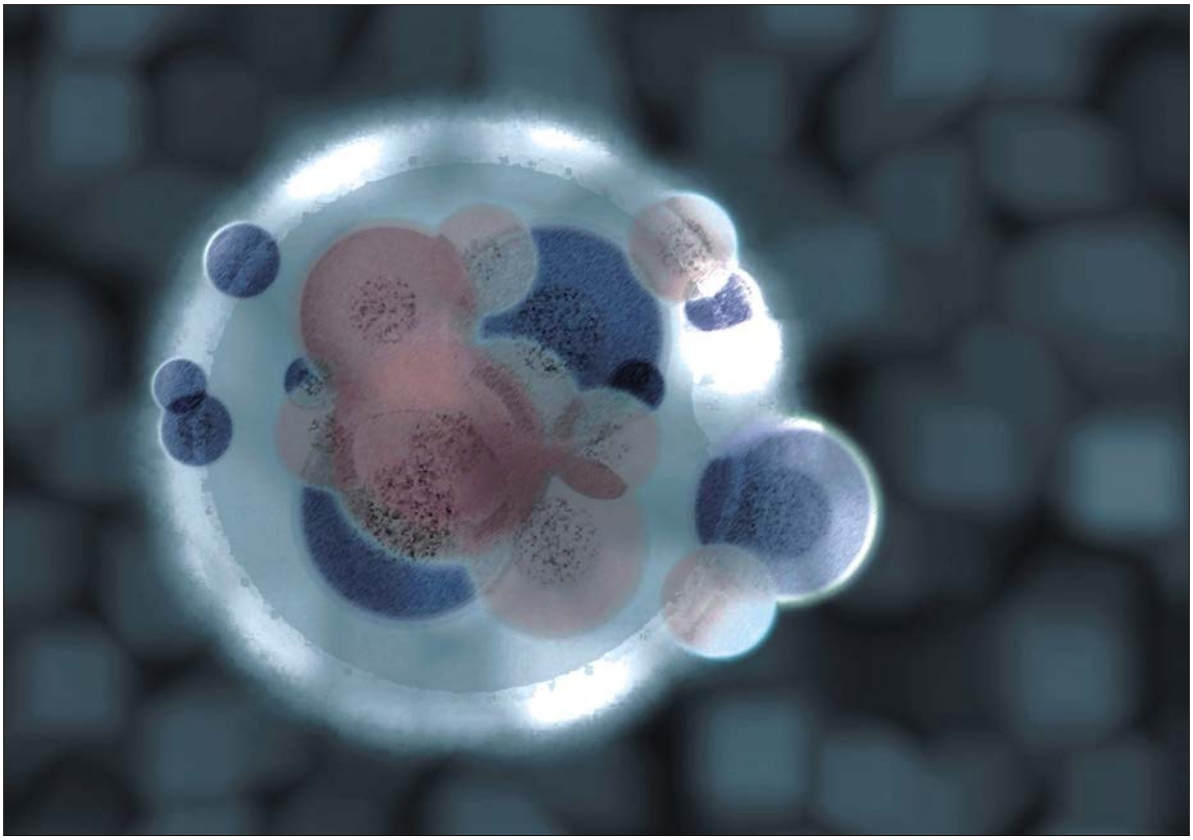

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In vitro Screening of 67 *Lycopersicon* accessions/Cultivars for Resistance to Two-Spotted Spider Mite

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Abstract: Sixty seven cultivars/accessions from different species of *Lycopersicon* including *L. esculentum, L. pimpinellifolium*, *L. peruvianum*, *L. parviflorum*, *L. hirsutum* and *L. pennellii* were screened for resistance to *Tetranychus urticae* Koch using thumbtack and leaf disk bioassays. Results indicated that more number of mites remaining on the tack of *L. hirsutum* and *L. pennellii* accessions compare to the other accessions, whereas there was no significant difference among the remaining accessions/cultivars. The lowest level of oviposition rate and leaf damage score were observed in *L. hirsutum* and *L. pennellii* accessions. Among the other *Lycopersicon* cultivars/accessions, Punjab Chhaura (*L. esculentum*) supported the highest level of oviposition (158.6 eggs/10 females/3 days) and *L. esculentum* (TLB-193, Arka Meghali and LA 2302) displayed highest level of damage whereas *L. esculentum* NDTVR-73 supported the lowest level of oviposition (4.14 egg/female/3 days) and damage score. The accessions/ cultivars were classified into five groups, based on the overall performance in leaf disk and thumbtack bioassays. *L. hirsutum* (LA 1740, LA 1777 and LA 2860) and *L. pennellii* (LA 2963 and LA 2580) appeared to be highly resistant, while *L. esculentum* (TLB 193, LA2302, Arka alok, Punjab Chhaura) appeared to be highly susceptible to two-spotted spider mite.

