Inheritance of Type IV Glandular Trichomes and Two-Spotted Spider Mite Resistance in an Interspecific Cross of Lycopersicon esculentum × L. pennellii ‘LA2580’

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ABSTRACT
An experiment was conducted to study the inheritance of type IV glandular trichomes and its relation to two-spotted spider mite (TSSM) resistance in cross Lycopersicon esculentum Mill × L. pennellii ‘LA 2580’. Parents and their F1 and F2 progenies were raised under natural conditions. Five weeks after transplanting, fully expanded young leaves were collected and used for estimating density of type IV glandular trichomes and TSSM resistance. Subsequently, two-month-old plants were challenged with TSSM (50 adults and 150 eggs). After two weeks, they were scored for damage index (0 to 6 scale). The estimated heritability in broad sense was high (93%) for density of type IV glandular trichomes. Statistical analyze showed that two dominant unlinked genes control the presence of type IV trichomes in the hybrids. There was an over expression of type IV glandular trichomes in F1 plants. Density of type IV trichomes in F2 individuals showed a broad range of variability (0 to 35 trichomes/mm²). There was a significant positive relationship between density of type IV glandular trichomes and resistance to TSSM.

Keywords: Lycopersicon esculentum, L. pennellii, Glandular trichomes, Tetranychus urticae, Inheritance, Resistance

INTRODUCTION
The wild relative of cultivated tomato, Lycopersicon pennellii (Corr) D’Arcy, is a potential source of resistance to many arthropods (Goffreda and Mutschler, 1989; Juvik et al., 1994; Simmons et al., 2003). Resistance of Lycopersicon spp to insects and mites has been attributed to type, density and exudates composition of type IV and VI glandular trichomes (Williams et al., 1980; Eigenbrode and Trumble, 1993; Weston et al., 1989). Exudates of these trichomes can physically entangle the pest (Simmons et al., 2003) and are usually associated with toxic or repellent chemicals (Williams et al., 1980; Goffreda and Mutschler, 1989). Some accessions of L. pennellii have been reported as the source of resistance to Macrosiphum euphorbiae (Thomas) and Myzus persicae.
Individual mites used for bioassays were collected and transferred to plants using a fine camel hair brush.

Plant materials, crossing and environmental conditions

*L. pennellii* ‘LA 2580’ was obtained from Nunhems ProAgro seeds, Pvt.Ltd., Bangalore, India and *L. esculentum* varieties (‘Nandi’ and ‘Sankranthi’) were obtained from Dept. of Genetics and Plant Breeding, UAS, Bangalore. *L. esculentum* was selected as maternal parent and *L. pennellii* was used as the paternal parent. Plant culture and crossing were done based on the methods described by Saeidi *et al.* (2007). Seeds of F₁ plants were used to produce F₂ generations. Plants were raised in an insectary enclosed with net under natural conditions of photoperiod, temperature and humidity. Study was carried out at GKVK campus, UAS, Bangalore during the period September to October 2005. Maximum to minimum temperature and humidity during September were 19.2-27.6°C and 53-93%, during October 18.8-26.8°C and 58-94% and during November 16.1-25°C and 56-92%, respectively.

**Leaf sampling**

Fully expanded young leaves (third leaf below the apical meristem) were collected and used for leaf disk bioassay and estimating density of type IV glandular trichomes.

Mite resistance and density of type IV glandular trichomes

Sixteen individuals of each parent, 16 of each F₁, 144 individuals of F₂ hybrid *L. esculentum* ‘Sankranthi’ × *L. pennellii* ‘LA2580’ and 136 individuals of F₂ hybrid *L. esculentum* ‘Nandi’ × *L. pennellii* ‘LA2580’ were evaluated for mite resistance and density of type IV glandular trichomes.
resistance and density of type IV glandular trichomes. The study was carried out in two experiments.

**Experiment 1**

Five weeks after transplanting, mite response to the host (oviposition and mortality) and host response to the mite (damage score) were evaluated simultaneously as overall performance of generations using leaf disk bioassay described by Gimenez-Ferrer et al., 1993. One leaf disk (2 cm in diameter) was obtained from each individual and infested with five adult female mites (3-5 days in age). Disks were kept in an incubator at 26±1°C and 16:8 h (light:darkness) photoperiod. After 72 h, mite oviposition and mite mortality were recorded on each disk. Then, after 96 h, leaf damage was estimated based on the intensity of damage (Nihoul et al., 1991; Gimenez-Ferrer et al., 1993) 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.

Presence and density of type IV glandular trichomes (number/mm²) were studied on abaxial leaflet surface of the F₁ and F₂ plants and compared with their parents. Density of the trichomes was determined from three regions of the abaxial surface and averaged for all three spots (Luczynski et al., 1990). Trichomes were counted under a stereo binocular microscope at 100X magnification.

