Dimock, 1983; Carter *et al.*, 1989) and *Helicoverpa zea* (Boddie) (Farrar & Kennedy, 1987). Type VI glandular trichomes of *L. hirsutum* accessions secrete methyl ketones or sesquiterpenes which are toxic or repellent for several arthropod pests (Williams *et al.*, 1980; Weston *et al*., 1989). The resistance of *L. pennellii* accessions, to the pests is mostly related to the chemistry and density of type IV glandular trichomes that cover all parts of the plant. Some accessions of *L. pennellii* are known as the sources of resistance to *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) (Goffreda & Mustchler, 1989; Simmons *et al*., 2003), *H. zea* and *S. exigua* (Juvik *et al*., 1994), *Helicoverpa armigera* (Hübner) (Simmons *et al*., 2004), *Bemisia argentifolii* Bellows & Perring (Liedl *et al*., 1995) and *T.*

urticae (Resende *et al*., 2002; Saeidi & Mallik, 2006). Acylsugars are the major secretion of type IV glandular trichomes of *L. pennellii* (Goffreda & Mustchler, 1989; Juvik *et al*., 1994).

This study was intended to evaluate the survival, oviposition and avoidance strategy of TSSM on different hosts, as well as the response of different *Lycopersicon* species (damage score) to the mite and finally the role of type IV and VI glandular trichomes in resistance of *Lycopersicon* species to TSSM.

Materials and methods

Plant material and leaf sampling

The germplasms used in this study were four varieties (Sankranthi, Nandi, Vybhav and Pusa ruby) and one accession (NDTVR-73) of *Lycopersicon esculentum* Mill., two accessions of *L. pimpinellifolium* (LA0373 and LA2533), two accessions of *L. peruvianum* (LA643 and LA2152), three accessions of *L. hirsutum* (LA1740, LA1777 and LA2860) and two accessions of *L. pennellii* (LA2580 and LA2963). *Lycopersicon esculentum* varieties were obtained from Department of Genetic and Plant Breeding, UAS, Bangalore, Karnataka, India, and wild accessions provided by Nunhems ProAgro seeds, Pvt. Ltd., Bangalore, Karnataka, India. Seeds germinated in petri dishes at room temperature. Small seedlings were transferred to plastic trays (26 × 52 cm) with 50 cells containing sterilized vermicompost. After three weeks seedlings were transferred to 25 × 25 cm earthen pots with equal portion of vermicompost and soil and irrigation daily. Plants were placed in an insectarium enclosed with net under natural conditions of photo period, temperature and humidity. Fully expanded young tomato leaves (third leaves below the apical meristem) were used for *in vitro* studies.

Maintenance of the mite stock culture

The strain of TSSM used in this study originated from the infested tomato fields at Hebbal Campus, University of Agricultural Science, Bangalore, Karnataka, India in September 2003. Mite rearing was carried out on susceptible variety *L. esculentum* Sankranthi in the greenhouse (25 ± 5°C, 60 ± 10 RH). The individual mites used for the bioassays were collected and transferred on the plants using a fine painting brush.

Mite avoidance on different accessions/cultivars

Mite avoidance was measured using thumbtack bioassay with 10 replications according to Weston & Snyder (1990) method. Ten adult female mites were put on top of each thumbtack and after 2 h, the number of remaining mites was recorded, while the travelling distance on the leaf surface scored as 1 (less than 5 mm), 2 (between 5 to 10 mm) or 3 (beyond 10 mm) for each mite.

Mite oviposition, mite mortality and leaf damage score on the accessions/cultivars

Mite oviposition, mite mortality and leaf damage score were studied using leaf disk bioassay (Gimenez-Ferrer *et al*., 1993). Five leaf disks (2 cm in diameter) of each cultivar placed on a layer of absorbent cotton in a plastic petri dish (12 cm in diameter) together with five adult female mites (3-5 days old). Samples were kept in an incubator at 26 ± 1ºC, 60 ± 5% RH and a photoperiod of 16: 8 h (L: D). After 72 h, oviposition, mortality and the number of mites that moved into water were recorded on each disk. Leaf damage was calculated on the intensity of damage (Nihoul *et al*., 1991; Gimenez-Ferrer *et al*., 1993) after 96 h on a 0 to 6 scale as: no damage, feeding patches < 10%, 10-25%, 26-40%, 41-60%, 61-80% and 81-100% of leaf area, respectively.

