Resistance of Certain Wild Tomato (Solanum spp.) Accessions to Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) Based on Choice and No-Choice Bioassays

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Resistance of certain wild tomato (*Solanum* spp.) accessions to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) based on choice and no-choice bioassays

Asif Ali¹², Mohamed Rakha², Farid Asif Shaheen¹, and Ramasamy Srinivasan²*¹

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**Abstract**

The development of cultivars resistant to tomato fruit borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), are very limited in Pakistan and other parts of the world because of the lack of good sources of resistance for breeding. Insect-resistant cultivars would be beneficial to decrease the indiscriminate use of pesticides on tomato crops. Seven wild tomato accessions, including *Solanum galapagense* Darwin & Peralta, *Solanum pimpinellifolium* L., *Solanum cheesmaniae* (Riley) Fosberg, and a susceptible check (*Solanum lycopersicum* L.) (all Solanaceae), were evaluated for resistance to tomato fruit borer based on oviposition preference and feeding in the choice and no-choice bioassays. In the oviposition preference test, *S. cheesmaniae* (VI037240) and *S. galapagense* (VI063174) recorded the lowest mean number of eggs, 4.40 and 7.00 per plant, respectively. The highest mortality of *H. armigera* larvae (90%) was recorded on *S. pimpinellifolium* (VI030462) compared to the susceptible check (15%). The lowest pupal weight (89.67 mg) resulted from *S. galapagense* (VI063174), but it was not significantly different from the *S. pimpinellifolium* and *S. cheesmaniae* accessions. Among all tested accessions, *S. pimpinellifolium* (VI030462) was found to be highly resistant to *H. armigera* based on larval mortality, pupal weight, and pupation percentage. These results can pave the way to enhance the resistance in cultivated tomato varieties to control this notorious fruit borer.

**Key Words:** tomato fruitworm; wild species; resistance; oviposition; antibiosis; mortality

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**Resumen**

El desarrollo de cultivares resistentes al barrenador de la fruta del tomate, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), es muy limitado en Pakistán y otras partes del mundo debido a la falta de buenas fuentes de variedades resistentes para el fitomejoramiento. Los cultivares resistentes a los insectos serían beneficiosos para disminuir el uso indiscriminado de pesticidas en los cultivos de tomate. Se evaluaron siete accesiones de tomates silvestres, incluyendo *Solanum galapagense* Darwin y Peralta, *Solanum pimpinellifolium* L., *Solanum cheesmaniae* (Riley) Fosberg y un control susceptible (*Solanum lycopersicum* L.) (todas Solanaceae), para determinar la resistencia al barrenador de la fruta del tomate en base de la preferencia de oviposición y alimentación en bioensayos de elección y no elección. En la prueba de preferencia de oviposición, *S. cheesmaniae* (VI037240) y *S. galapagense* (VI063174) recibieron el número promedio más bajo de huevos, 4.40 y 7.00 por planta, respectivamente, en bioensayos de elección y de no elección. La mortalidad más alta de larvas de *H. armigera* (90%) se registró en *S. pimpinellifolium* (VI030462) en comparación con el control susceptible (15%). El peso pupal más bajo (89,67 mg) resultó de *S. galapagense* (VI063174), pero no fue significativamente diferente de las accesiones de *S. pimpinellifolium* y *S. cheesmaniae*. Entre todas las accesiones analizadas, se encontró que *S. pimpinellifolium* (VI030462) es altamente resistente a *H. armigera* en función de la mortalidad de las larvas, el peso de las pupas y el porcentaje de pupación. Estos resultados pueden ser un camino para mejorar la resistencia en las variedades de tomate cultivadas para controlar este notorio perforador de frutas.

**Palabras Clave:** gusano de tomate; especies silvestres; resistencia; oviposición; antibiosis; mortalidad
(Shaheen 2008). However, management of *H. armigera* with pesticides is harmful to human beings, as well as beneficial insects. The dependence on chemical pesticides have several adverse effects, including insect resurgence, outbreak of secondary pests, environmental contamination, and resistance development (Kranthi et al. 2002). For instance, this pest has developed resistance to chemical pesticide groups including organophosphates, organochlorines, pyrethroids, and carbamates (Ahmad et al. 2001).