Key words: *Lycopersicon*, resistance, two-spotted spider mite

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important and widely grown vegetable crops in the world. Among the wide range of pests which attack this crop, two-spotted spider mite (*Tetranychus urticae* Koch) is one of the most important pests. The short life span, high fecundity and its ability to develop resistance to many acaricides have made chemical control of this mite particularly difficult (Luczynski *et al*., 1990). Host plant resistance to arthropods mediated by mechanisms that reduce pest feeding on the plants can be an important component of integrated management of *T. urticae* (Wetson and Snyder, 1990).

Host plant resistance to two-spotted spider mite (TSSM) has been reported in different crops such as strawberry (Gimenez- Ferrer *et al*., 1993, 1994), Raspberry (Wilde *et al*., 1991) and *Lycopersicon* species (Ania *et al*., 1972; Kennedy and Dimock, 1983; Weston *et al*., 1989; Goffreda and Mustschler, 1989; Simmons *et al*., 2003). Weston and Snyder (1990) described a quick method (thumbtack bioassay) for measuring plant resistance to TSSM. Various studies have shown that resistance of *Lycopersicon* sp. to insects and mites depends on type, density and exudate composition of type IV and VI glandular trichomes (Williams *et al*., 1980; Kennedy and Dimock, 1983; Good and Snyder, 1988; Eigenbrode and Trumble, 1993; Goffreda and Mutschler, 1989; Weston *et al*., 1989; Simmons *et al*., 2003). Exudates of these trichomes can physically entangle the pest (Simmons *et al*., 2003, 2004) and are usually associated with toxic or repellent chemicals (Williams *et al*., 1980; Kennedy and Dimock, 1983; Goffreda and Mutschler, 1989).

The objective of this study was, screening of wild and cultivated tomato accessions for resistance to TSSM in order to use resistant ones in future plant breeding programs.

MATERIALS AND METHODS

Plant material and leaf sampling: The germplasms used in this study were collected from Dept. of Genetics and Plant Breeding, University of Agricultural Science, Bangalore, India, Indian Institute of Horticultural Science, Bangalore, India and Nunhems Proagro seeds, Pvt. Ltd. Bangalore, India. They were 41 varieties and hybrids of *L. esculentum*, 14 accessions of *L. pimpinellifolium*, 6 of

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L. peruvianum, 1 of *L. parviflorum*, 3 of *L. hirsutum* and 2 of *L. pennellii*. Plants were raised in an insectary enclosed with sheet net under natural conditions of photo period, temperature and humidity. Tomato leaflets were collected from equivalent positions. Fully expanded young leaves (third leaves below the apical meristem) were collected and used for *in vitro* studies.

Maintenance of the mite stock culture: The strain of *T. urticae* used in this study originated from infested leaves of tomato collected from Hebbal Campus of University Agricultural Science, Bangalore in September 2003. Mite rearing was carried out on susceptible variety Sankranthi of *L. esculentum* under green house conditions. The individual mites used for the bioassays were collected and transferred to the plants using a fine camel hair brush.

Screening of germplasms for resistance to two-spotted spider mite: Screening for resistance to TSSM was conducted using thumb tack and leaf disk bioassays based on Randomize Complete Design with five replications.