Developmental time of TSSM on different accessions/cultivars

Five leaf disks (2 cm in diameter) of each accession or cultivar were placed on a layer of absorbent cotton in a plastic petri dish. Four adult female mites were transferred on each disk for a 12 h period and then removed, leaving 25 eggs on each disk. There was no egg on *L. hirsutum* and *L. pennellii* accessions. The eggs on the susceptible variety, *L. esculentum* Sankranthi, were moved and after 6-10 days the number of emerged adults recorded daily and their mean developmental time and percentage mortality of juveniles calculated for each accession or cultivar.

Population build-up of TSSM on the accessions and cultivars in the greenhouse

The seeds germinated at room temperature and small seedlings were placed into 250 ml plastic cups containing sterilized vermicompost and soil (1:1 ratio). The experiment was conducted at temperature 26 ± 2ºC, 55 ± 10% RH and a photoperiod of 16: 8 h (L: D). The light source was sunlight along with five condensed fluorescent lamps (750 Lux each) fixed one meter above the plant canopy.

The experiment was carried out in a completely randomized design with three replicates (each replicate contained 2 plants). The plant foliage was pruned to keep only two fully expanded leaves for 10 adult female mites (3-5 days old) on each plant. Population density for all developmental stages of the mite was recorded using a stereomicroscope. The damage on each plant was scored on a 0 to 6 scale (as described above).

Density and droplet size of type IV and VI glandular trichomes

The density of trichomes (number per mm²) was determined from three regions of abaxial and adaxial surfaces of leaflets and averaged for all three spots (Luczynski *et al*., 1990). Eight and six leaflets were examined for the density of type VI and IV glandular trichomes, respectively.

The droplet size of type IV and VI glandular trichomes of six resistant accessions was evaluated for *L. hirsutum* (LA1740, LA1777 and LA2860), *L. pennellii* (LA2963 and LA2580) and *L. esculentum* NDTVR-73 and the susceptible variety (*L. esculentum* Sankranthi). Four leaflets of each accession were sampled and thin cross sections were made to measure the droplet size of 20 trichomes using a phase microscope.

Statistical analysis

Data were analyzed by SAS software (SAS Institute, 1996). Analysis of variance (Proc ANOVA) revealed significant differences among the accessions. Mean comparison was done by Tukey's Studentized Range (HSD) Test. Kruskal Wallis test was used to compare the non-parametric data. Percentage’s data were normalized using Arcsin $\sqrt{x}$ formulas and correlation (Proc Corr) was used to describe the linear relation between variables.

Results

Mite avoidance

The remaining mites on the tack and their travelled distance differed significantly among the accessions/cultivars (ANOVA: $F_{13/98}$: 122.48, $P < 0.0001$). *Lycopersicon hirsutum* and *L. pennellii* accessions held higher number of mites on the tack whereas on the other species, all mites moved on to the leaf surface after 2 h. On this basis, accessions/cultivars were classified into four groups (table 1).

Mite oviposition and mortality

Mite oviposition was significantly different among the accessions/cultivars (ANOVA: $F_{13/98}$: 54.60, $P < 0.0001$). TSSM females did not lay egg or laid a few number of eggs on the leaf disks of *L. hirsutum* and *L. pennellii* accessions. The highest number of eggs was on leaf disks of *L. pimpinellifolium* LA2533, followed by *L. esculentum* (varieties Nandi and

Sankranthi). Among the *L. esculentum*, *L. pimpinellifolium* and *L. peruvianum* cultivars/ accessions, NDTVR-73 (an accession of *L. esculentum*) supported the least number of eggs and the highest mite mortality (table 1). Mortality on the leaf disks of *L. pennellii* and *L. hirsutum* accessions was very high (100%), suggesting the possibility of presence of factors which killed or entrapped TSSM.

Host response to the mite (damage score)

Kruskal Wallis test was significantly different among the accessions/cultivars (χ^2 = 98.62, df = 13, $P < 0.0001$). TSSM did not cause damage to the leaf disks of *L. hirsutum* and *L. pennellii* accessions. Damage to leaf disks of *L. esculentum* (accessions NDTVR-73 and cultivar Vybhav) was relatively low (mean damage score 2.12 and 2.37, respectively), but damage to the leaf disks of other cultivars was high (feeding patch more than 50% of leaf area) (table 1).