Development of *H. armigera* resistant cultivars has remarkable potential in integrated pest management. However, there are no such tomato cultivars with high resistance to *H. armigera* available globally. Screening of wild tomato accessions and identification of new sources of resistance are the first steps toward developing resistant cultivars for control of herbivores. Resistant sources to *H. armigera* already have been reported in *Solanum pennellii* Correll (Solanaceae), *Solanum habrochaites* Knapp & Spooner (Solanaceae), *S. pimpinellifolium*, and *S. galapagense* accessions (Selvanarayanan & Narayanasamy 2006; Talekar et al. 2006; Silva et al. 2016). The resistance in these species was related to the presence of glandular trichomes (Bleeker et al. 2012). The glandular trichomes produce and exude allelochemicals. For instance, *S. pennellii* accessions contain acyl sugars that constitute 90% of the substances secreted by type IV trichomes (Mutschler et al. 1996). These compounds have been associated with antibiosis and antixenosis resistance mechanisms (Maluf et al. 2010; Dias et al. 2016). Several yr ago, some accessions of *S. galapagense* were identified as the best whitefly resistant source, which were associated with the presence of type IV trichomes (Simmons & Gurr 2005; Firdaus et al. 2013).

Although development of insect-resistant cultivars is much more difficult than disease-resistant cultivars, due to linkage drag (the association between insect-resistance genes with other genes from wild species-conditioning poor horticultural traits) (Kohler & St. Clair 2005), tomato wild species including *S. galapagense*, *S. pimpinellifolium*, and *S. cheesmaniae* have high potential in insect-resistant breeding programs because of their genetic and morphological characteristics, as well as producing fruits with desirable colors (Darwin et al. 2003; Liedl et al. 2013). In addition, these species are closely related to the cultivated tomato, which may facilitate rapid introgression of the resistance components. In cultivated tomato, 3 genotypes, namely ‘Sahil,’ ‘Pakiti,’ and ‘Nova Mech,’ were identified as the least infested (12.30–13.96%) by *H. armigera* in Pakistan (Sajjad et al. 2011). A subsequent study also found 3 other genotypes, ‘Chinar,’ ‘Sourabhi,’ and ‘Sultan,’ had a minimum number of infested fruits (21.40–25.43%) by *H. armigera* (Usman et al. 2013). However, these tomato genotypes cannot be considered as resistant based on the recorded fruit damage of 12 to 25%. Hence, it has become highly imperative to identify tomato genotypes with appreciable levels of resistance to *H. armigera*.

A total of 8 wild tomato accessions, including *S. cheesmaniae*, *S. galapagense*, and *S. pimpinellifolium* were identified recently as resistant to whitefly (*Bemisia tabaci* Genn.; Hemiptera: Aleyridae), the two-spotted spider mite (*Tetranychus urticae* Koch; Prostigmata: Tetranychidae), and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Rakha et al. 2017a, b, c). Hence, the objective of this study was to screen selected wild tomato accessions that are resistant to whitefly and spider mite against *H. armigera*.

Materials and Methods

**PLANT MATERIALS**

Eight accessions including 5 accessions of *S. galapagense*, 1 accession each of *S. pimpinellifolium* and *S. cheesmaniae*, and a susceptible check (*S. lycopersicum*) were examined in the present study. These accessions have been confirmed already for their resistance to *B. tabaci*, *T. urticae*, and *T. absoluta* (Rakha et al. 2017a, b, c). The seeds were obtained from the Tomato Breeding Unit of the World Vegetable Center. Seeds were sown in 72-plug nursery trays with 40 mL of peat moss per cell. Three weeks after sowing, seedlings of each accession were transplanted into 14 cm pots with potting soil. Plants were watered daily and fertilized with N-P-K 15-15-15 once per wk. Six-wk-old plants were tested for *H. armigera* for oviposition and feeding bioassays during Nov and Dec 2015 at the World Vegetable Center’s growth rooms in Shanhua, Tainan, Taiwan.