Thumb tack bioassay: The bioassay consists of a thumbtack inserted through a test leaf and into a light weight material such as Styrofoam or cork (Weston and Snyder, 1990). In this study a thin layer of cotton was spread on the Styrofoam (20×20 cm). The cotton layer was saturated with water; the wet cotton was able to keep the leaves fresh for several hours. For bioassay fully expanded tomato leaflets (as mentioned above) were selected. Ten adult female mites were transferred with a fine camel hair brush on to the head of the thumbtack and after 2 h, the number of mites remaining on the tack was recorded. In addition, the distances traveled by mites on the leaf surface were measured from the edge of the thumbtack nearest to the mite and scored from 1 to 3 scales as below.

1 = distance traveled by mites less than 5 mm.
2 = distance traveled by mites 5-10 mm
3 = distance traveled by mites beyond 10 mm

Leaf disk bioassay: leaf disks (2×2 cm) were obtained from either side of the main vein and placed with abaxial surface facing up on a water- saturated none sterile cotton wad in a plastic Petri plate (12.5 cm in diameter). For bioassay 5 leaf disks of each accession/cultivar were infested with 10 adult female mites (3-5 days in age). Leaf disks were kept in an incubator (at maintaining 26±1 °C). Mite behavior and response on different genotypes were assessed by 2 parameters (Gimenez-Ferrer *et al*., 1993).

- Counting the number of eggs laid on the disk after 72 h.
- Recording the leaf damage index: LDI scored on a 0 to 6 scale after 96 h, based on the intensity of damage described by Nihoul *et al*. (1991) and Gimenez-Ferrer *et al*. (1993) as given below:

0- no damage
1- Few small feeding patches<10% of leaf area
2- Feeding patches 10-25% of leaf area
3- Feeding patches 26-40% of leaf area
4- Feeding patches 41-60% of leaf area
5- Feeding patches 61-80% leaf area
6- Feeding patches 81-100% of leaf area

Presence and density of type IV glandular trichomes: Abaxial and adaxial surfaces of leaflets were observed for presence of type IV glandular trichomes. Density of type IV glandular trichomes in *L. hirsutum* and *L. pennellii* was determined in 1 mm^2 from 3 regions of both sides and averaged.

Statistical analysis: Data were analyzed using SAS (1996) software. Analysis of variance (Proc ANOVA) was performed to identify significant differences among the accessions/cultivars. Correlation (Proc Corr) analysis was used to describe relationships between variables. Grouping of accessions/varieties was done using Statgraph software.

RESULTS

Thumbtack bioassay (mite avoidance): The number of mites remaining on the thumbtack and the distance traveled by mites differed significantly among accessions/cultivars of *Lycopersicon* genus (Table 1). *L. hirsutum* and *L. pennellii* accessions had more number of mites remaining on the thumbtack. There was no significant difference among *L. esculentum*, *L. peruvianum*, *L. pimpinellifolium* and *L. parviflorum* cultivars/accessions. Among *L. hirsutum* and *L. pennellii* accessions the highest number of mites remained on the tack of *L. hirsutum* LA 1740 followed by *L. pennellii* accessions (Table 1).