Developmental time and survival of TSSM on leaf disks of different accessions/cultivars

Mean developmental time of TSSM varied significantly on different cultivars/accessions (ANOVA: $F_{13/61}$: 578.57, $P < 0.0001$). On the leaf disks of *L. hirsutum* and *L. pennellii* accessions, no larvae completed their developmental stages (table 2). Among the other *Lycopersicon* species, developmental time of TSSM was the longest on leaf disks of NDTVR-73 (an accession of *L. esculentum*) and shortest on *L. pimpinellifolium* LA2533 followed by *L. esculentum* Sankranthi. The highest and lowest mortality (ANOVA: $F_{13/61}$: 7.89, $P < 0.0001$) occurred on *L. esculentum* NDTVR-73 and *L. esculentum* Nandi, respectively (table 2).

Greenhouse study

To confirm the results of *in vitro* bioassays, accessions/cultivars were studied in the greenhouse. The build up of TSSM population and increasing plant damage showed significant differences among the accessions/cultivars (ANOVA: egg, $F_{13/28}$: 46.30, $P <$ 0.0001; nymph, $F_{13/28}$: 29.15, $P < 0.0001$; adult, $F_{13/28}$: 57.29, $P < 0.0001$). On *L. hirsutum* and *L. pennellii* accessions, the mite was not successfully established. Among the *L. esculentum*, *L. pimpinellifolium* and *L. peruvianum* cultivars/accessions, Nandi and Sankranthi (from *L. esculentum*) accommodated the highest population (eggs, nymphs and adults) of TSSM and plant damage score, followed by *L. pimpinellifolium* LA2533. The lowest mite population and plant damage were recorded on NDTVR-73 followed by Vybhav (fig. 1).

Table 1. Mean (± SE) two-spotted spider mite response to the host (oviposition, mortality, number on the tack) and host response to the mite (leaf damage score) on 14 accessions/cultivars of *Lycopersicon*.

Lycopersicon species	Accession/cultivar	Leaf disk bioassay				Thumbtack bioassay	
		Eggs / female / day	Damage score *	Dead on leaf [1]	Dead in water [1]	No. on tack [2]	Distance traveled (score) *
L. hirsutum	LA2860	0.15± 0.082 gf	0 c	1.37 ± 0.18 b	2.62 ± 0.41 a	5.25 ± 1.11 b	2
	LA1777	0 g	0 c	5 ± 0 a	0 c	8.62 ± 0.37 a	1
	LA1740	0 g	0 c	5 ± 0 a	0 c	6.75 ± 0.45 b	1
L. pennellii	LA2963	0 g	0 c	5 ± 0 a	0 c	9.12 ± 0.29 a	1
	LA2580	0 g	0 c	4.75 ± 0.16 a	0.25 ± 0.16 c	8.5 ± 0.27 a	1
L. esculentum	Nandi	4.65 ± 0.33 ab	4.37 ± 0.18	0.12 ± 0.12 e	0 c	0 c	3
	Sankranthi	4.73 ± 0.32 ab	4 ± 0.26	0.25 ± 0.16 e	0 c	0 c	3
	Vybhav	2.24 ± 0.18 de	2.37 ± 0.18	1.12 ± 0.29 bcd	0.12 ± 0.10 c	0 c	3
	NDTVR-73	1.51 ± 0.11 ef	2.12 ± 0.22	1.25 ± 0.31 bc	1.12 ± 0.35 b	0 c	3
	Pusa ruby	3.51 ± 0.24 bcd	4.37 ± 0.18	0.12 ± 0.10 e	0 c	0 c	3
L. pimpinellifolium	LA2533	5.15 ± 0.48 a	4.62 ± 0.18	0 e	0 c	0 c	2.75
	LA0373	2.93 ± 0.24 cd	4 ± 0.26	0.5 ± 0.18 cde	0 c	0 c	2.75
L. peruvianum	LA643	3.81 ± 0.63 abc	4.12 ± 0.29	0.37 ± 0.18 de	0 c	0 c	3
	LA2152	4.64 ± 0.25 ab	4 ± 0.26	0.25 ± 0.16 e	0 c	0 c	3

Means with the same letter at each column are not significantly different at $P = 0.05$ using Tukey's Studentized Range (HSD) Test.

[1] number out of 5 mites.

[2] number out of 10 mites.