**INSECT REARING**

Larvae of *H. armigera* were reared on a semi-synthetic diet using the diet of a polyphagous insect, beet armyworm, *Spodoptera exigua* Hübnner (Lepidoptera: Noctuidae) (Product #F9219B; Bio-Serv, French Town, New Jersey, USA). Insects were reared in controlled conditions under 14:10 h (L:D) photoperiod cycle at 27 ± 1 °C and 70 ± 10% RH. After emergence, larvae were placed in polystyrene cups (22 × 15 × 4 cm) (Hwa Hsing Chemical Co., Taichung, Taiwan) with artificial diet until the second or early third instar. They were then individually reared in cups (4.5 cm high × 4 cm wide) with lids until pupation. Pupae were kept in acrylic cylinders (30 cm height × 15 cm diam) (Kaohsiung Monomer Company, Kaohsiung, Taiwan) for adult emergence. Each acrylic cylinder contained 1 tomato plant with a total of 10 pairs of adults, and included a cotton plug containing 10% honey solution. Emerged adults were allowed to mate and produce further generations. Larvae and adults of *H. armigera* were used for choice and no-choice bioassays. Adults of *H. armigera* used in choice and no-choice bioassays were allowed to mate for 24 h in insect cages provided with 10% honey solution.

**OVIPOSITION BIOASSAYS**

Choice bioassays were conducted in Oct and Nov 2015 using 6-wk-old tomato plants. Five plants of each accession and the susceptible check were transferred to the growth room. All the plants were arranged in a completely randomized design with 1 plant per trial unit. The trial was carried out on steel benches with a plant spacing of 20 cm. The temperature of the plant growth chamber was reduced slowly from 30 °C to 26 °C for the plants to adjust to suitable optimum temperature (27 ± 1 °C) and the inside environment (70% RH, 16:8 h [L:D] cycle). Forty pairs of mated adults (within 24 h) were released into the growth room for oviposition. The cotton plugs soaked with honey solution were provided in the growth rooms continuously until the end of the experiment. After 3 d of egg laying, the number of eggs on stem, adaxial, and abaxial leaf surfaces was recorded for each plant using a hand lens with an appropriate magnification power (Srinivasan 2003).

**FEEDING BIOASSAY**

No-choice bioassays were carried out using plastic jars (30 cm length × 8 cm diam) (Hwa Sheng Plastic Industry Co., Ltd., Tainan, Taiwan) covered with muslin cloth. Six-wk-old plants were moved to the growth room in plastic jars. Five replications of each accession were maintained to conduct the experiment following the completely randomized design. The growth room conditions were 27 ± 1 °C temperature, 70% RH, and 16:8 h (L:D) cycle. One d before insect release, adults of *H. armigera* were allowed to mate in separate insect cages (45 × 45 × 45 cm) and provided with 10% honey solution. After 24 h, 1 pair was released into each plastic jar, and provided with 10%
honey solution continuously until the end of the experiment. After 3 d, adults of *H. armigera* were removed from the plants, and the egg numbers on adaxial and abaxial leaf surfaces were recorded for each plant.

**FEEDING BIOASSAYS (NO-CHOICE)**

Second instar larvae of *H. armigera* were obtained from the insect colony and placed into the tagged polystyrene cups individually. Fresh leaves from each wild accession and the susceptible check were provided each d to the caterpillars until pupation. Caterpillars were shifted to new sterilized polystyrene cups individually every 2 d. Twenty larvae were placed separately for each replication, and 3 replications per accession were maintained. Data on larval mortality was recorded at 3 d intervals until pupation. Pupal weight and pupation percentage were recorded. Experiments were performed in a controlled environment, 16:8 h (L:D) cycle at 27 ± 1 °C and 70 ± 10% RH (Sharma et al. 2005).