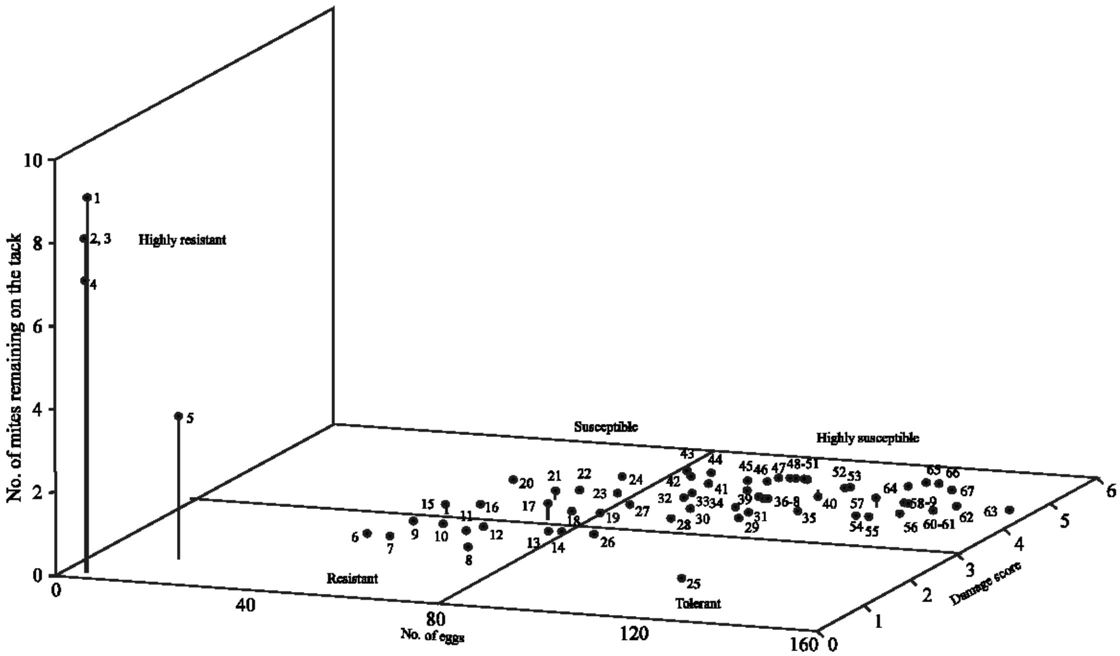
Leaf disk bioassay

Ovipositional rate: Accessions/cultivars significantly influenced the ovipositional response of two-spotted spider mite females. *L. hirsutum* and *L. pennellii* accessions did not support the females to lay eggs as the number of eggs laid by 10 females in 3 days was 0-13 (Table 1). In contrast there were significant differences among accessions/cultivars of other species. Among

Table 1: Screening 67 accessions/cultivars of *Lycopersicon* sp. for resistance to *T. urticae* using leaf disk and thumbtack bioassays

		Leaf disk bioassay		Thumbtack bioassay	
Species	Cultivar/ Accession	No. eggs laid by (10♀/3 days)	Damage score (after 4 days)	No. of mites on tack (after 2h)	Distance traveled (score)
L. esculentum	TLB-148	78.8h-p	2.8g-j	0e	3
	TLB-205	70.6k-s	5.0a-d	0e	3
	BWR-1	100.6c-m	4.8a-e	0e	3
	Arka vikas	103.6b-n	4.2b-f	0e	3
	ATY-14	52.8o-t	3.6e-h	0e	3
	CO-1	65.6l-s	2.2i-k	0e	3
	Nandi	96.6d-o	5.2a-c	0e	3
	TLB-191	119.4a-i	5.2a-c	0e	3
	BWR-5	101.0c-n	5.2a-c	0e	3
	Vybhav	101.6c-n	5.4ab	0e	3
	TLB-183	86.6g-r	5.4ab	0e	3
	TLB-196	106.0b-m	4.6a-e	0e	3
	TILB-195	90.2f-q	4.6a-e	0e	2.66
	TLB-193	132.4a-f	5.6a	0e	3
	Sankranthi	104.4b-m	5.4ab	0e	3
	LA7996	84.0i-s	5.2a-c	0e	3
	Arka meghali	105.6b-m	5.4ab	0e	3
	TLB-192	81.2h-p	5.4ab	0e	3
	LA 2308	50.4q-t	4.6a-e	0e	3
	NDTVR-73	41.4s-t	2.2i-k	0e	2.66
	AMR	52.4p-t	2.8g-j	0e	3
	Pusa ruby	65.6l-s	4.0c-g	0.2e	3
	LA 2302	135.4a-e	5.6a	0e	3
	LA 2301	108.0b-l	5.4ab	0e	3
	LA 550-3	46.4q-t	2.2i-k	0e	3
	LA 915	131.0a-g	4.6a-e	0.2e	3
	LA 2306	130.4a-h	5.4ab	0e	3
	Swaraksha	75.6h-p	4.4a-f	0e	3
	NS2535	135.4a-e	4.8a-e	0e	3
	UTSAV	118.4a-i	5.2a-c	0e	3
	NS 816	107.2b-l	5.4a-b	0e	3
	Arka abhay	105.0b-m	4.6a-e	0e	3
	PKM-1	61.4m-s	2.8g-j	0e	3
L. esculentum	Arka alok	140.0a-d	5.4a-b	0e	3
	BWT-2	90.0f-q	5.0a-d	0e	3
	LA 90	75.6i-s	2.8g-j	0e	3
	Buny bert	136.4a-e	4.8a-e	0e	3
	Rudex	95.2e-p	3.6e-h	0e	3
	TVK-1	86.0h-r	2.8g-j	0e	3
	Kesri	133.4a-f	4.2b-f	0e	3
	Punjab chhaura	158.6a	4.8a-e	0e	3
L. peruvianum	LA 643	59.4n-s	2.6h-j	0e	3
	LA 634	71.8k-s	3.2f-i	0.4e	3
	LA 733	79.4h-p	3.6e-h	0e	3
	LA 2152	138.2a-e	4.4a-f	0e	3
	LA 2163	119.6a-i	1.4l-k	0e	3
	LA 2744	67.0l-s	4.4a-f	0e	3
L. pimpinellifolium	LA 4395	90.4f-q	4.4a-f	0e	3
	LA 4394	95.6d-p	4.0c-g	0e	3
	AC 18815	49.0q-t	3.2f-i	0.2e	3
	LA 0373	45.8r-t	2.8g-j	0e	3
	LA 0411	108.2b-l	3.8d-g	0e	3
	LA 1279	143.8a-c	4.6a-e	0e	3
	LA 1301	116.2a-k	4.8a-e	0e	3
	LA 1478	108.4b-l	4.0c-g	0e	3
	LA 1578	144.0a-c	4.6a-e	0e	3
	LA 1617	107.2b-l	4.6a-e	0e	3
	LA 1924	82.2i-s	4.0c-g	0e	3
	LA 2181	117.6a-j	4.2b-f	0e	3
	LA 2183	73.2j-s	3.6e-h	0e	3
	LA 2533	147.0ab	4.8a-e	0e	3
L. parviflorum		130.4a-g	4.2b-f	0e	3
L. hirsutum	LA 1740	0.4u	0.0m	9a	1
	LA 1777	0.0u	0.0m	7c	1
	LA 2860	13.0tu	0.8l-m	3.8d	2.66
L. pennellii	LA 2580	0.0u	0.0m	8b	1
	LA 2963	0.0u	0.0m	8b	1