* Kruskal Wallis test showed significant difference among the treatments (damage score, $\chi^2 = 98.62$, df = 13, $P < 0.0001$; distance traveled, $\chi^2 = 58.62$, df = 13, $P < 0.0001$).

Table 2. Mean (± SE) developmental time and survival of two-spotted spider mite on the leaf disks of *Lycopersicon* accessions.

***Lycopersicon* species**	**Accession/cultivar**	**Mean developmental time**	**% Mortality**
L. hirsutum	LA2860	–	–
	LA1777	–	100 a
	LA1740	–	100 a
L. pennellii	LA2963	–	100 a
	LA2580	–	100 a
L. esculentum	Nandi	8.01 ± 0.35 bc	31 ± 5.57 d
	Sankranthi	7.84 ± 0.06 bc	44 ± 6.06 cd
	Vybhav	7.56 ± 0.43 cd	41 ± 6.61 cd
	NDTVR-73	8.61 ± 0.18 a	81 ± 6.58 b
	Pusa ruby	8.12 ± 0.08 ab	47 ± 10.31 cd
L. pimpinellifolium	LA2533	7.18 ± 0.51 d	37 ± 5.50 d
	LA0373	8.18 ± 0.20 ab	55 ± 6.56 c
L. peruvianum	LA643	7.88 ± 0.18 bc	43 ± 7.52 cd
	LA2152	8.17 ± 0.11 ab	56 ± 6.73 c

Means with the same letter at each column are not significantly different at P = 0.05 using Tukey's Studentized Range (HSD) Test.

Density and droplet size of type VI and type IV glandular trichomes

Density of type VI glandular trichomes on both abaxial and adaxial leaf surfaces were significantly different among the accessions/cultivars (ANOVA: abaxial, $F_{13/98}$: 27.39, P < 0.0001; adaxial, $F_{13/98}$: 42.04, P < 0.0001). The highest density of type VI glandular trichomes was observed on abaxial (43.37 ± 2.76 trichomes/mm²) and adaxial (67.33 ± 6.34 trichomes/mm²) leaf surfaces of *L. esculentum* NDTVR-73, followed by *L. esculentum* Vybha (29.91 ± 3.04 and 55.33 ± 4.95 trichomes/mm² on abaxial and adaxial leaf surfaces, respectively), while the lowest density of type VI trichomes was found on abaxial leaf surfaces of *L. pennellii* LA2963 (0.79 ± 0.15 trichomes/mm²) and LA2580 (3.29 ± 0.68 trichomes/mm²). Within the *L. esculentum* varieties, the highest and the lowest density of type VI glandular trichomes were on NDTVR-73 and Sankranthi, respectively (table 3).

There was no relation between the type VI glandular trichomes density and resistance to TSSM. Density of type VI glandular trichome was greater on susceptible cultivars Nandi and Sankranthi comparing to the highly resistant accessions of *L. hirsutum* and *L. pennellii*. The density of type VI trichomes of *L. esculentum* cultivars indicated a strong positive relation between density of type VI glandular trichomes and resistance to TSSM (tables 4 and 5). As the density of type VI trichomes increased, resistance to TSSM also increased. Density of type VI glandular trichomes on NDTVR-73 (a resistant accession) was more than three times greater than Sankranthi (a susceptible variety) (table 3). It was showed that the density of type VI glandular trichomes cannot be a major criterion for screening the resistant accessions/

cultivars from the non-resistant species of *Lycopersicon*. But within the *L. esculentum* cultivars/accessions, it can be an important factor for separating the resistant germplasms.