**STATISTICAL ANALYSIS**

Statistical software SAS (version 9.1; SAS Institute, Cary, North Carolina, USA) was used to carry out analysis of variance (ANOVA) on data collected from oviposition and feeding bioassays. Data on number of eggs from the no-choice and choice bioassays were transformed using natural logarithm before analysis. Larval mortality and pupation percentage data in feeding bioassays were transformed using the arc-sine transformation. Data on pupal weight was transformed using square-root transformation. After data transformation, data on pupal weight and pupation percentage were recorded. Data on larval mortality were transformed using natural logarithm before analysis. Larval mortality and pupation percentage data in feeding bioassays were transformed using the arc-sine transformation. Data on pupal weight was transformed using square-root transformation. After data transformation, data were analyzed using ANOVA with the PROC GLM procedure of SAS®. When significant treatment differences were indicated by the *F*-test at *P* ≤ 0.05, means were separated by Tukey’s Honest Significant Difference (HSD).

Table 1. Mean number of *Helicoverpa armigera* eggs on selected wild tomato accessions compared to the susceptible check CL5915 under choice and no-choice conditions.

<table>
<thead>
<tr>
<th>Tomato species and accession code</th>
<th>Other codes</th>
<th>Choice test</th>
<th>No-choice test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum galapagense</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI037239</td>
<td>LA 436</td>
<td>18.60</td>
<td>18.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.48) bc</td>
<td>(1.54) a</td>
</tr>
<tr>
<td>VI037241</td>
<td>LA 526</td>
<td>36.00</td>
<td>24.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.17) b</td>
<td>(2.02) a</td>
</tr>
<tr>
<td>VI045262</td>
<td>LA 1141</td>
<td>39.20</td>
<td>89.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.49) ab</td>
<td>(3.96) a</td>
</tr>
<tr>
<td>VI063174</td>
<td>LA 0438</td>
<td>12.80</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.55) bc</td>
<td>(1.45) a</td>
</tr>
<tr>
<td>VI063177</td>
<td>LA 0530</td>
<td>31.00</td>
<td>87.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.09) b</td>
<td>(3.39) a</td>
</tr>
<tr>
<td><em>Solanum cheesmaniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI037240</td>
<td>LA 483</td>
<td>4.40</td>
<td>49.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.13) c</td>
<td>(3.23) a</td>
</tr>
<tr>
<td><em>Solanum pimpinellifolium</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI030462</td>
<td>PI 390519</td>
<td>67.60</td>
<td>28.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.99) ab</td>
<td>(2.33) a</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL5915</td>
<td>Check</td>
<td>160.60</td>
<td>48.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.04) a</td>
<td>(3.00) a</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>8.51**</td>
<td>1.28**</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt; 0.0001</td>
<td>0.292</td>
</tr>
</tbody>
</table>

Means followed by different letter(s) within columns are significantly different based on Tukey’s HSD (*P* = 0.05). *These mean values are transformed for normalizing the data. **Values are highly significantly different. ns = non-significant.

**RESULTS**

**OVIPPOSITION BIOASSAY**

Significant differences (*P* < 0.0001) in the number of eggs were found among 7 tested accessions and check in the choice assay (Table 1). The lowest mean number of eggs (4.40 per plant) by *H. armigera* female moths was recorded on *S. cheesmaniae* accession (VI037240), compared to the maximum mean number of eggs (160.60 per plant) on the susceptible check (CL5915) (Table 1). Among the accessions of *S. galapagense*, the lowest mean number of eggs (12.80 per plant) was recorded on VI063174 in contrast to 39.20 eggs per plant on VI045262, which was on par with *S. pimpinellifolium* accession VI030462. Thus, the *S. cheesmaniae* accession VI037240 was found to be highly resistant, followed by *S. galapagense* accessions VI037239 and VI063174. However, the number of eggs laid by *H. armigera* female moths did not differ significantly among tested accessions and the check in no-choice assays (Table 1).

**FEEDING BIOASSAY**

The larval mortality in the feeding bioassay was significantly different among the accessions and the check (*P* < 0.0001) (Table 2). Larval mortality of *H. armigera* after 16 d of feeding on various wild tomato accessions was significantly higher on *S. pimpinellifolium* (VI030462), followed by *S. cheesmaniae* (VI037240), and *S. galapagense* accessions (VI063174 and VI037241). The lowest larval mortality was recorded on the susceptible check CL5915. Larval mortality values for the remaining *S. galapagense* accessions (VI037239, VI063177, and VI045262) were intermediate and similar to either of the extremes. The result revealed that the pupal weight of *H. armigera* did not differ significantly among the accessions and the check (Table 2). Lowest pupation percentage was observed in *S. pimpinellifolium* (VI030462), and it was followed...
by *S. cheesmaniae* (VI037240) and all *S. galapagense* accessions except VI045262, which was on par with the susceptible check CL5915 (Table 2). Thus, the feeding assay also confirmed *S. pimpinellifolium* (VI030462) as a highly resistant accession among tested accessions.