Mean values with the same letter are not significantly different at p = 0.01, using Duncan's Multiple Range test



L. esculentum				*L. pimpinellifolium*		
6 = NDTVR-73	24 = TLB-205	44 = TLB-183	57 = LA 915	9 = LA 0373	60 = LA 1279	54 = *L. parviflorum*
7 = 550-3	26 = TVK-1	45 = Nandi	58 = NS-2533	15 = AC 18815	61= LA 1578	
8 = CO-1	28 = Rudex	46 = BWR-5	59 = Buny bert	18 = LA 2183	62 = LA 2533	*L. hirsutum*
10 = AMR-73	33 = TLB-195	47 = Vybhav	63 = Punjab chaura	27 = LA 1924		1 = LA 1740
12 = PKM-1	34 = Arka vikas	48 = Sankranthi	64 = LA 2306	29 = LA 0411	*L. peruvianum*	4 = LA 1777
13 = LA 90	36 = Arka abhay	49 = NS-816	65 = TLB-193	30 = LA 4394	11 = LA643	5 = LA2860
14 = TLB-148	37 = TLB-191	50 = Arka meghali	66 = = LA 2302	31 = LA 1478	17 = LA634	
16 = ATY-14	39 = BWR-1	51 = LA 2301	67 = Arka alok	32 = LA 4395	19 = LA733	*L. pennellii*
20 = LA 2308	41 = BWT-2	52 = UTSAV		35 = LA 2181	22 = LA2744	2 = LA 2580
21 = Pusa ruby	42 = L7996	53 = TLB-191		38 = LA 1617	25 = LA2163	3 = LA 2963
23 = Swaraksha	43 = TLB- 192	55 = Kesri		40 = LA 1301	56 = LA2152	

Fig. 1: Grouping 67 *Lycopersicon* accessions/cultivars via number of mites remaining on the tack, number of eggs laid by the mites and leaf damage score

other *Lycopersicon* accessions/cultivars, Punjab Chhaura (*L. esculentum*) supported the highest level of oviposition (158.6 eggs/10♀/3 days) whereas *L. esculentum* NDTVR-73 supported the lowest level of oviposition (41.4 eggs/10♀/3 days) (Table 1).