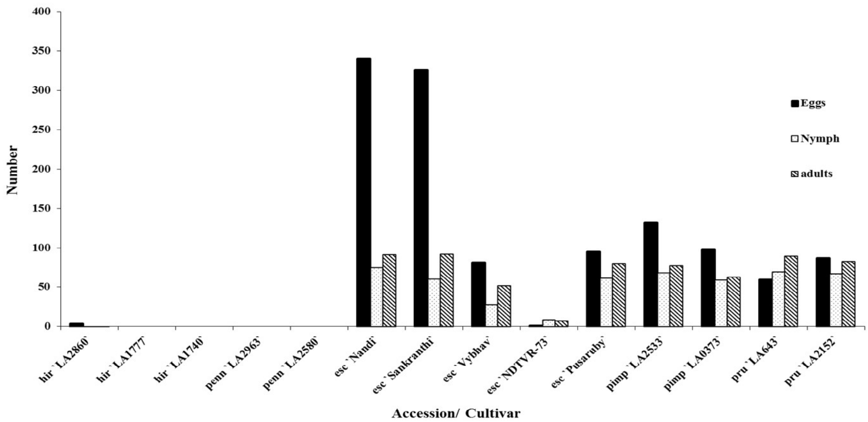

Figure 1. Mean number of eggs, nymphs and adults of two-spotted spider mite and plant damage score on different accessions/cultivars of *Lycopersicon* in greenhouse. hir = *hirsutum*, penn = *pennellii*, esc = *esculentum*, pimp = *pimpinellifolium*, pru = *peruvianum*.

Density of type IV glandular trichomes varied significantly among the accessions/ cultivars (ANOVA: abaxial, $F_{6/35}$: 287.2, $P < 0.0001$; adaxial, $F_{6/35}$: 27.95, $P < 0.0001$). Type IV glandular trichomes were observed only on *L. hirsutum* and *L. pennellii* accessions. *Lycopersicon esculentum*, *L. pimpinellifolium* and *L. peruvianum* accessions/cultivars did not possess type IV glandular trichomes on their foliage. Among the *L. hirsutum* and *L. pennellii* accessions, the highest density of type IV glandular trichomes was recorded on *L. hirsutum* LA1777 (86.72 ± 4.16 and 34.44 ± 5 trichomes/mm² on abaxial and adaxial leaf surfaces, respectively) and the lowest was observed on *L. hirsutum* LA2860 (6.94 ± 0.57 and 4.83 ± 0.77 trichomes/mm² on abaxial and adaxial leaf surfaces, respectively). Density of type IV glandular trichomes was greater on abaxial than adaxial leaf surface, especially on *L. hirsutum* accessions. The type IV glandular trichomes density was higher than type VI glandular trichomes on *L. hirsutum* and *L. pennellii* accessions.

Table 3. Mean (± SE) density and droplet size of type VI and type IV glandular trichomes on abaxial and adaxial leaf surfaces of *Lycopersicon* accessions/cultivars.

Lycopersicon species	Cultivar/accession	Density				Droplet size (µm)	
		Type VI glandular trichomes		Type IV glandular trichomes		Type VI glandular trichomes	Type IV glandular trichomes
		Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface		
L. hirsutum	LA2860	17 ± 1.22 cde	14.62 ± 1.26 cd	6.94 ± 0.57 d	4.83 ± 0.77 c	5.06 ± 0.03 bc	1.02 ± 0.01 b
	LA1777	20.21 ± 2.41 bcd	20.54 ± 2.46 c	86.72 ± 4.16 a	34.44 ± 5.00 a	5.36 ± 0.18 bc	1.13 ± 0.05 b
	LA1740	16.87 ± 1.23 cde	16.33 ± 1.60 cd	74.78 ± 3.32 b	29.94 ± 4.37 ab	4.88 ± 0.19 c	1.07 ± 0.05 b
L. pennellii	LA2963	0.79 ± 0.15 f	4.42 ± 0.91 d	24.88 ± 0.64 c	21.06 ± 2.36 b	7.54 ± 0.22 a	2.08 ± 0.05 a
	LA2580	3.29 ± 0.68 f	10.17 ± 1.98 cd	25.72 ± 1.32 c	20.28 ± 0.71 b	7.56 ± 0.16 a	2.07 ± 0.04 a
L. esculentum	Nandi	22 ± 2.61 bc	45.87 ± 3.18 b	0 d	0 c	–	–
	Sankranthi	11.16 ± 0.88 def	21.91 ± 1.51 c	0 d	0 c	5.83 ± 0.25 b	–
	Vybhav	29.91 ± 3.04 b	55.33 ± 4.95 ab	0 d	0 c	–	–
	NDTVR-73	43.37 ± 2.76 a	67.33 ± 6.34 a	0 d	0 c	5.5 ± 0.29 bc	–
	Pusa ruby	18.75 ± 1.73 cd	42.58 ± 4.98 b	0 d	0 c	–	–
L. pimpinellifolium	LA2533	23.41 ± 4.82 bc	10.87 ± 2.29 cd	0 d	0 c	–	–
	LA0373	14.62 ± 1.61 cde	14.47 ± 3.63 cd	0 d	0 c	–	–
L. peruvianum	LA643	6.83 ± 0.92 ef	7.12 ± 0.87 cd	0 d	0 c	–	–
	LA2152	6.62 ± 0.89 ef	5.21 ± 1.24 d	0 d	0 c	–	–

Means with the same letter at each column are not significantly different at $P = 0.05$ using Tukey's Studentized Range (HSD) Test.