**Experiment 2**

Six weeks after transplanting, plants were artificially infested with TSSM. Tomato leaves containing about 50 adults and 150 eggs were placed on the third apical leaf of each individual and after two weeks the damage was scored on a 0 to 6 scale (as described above).

**Statistical Analysis**

Data were analyzed using SAS (1996) software. Analysis of variances (Proc ANOVA) was performed to identify significant differences among the generations. Correlation (Proc Corr) was used to describe relationship between variables. Heritability in broad sense was estimated using formula of Hanson et al., (1956).

**RESULTS**

The estimated broad sense heritability was high (93%) for density of type IV glandular trichomes in both crosses, indicating that 93% of the total phenotypic variation can be explained by the genetic effects (Table 1). Chi-square test for goodness to fit duplicate dominant gene for presence of type IV glandular trichomes in tomato crosses showed that the presence of type IV trichomes is controlled by two dominant unlinked genes, with either gene conferring the presence of type IV glandular trichomes in the hybrids (Table 1).

All F₁ plants possessed type IV glandular trichomes. F₂ data showed a goodness fit ratio (15:1) in both crosses. Thus, the presence of type IV trichomes is simply inherited in L. esculentum × L. pennellii ‘LA 2580’. Density of type IV glandular trichomes showed a wide range of variability. L. pennellii had a high density of type IV trichomes (25.7/mm²), whereas type IV trichomes were absent on L. esculentum varieties. Mean type IV glandular trichomes density in F₁ was higher than resistant parent (32.4 and 34.00/mm² in hybrids L. esculentum ‘Sankranthi’ and ‘Nandi’ × L. pennellii ‘LA 2580’, respectively) (Table 1). A broad range of trichome density was observed in F₂ populations (0 to 35 trichomes/mm²). The mean densities of type IV glandular
trichomes on F₂ plants with either tomato cultivars were intermediate between cultivated tomato and L. pennellii ‘LA 2580’ (Table 1). Estimated broad sense heritability for type IV trichomes density indicated a high heritability for this trait (Table 1).

Treatments significantly influenced the mite responses to the host (oviposition and mortality) and host response to the mite (plant damage). L. esculentum varieties supported the highest mite oviposition and plant damage beside lowest mite mortality. There were no significant differences in mite response to the host and host response to the mite among the L. pennellii ‘LA 2580’, F₁ and F₂ individuals, which indicates that most of the F₂ plants exhibited the same level of resistance (Table 2). Relationship between density of type IV glandular trichomes and resistance to TSSM was strongly positive. Correlation between density of type IV glandular trichomes and mite mortality was strongly positive; whereas it was strongly negative with mite oviposition and damage score (Table 2).

Table 1. Broadsense heritability of type IV glandular trichomes density and chi-square test for goodness of fit for duplicate dominant genes for presence of type IV trichomes in cross L. esculentum × L. pennellii ‘LA2580’

<table>
<thead>
<tr>
<th>Generation</th>
<th>Pedigree</th>
<th>No. plants sampled</th>
<th>Density of type IV trichomes</th>
<th>Presence of type IV trichomes</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range Min-Max</td>
<td>Mean ± SD</td>
<td>h² (%)</td>
</tr>
<tr>
<td>P₁</td>
<td>Sankranthi</td>
<td>16</td>
<td>≤ 0 d</td>
<td>25.7±2.5 b</td>
<td>16 0</td>
</tr>
<tr>
<td>P₂</td>
<td>LA2580</td>
<td>16</td>
<td>23-29</td>
<td>32.4±1.9 a</td>
<td>16 0</td>
</tr>
<tr>
<td>F₁</td>
<td>P₁ × P₂</td>
<td>16</td>
<td>28-34</td>
<td>11.4±6.9 c</td>
<td>93 134</td>
</tr>
<tr>
<td>F₂</td>
<td>F₁ × F₁</td>
<td>144</td>
<td>0-33</td>
<td>10.1±6.4 c</td>
<td>93 129</td>
</tr>
</tbody>
</table>

Chi-square test was done against 15:1 ratio
** (p<0.01)
Table 2. Leaf damage score, mite oviposition and mortality on *L. esculentum* (‘Nandi’ and ‘Sankranthi’), *L. pennellii* ‘LA2580’ and their hybrids and their relationship with density of type IV glandular trichomes