Damage score: The mites did not cause any damage on leaf disk of *L. hirsutum* (LA 1740, LA 1777) and *L. pennellii* (‘LA 2580’ and ‘LA 2963’) accessions. Leaf disks of *L. hirsutum* ‘LA 2860’ were slightly damaged (feeding patch were <10% of leaf area). On the leaf disks of other accessions/cultivars, damage score was moderate to high. Among the *L. esculentum*, *L. pimpinellifolium*, *L. peruvianum* and *L. parviflorum* accessions/cultivars, leaf disks of *L. peruvianum* LA 2163 and *L. esculentum* (NDTVR-73, CO-1 and 550-3) were least damaged suggesting an expression of resistance in these accessions, whereas *L. esculentum* TLB-193, Arka meghali and LA 2302 displayed highest level of damage (Table 1).

Overall performance: correlation between the number of eggs and damage score was strong and positive whereas, it was negative between number of mites on tack and number of eggs or damage score (Table 2). The accessions/cultivars were classified into five groups, based on the overall performance in leaf disk and thumbtack bioassays. Mite response to host (oviposition and avoidance) and plant response to the mite (host damage score) were considered simultaneously as a measure of overall a cessions/cultivar performance (Fig. 1).

Presence and density of type IV glandular trichomes: Type IV glandular trichomes were observed only on *L. hirsutum* and *L. pennellii* accessions. *L. 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 abaxial and

Table 2: Correlation between number of eggs, damage score and number of mites on the tack among 68 accessions/cultivars of *Lycopersicon* species

	No. of eggs	Damage score	No. of mite on tack
No. of eggs	1.00		
Damage score	0.69 **	1.00	
No. of mite on tack	-0.56 **	-0.67 **	1.00

** Significant at p = 0.01

Table 3: Presence and density of type IV glandular trichome on abaxial and adaxial leaf surfaces of *Lycopersicon* accessions

		Type IV glandular trichomes	
Species	Accession	Abaxial surface	Adaxial surface
L. hirsutum	LA 2860	6.94d	4.83c
	LA 1777	86.72a	34.44a
	LA 1740	74.78b	29.94ab
L. pennelli	LA 2963	24.88c	21.06b
	LA 2580	25.72c	20.28b
L. peruvianum	All accessions	absent	absent
L. pimpinellifolium	All accessions	absent	absent
L. parviflorum	-	absent	absent
L. esculentum	All accessions	absent	absent

Means with the same letter are not significantly different at p = 0.05, using Duncan's Multiple Range Test

adaxial leaf surfaces of *L. hirsutum* LA 1777 and the lowest type IV trichomes density was observed on abaxial and adaxial leaf surfaces of *L. hirsutum* LA 2860 (Table 3).

DISCUSSION

Several authors have reported differences in host suitability for survival, oviposition and avoidance of TSSM in different crops. Rasmy (1985) studied the difference in suitability of *Lycopersicon* species for oviposition of TSSM and suggested the existence of a genetic component that determines the suitability of the host plant for TSSM oviposition and survival. In the present study sixty seven accessions/cultivars of *Lycopersicon* genus were screened using two methods (Leaf disk and thumbtack bioassays) which have been used successfully to quantify resistance to two-spotted spider mite in several crops. Leaf disk bioassay, based on oviposition rate (eggs/♀/day) and damage score has been used for *in vitro* screening of 76 straw berry (*Fragaria* sp.) cultivars for TSSM resistance by Gimenez-Ferrer *et al*. (1993). Wilde *et al*. (1991) evaluated resistance of 18 raspberry (*Rubus idaens* L.) cultivars and Ania *et al*. (1972) studied characterizing resistance in tomato lines to TSSM using same method.