Table 4. Pearson correlation coefficients between the density of type IV and type VI glandular trichomes in *Lycopersicon* species and response of two-spotted spider mite.

Mite response	Type VI glandular trichomes				Type IV glandular trichomes			
	Abaxial surface		Adaxial surface		Abaxial surface		Adaxial surface	
	r	p	r	p	r	p	r	p
No. eggs / ♀ / day	0.075	0.84	0.079	0.79	-0.66	0.009	-0.70	0.001
Damage score	0.11	0.66	0.15	0.56	-0.67	0.005	-0.76	0.0004
No. dead mites / leaf	-0.25	0.33	-0.28	0.33	0.84	0.0002	0.95	0.0001
No. mites dead in water	0.17	0.35	0.06	0.66	-0.09	0.62	-0.12	0.67
No. mites on the tack	-0.36	0.15	-0.37	0.13	0.76	0.001	0.91	0.0001
Mortality of juveniles	-0.19	0.53	0.40	0.35	0.68	0.007	0.79	0.007
Developmental time	0.34	0.66	-0.24	0.36	–	–	–	–

Table 5. Pearson correlation coefficients between the density of type VI glandular trichomes on abaxial leaf surface of *L. esculentum* and response of two-spotted spider mite.

Mite response	Type VI glandular trichomes			
	Abaxial leaf surface		Adaxial leaf surface	
	r	p	r	p
No. eggs / ♀ / day	-0.64	0.01	-0.56	0.05
Damage score	-0.64	0.03	-0.49	0.01
No. dead mites / leaf	0.45	0.01	0.33	0.02
No. mites dead in water	0.49	0.02	0.29	0.42
Mortality of juveniles	0.26	0.15	0.25	0.30
Developmental time	0.43	0.03	0.28	0.33

In contrast to type VI glandular trichomes, density of type IV glandular trichomes was strongly related to TSSM resistance in *Lycopersicon* species (table 4), so the presence and density of type IV glandular trichomes serve as a factor for separating the resistant accessions/ varieties to TSSM across *Lycopersicon* species.

Droplet size of type VI glandular trichomes significantly varied among the accessions/ cultivars (ANOVA: type VI, $F_{6/21}$: 30.08, $P < 0.0001$; type IV, $F_{4/15}$: 158.35, $P < 0.0001$). *Lycopersicon pennellii* accessions displayed greater droplet size comparing to *L. hirsutum* and *L. esculentum* accessions/cultivars. Droplet size of type IV glandular trichomes was significantly different between *L. pennellii* and *L. hirsutum* accessions. Droplet size of *L. pennellii* accessions was 1.93 times greater than *L. hirsutum* (table 3).

Discussion

Mite response to the host and host response to the mite

There are reports about the differences in host suitability for survival, oviposition and avoidance of TSSM in different crops. Rasmy (1985) believed that a genetic component in *Lycopersicon* species determines the suitability of the host plant for TSSM oviposition and survival.

In the course of this study, 14 cultivars and accessions of *Lycopersicon* were examined, using two methods (leaf disk and thumbtack bioassays) to quantify their resistance to TSSM (Weston *et al.*, 1989; Weston & Snyder, 1990; Nihoul *et al*., 1991; Gimenez-Ferrer *et al*., 1993; Simmons *et al.*, 2003). The accessions of *L. hirsutum* and *L. pennellii* were highly resistant to TSSM, whereas other *Lycopersicon* species showed a wide range of susceptibility to TSSM. Many researchers have reported resistance of *L. hirsutum* and *L. pennellii* accessions to arthropods (Snyder & Carter, 1984; Fobes *et al*. 1985; Carter & Snyder 1986; Weston *et al*, 1989; Eigenbrode & Trumble, 1993; Simmons *et al*., 2003, 2004) Among the accessions, only *L. hirsutum* LA1777 had reported as a source of resistant to beet army worm, *S. exigua*, (Eigenbrode & Trumble, 1993) and whitefly *B. argentifolii* (Muigai *et al*., 2003).