<table>
<thead>
<tr>
<th>Generation</th>
<th>Pedigree</th>
<th>No. of eggs¹</th>
<th>No. dead mites ²</th>
<th>Damage (leaf disks)</th>
<th>Damage (plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>P₁</td>
<td>Sankranthi</td>
<td>71 ± 13.6 a</td>
<td>0.25 ± 0.5 b</td>
<td>4.2± 0.7 a</td>
<td>5.5± 0.53 a</td>
</tr>
<tr>
<td>P₂</td>
<td>LA2580</td>
<td>0 b</td>
<td>5.00± 0 a</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>F₁</td>
<td>P₁ × P₂</td>
<td>0 b</td>
<td>4.9± 0.35 a</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>F₂</td>
<td>F₁ × F₁</td>
<td>11.2± 2.6 b</td>
<td>3.9± 1.6 a</td>
<td>0.9± 1.7 b</td>
<td>0.8± 1.6 b</td>
</tr>
<tr>
<td>P₃</td>
<td>Nandi</td>
<td>69.9±14.2 a</td>
<td>0.12± 0.35 b</td>
<td>4.6± 0.5 a</td>
<td>5.5± 0.5 a</td>
</tr>
<tr>
<td>P₂</td>
<td>LA2580</td>
<td>0 b</td>
<td>5.00± 0 a</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>F₁</td>
<td>P₃ × P₂</td>
<td>0 b</td>
<td>5.00± 0 a</td>
<td>0 b</td>
<td>0 b</td>
</tr>
<tr>
<td>F₂</td>
<td>F₁ × F₁</td>
<td>12.5± 2.1 b</td>
<td>3.8± 1.7 a</td>
<td>1.02± 1.8 b</td>
<td>0.99± 1.8 b</td>
</tr>
</tbody>
</table>

Linear correlations (r)

<table>
<thead>
<tr>
<th>Density of type IV glandular trichomes</th>
<th>LA2580 × Sankranthi</th>
<th>LA2580 × Nandi</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA2580 × Sankranthi</td>
<td>-0.60**</td>
<td>0.65**</td>
</tr>
<tr>
<td>LA2580 × Nandi</td>
<td>-0.57**</td>
<td>0.65**</td>
</tr>
</tbody>
</table>

Means with the same letter in each column are not significantly different at p < 0.05 using Duncan Multiple Range Test

¹ Number egg / 5 females /3day  ² number dead out of 5 mites

**(p<0.01)

**DISCUSSION**

F₂ populations showed a goodness to fit ratio 15:1, suggesting that two dominant unlinked genes control the presence of type IV glandular trichomes. These data support the conclusions of Lemke and Mutschler (1984) and Goffreda and Mutschler (1989). High heritability of the type IV trichomes density indicated the large effect of genetic variance on heredity of this trait. Steady state of environmental conditions during the experiment might have decreased the role of environment.

It was found over expression of type IV glandular trichomes in F₁ hybrids and that was in contrast with findings of Goffreda and Mutschler (1989) and Fernandez-Munoz *et al.* (2003) who reported that the mean type IV trichomes density on F₁ plants were intermediate between *L. esculentum* and *L. pennellii*. Density of type IV glandular trichomes in F₂ plants with either tomato cultivars were intermediate between parents and that was similar to finding of Goffreda and Mutschler (1989) and Fernandez-Munoz *et al.* (2003). Significant and positive relationship between density of type IV glandular trichomes and resistance to TSSM (Table 2) indicated that selection based on the density of type IV glandular trichomes is very useful to identify resistant individuals to TSSM within F₂ populations. Similar finding was reported by Carter and Snyder (1985) in cross *L. esculentum × L. hirsutum* and by Saeidi
et al. (2007) in cross *L. esculentum × L. pennellii* ‘LA 2963’. Aforementioned obtained results was in contrast with finding of Goffreda and Mutschler (1989), who have not observed significant relationship between type IV trichomes density and resistance to aphids in segregating F2 population of *L. esculentum × L. pennellii* ‘LA 716’. One possible explanation is variability in pest resistance of hybrids derived from different *L. pennellii* accessions and *L. esculentum* varieties.

Density and secretion (Weston et al., 1989; Goffreda and Mutschler, 1989) of type IV glandular trichomes has been reported to be the basis for resistance in *Lycopersicon* species to pests. Simmons et al. (2003) reported a significant positive relationship between density of type IV glandular trichomes in *L. pennellii* and mortality of *M. persicae*. Good and Snyder (1988) suggested that, in F2 progenies of *L. esculentum × L. hirsutum*, density of type IV trichomes is the main factor responsible for host plant resistance to the mites.

Collected data and obtained results showed possibility of increasing the level of pest resistance in cultivated tomato by introgression with *L. pennellii*. It has proven that glandular trichomes play role in non-preference of tomato by white fly and consequent management of tomato leaf curl menace, therefore the present study would pave the way for developing multiple pest resistance in tomato. Resistant cultivars can contribute to the stability of tomato production and serve as the foundation of integrated pest management.

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**REFERENCES**