The tested accessions/cultivars were classified into five groups (highly susceptible, susceptible, tolerant, resistant and highly resistant) according to mite response to the host (oviposition, mortality and avoidance) and host response to the mite (damage score). Determining relative resistance among the cultivars could be more clearly established when mite response to host and host response to mite were considered simultaneously as a measure of overall cultivar performance (Gimenez-Ferrer *et al*., 1993). Differences in mite response to the host and vice versa, indicated the presence of antibiotic, antixenotic and tolerance factors. *L. hirsutum* and *L. pennellii* accessions which studied here, were highly resistant to TSSM, but other *Lycopersicon* species showed different range of susceptibility to TSSM (Table 1). Several authors have reported resistance of *L. hirsutum* and *L. pennellii* accessions to many pests (Snyder and Carter, 1984; Carter and Snyder 1986; Weston *et al*., 1986; Weston *et al*., 1989; Fobes *et al*., 1984; Eigenbrode and Trumble, 1993; Simsons *et al*., 2003 and 2004). But accessions which used in the present study were differed from those reported earlier. Among the accessions which were tested, only *L. hirsutum* LA 1777 has already been reported as resistant to beet army worm, *Spodoptera exigua* (Hubner) (Eigenbrode and Trumble, 1993) and whitefly *Bemisia argentifolii* Bellow (Muigai *et al*., 2003). These accessions can be used as a source of resistance to TSSM in future plant breeding programmes. More over varieties and accessions of *L. esculentum* which were grouped as resistant (Fig. 1) are useful in integrated management of TSSM. Among them 'NDTVR-73' showed the highest level of resistance to TSSM, on which mite fecundity and plant damage were lower while mite mortality was high compared to the other accessions/varieties (Table 1).

Comparison of thumbtack and leaf disks bioassays: *In vitro* methods have the following two advantages: Firstly, they are relatively inexpensive and can be conducted more rapidly than field test, thus the number of genotypes evaluated in a given test period can be expanded and secondly, they minimize effects of environmental variation on test results (Gimenez-Ferrer *et al*., 1993). Thumbtack bioassay provides a direct measure of host repellency to mite. On resistant accessions, mites turned toward the center of the thumbtack after they approached the edge of the thumbtack and touched the leaf surface. Similar observation has been reported by Weston and Snyder (1990). The turning behaviour indicates repellency of the plant foliage (Weston and Snyder, 1990).

Leaf disk bioassay based on the mite response to the host (oviposition and mortality) and host response to the mite (damage score) can be an effective method for studying resistance to TSSM. In comparison, leaf disk bioassay is more effective method than thumbtack bioassay for measuring resistance in *Lycopersicon* accessions/cultivars. The thumbtack bioassay measures resistance mediated by antixenosis only, therefore, other bioassays might be necessary to completely measure the resistance of plants to mite (Weston and Snyder, 1990).

In the present study it was found that thumbtack bioassay could measure only resistance to TSSM among the *L. hirsutum* and *L. pennellii* accessions. There was significant relation between efficiency of thumbtack bioassay and presence of type IV glandular trichomes. As type IV glandular trichomes are present in *L. hirsutum* and *L. pennellii* accessions only, Therefore, using of thumbtack bioassays to study resistance among other *Lycopersicon* species cultivars (such as *L. esculentum*, *L. pimpinellifolium L. peruvianum*) which do not have IV glandular trichomes is questionable. In contrast leaf disk bioassay clearly indicated differences in resistance among the tested accessions/cultivars. Hence, it is suggested using thumbtack bioassay to study resistance of *L. pennellii* and *L. hirsutum* accession only, as an evidence, *L. hirsutum* LA 2860 which had less number of type IV trichomes compared to other accessions, supported less number of mites on the tack (Table 1 and 3). It is also suggested using of leaf disk bioassay to study resistance to TSSM among the other species of *Lycopersicon*. Compare to the field or green house studies, this is an effective method to gather the data in a temporally, spatially, economically and labour-efficient manner (Gimenez-Ferrer *et al*., 1993). However, because the host environment relationship is extremely complex, selected germplasms by *in vitro* tests have to be studied in green house or field conditions.

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