The developmental time and survival of TSSM using leaf disk bioassay proved the existence of strong resistance factors on the foliage of *L. hirsutum* and *L. pennellii* accessions, which caused 100% mortality in the juveniles. Toxic chemicals (Williams *et al*., 1980; Juvik *et al*., 1994; Eigenbrode *et al*., 1996) or adhesive exudates (Simmons *et al*., 2003, 2004) of type IV and type VI glandular trichomes might be responsible for resistance in these accessions. Longer developmental time and greater mortality on *L. esculentum* NDTVR-73 is likely due to higher density of type VI glandular trichomes.

The complex interactions among host plant, pest and the environment, resistant cultivars in *in vitro* methods did not display the same level of resistance in the field and greenhouse (Gimenez-Ferrer *et al*., 1993). The accessions in the greenhouse indicated that most of them held the same level of resistance that was recorded during the *in vitro* bioassays.

Relation between density of type VI and IV glandular trichomes and resistance to TSSM

There was no relation between the density of type VI glandular trichomes and resistance to TSSM. Density of type VI trichomes was greater on susceptible cultivars of *L. esculentum* (Sankranthi and Nandi) than on highly resistant accessions of *L. hirsutum* and *L. pennellii* (tables 1 and 3). This is in agreement with the findings of Good & Snyder (1988) who concluded that the density of type VI trichomes in *Lycopersicon* interspecific hybrids was not correlated with the survival of TSSM. The level of toxic chemicals such as methyl ketones (Williams *et al*., 1980, Weston *et al*., 1989; Eigenbrode & Trumble, 1993) and sesquiterpenes (Eigenbrode *et al*., 1996) at the tip of type VI glandular trichomes of *L. hirsutum* were noticeably higher than in *L. esculentum* cultivars.

The density of type VI glandular trichomes cannot be a useful criterion for screening resistant accessions of *Lycopersicon* species; however, within *L. esculentum* cultivars it may indicate the ability of a variety to contribute to resistance against TSSM (tables 1, 3 and 5). Density of type VI glandular trichomes might have caused lower establishment rate of TSSM on NDTVR-73. Gimenez-Ferrer *et al*. (1994) reported that reduction of mite population growth could be through low fecundity of females, low egg hatchability and low survival rate of immature stages or a combination of these methods. Lower populations' build up of the mite on NDTVR-73 could be due to the combination of above-mentioned factors (tables 1 and 2; fig. 1).

Density of type IV glandular trichomes was probably suggesting resistance to TSSM among the *Lycopersicon* species. Density of type IV glandular trichomes had a negative correlation with the number of eggs / female / day and leaf damage, while positively correlated to the number of dead mites on the leaf disk as well as the number of remaining mites on the tack and also the morality of immature stages (table 4). Simmons *et al*. (2003) reported highly significant positive relationship between density of type IV glandular trichomes in *L. pennellii* and mortality of *M. Persicae*, in addition to the negative correlation between this type of trichomes and number of unaffected aphids. Density of type IV glandular trichomes has been reported as the basis of resistance to TSSM (Weston *et al*., 1989) and *S. exigua* (Eigenbrode &Trumble, 1993) in *L. hirsutum*. Good & Snyder (1988) found that TSSM survival in F_2 progenies of *L. esculentum* × *L. hirsutum* was highly correlated with type IV glandular trichomes density and discussed the high density of IV trichome as a mechanism for increasing host plant resistance to the mite.

The type IV glandular trichomes are the key factors that determined the number of remaining mites on the tack during the thumbtack bioassay. On *L. esculentum*, *L. pimpinellifolium* and *L. peruvianum* accessions/cultivars, where type IV glandular trichomes were absent, no mites remained on the tack after 2 h (tables 1 and 3). As the density of *L. hirsutum* accessions of type IV glandular trichomes increased, the number of remaining mites on the tack also increased (tables 1 and 3).

The density of type IV glandular trichomes is higher on leaves of *L. hirsutum* than on *L. pennellii* accessions that agrees with Eigenbrode & Trumble (1993). Droplet size of type IV glandular trichomes on *L. pennellii* accessions was about twice greater than on *L. hirsutum* that significantly increases the effectiveness of type IV glandular trichomes on *L. pennellii*.

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