### Discussion

Tomato fruit borer, *H. armigera*, is one of the most destructive pests of tomato in Asia, Africa, Europe, Oceania, and South America. Indiscriminate use of chemical pesticides to control *H. armigera* has led to development of resistance. Host plant resistance is the most efficient method to control *H. armigera*. However, cultivated tomato lines with appreciable levels of resistance to *H. armigera* are scanty. For instance, cultivated line V-29 was found to be moderately resistant to *H. armigera* in Bangladesh (Khanam et al. 2003). Furthermore, a high level of resistance was found in *S. pennelli* and *S. habrochaites* (Talekar et al. 2006), but the development of resistance has been hampered by the quantitative inheritance of resistance and by linkage drag. In the current study, it was determined that the highest level of *H. armigera* resistance is present in *S. pimpinellifolium* accession VI030462. This accession also was found to possess resistance to *T. absoluta*, whitefly, and spider mites (Rakha et al. 2017a, b, c). Our study also confirmed that appreciable levels of *H. armigera* resistance is present in *S. cheesmaniae* (VI037240) and 2 *S. galapagense* accessions (VI063174 and VI037241), besides *S. pimpinellifolium* accession VI030462. These accessions also were found to possess resistance to *T. absoluta*, spider mite, and whitefly (Rakha et al. 2017a, b, c). Thus, the current study revealed that most spider mite and whitefly-resistant accessions also were resistant to *H. armigera*.

Both glandular and non-glandular trichomes in tomatoes contribute toward insect resistance (Firdaus et al. 2013; Lucatti et al. 2013). Trichomes usually interfere with the oviposition of insect pests. For instance, high densities of type IV trichomes and low densities of type V trichomes in resistant tomato accessions were associated with reduced numbers of whitefly and spider mites (Rakha et al. 2017a, b). Hence, it is possible that the glandular trichomes contribute to *H. armigera* resistance as well in these accessions. Besides contributing to antixenosis (reduced oviposition), glandular trichomes also secrete toxic substances which contribute toward antibiosis. Increased mortality of *H. armigera* larvae, with substantial reduction in pupation percentage on resistant tomato accessions in the current study, could possibly be due to the presence of toxic substances produced by glandular trichomes. Rakha et al. (2017a) has demonstrated that all resistant accessions accumulated high levels of total acyl sugars, which were positively associated with type IV trichomes. There was a significant negative relationship between acyl sugar content and spider mite (*T. urticae*) egg numbers. Although ingestion of trichomes and their acyl sugar contents may not be toxic for neonate larvae, they adversely affect the larval growth and development (Weinhold & Baldwin 2011), thus leading to chronic larval mortality. It also was shown that acyl sugars present in the *S. pennelli* LA-716 accession conferred good levels of resistance to arthropod pests (Baier et al. 2015). Also, BARI Tomato-7 was classified as resistant (Amin et al. 2017) due to glandular and non-glandular trichomes, which we considered to be the most important pest resistance factors (Gurr & McGrath 2001; Simmons & Gurr 2005).

Although appreciable levels of insect resistance are present in wild tomato accessions, efforts to introgress this resistance into the cultivated tomato in order to combine it with acceptable fruit qualities were not successful in the past (Talekar et al. 2006). However, insect resistance in VI037240 and VI030462 is noteworthy because these species are closely related to cultivated tomato, and introgression of insect resistance should be relatively straightforward (Rakha et al. 2017b). Crosses between these accessions and cultivated tomato are being made to identify insect resistance genes, and to design markers to facilitate the introgression and pyramiding of insect-resistance genes into cultivated tomato.
Acknowledgments

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