# Development of tomato lines for resistance to sucking and feeding pests

Research Proposal

For

APSA-WorldVeg Vegetable Breeding Consortium

By

World Vegetable Center

World Vegetable Center

**Proposal Summary**

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| **Project title** | Development of tomato lines for resistance to sucking and feeding pests | |
| **Submitted to** | APSA-WorldVeg Vegetable Breeding Consortium Members | |
| **Main WorldVeg contact person** | Mandy Lin (mandy.lin@worldveg.org) | |
| **Main WorldVeg scientists** | Dr. Peter Hanson<br>Dr. Hamid Khazaei<br>Dr. Roland Schafleitner<br>Dr. Paola Sotelo-Cardona | |
| **Project duration** | 3 years (January 2023-December 2025) | |
| **Funding requested (US$)** | 600,000 | Minimum of 15 companies at 40,000 per company |
| | | Maximum of 25 companies at 24,000 per company |

**Objective**

Seed companies incorporate insect resistance into tomato cultivars that enable farmers to reduce pesticide use and produce high quality tomato crops

**Importance**

Tomato farmers rely heavily on pesticides to control insect infestations; unfortunately, the indiscriminate use of chemical pesticides to manage insect pests on tomato has led to the rapid development of pesticide resistance, as well as increased pesticide residues on fruits. Insect resistant tomato cultivars, if available, would allow farmers to substantially reduce pesticides and pesticide costs, lowering health risks for farmers and consumers, and making tomato farming more sustainable and environmentally friendly. Insect resistance, a breakthrough trait, would add great value to tomato cultivars.

**Background**

Tomato is attacked by a multitude of insect pests, some causing direct damage and others transmitting viruses (Kennedy, 2003; Srinivasan 2010). Insect pests can damage tomato plants and fruits by direct feeding, notably tomato fruitworm (*Helicoverpa armigera),* common armyworm (*Spodoptera litura*), beet armyworm (*Spodoptera exigua*), and the South American tomato pinworm (*Tuta absoluta*); or indirectly through transmission of virus diseases, especially whiteflies (*Bemisia tabaci*) which transmit *Begomoviruses* and *Criniviruses*, thrips (*Thrips palmi*, *Frankliniella occidentalis*) which vector Tospoviruses, and aphids *(Myzus persicae*) that vector Cucumber mosaic virus. Other important insect pests of tomato include spider mites (*Tetranychus urticae*, *T. cinnabarinus*, *T. evansi*), and the tomato leaf miner (*Liriomyza bryoniae*) (Srinivasan 2010).

Consequently, tomato farmers rely heavily on pesticides which constitute a major production cost and pose health risks for farmers, consumers and the environment. In addition, the repetitive use of the same active ingredients has led to the development of insecticide resistance against organophosphates and pyrethroids in whiteflies (Naveen et al. 2017). Moreover, field populations of *T. absoluta* are showing resistance to diamides (i. e. chlorantraniliprole), one of the few effective classes of insecticides in use for its management (Silva et al. 2019). Insecticide resistance has also been detected long ago in *H. armigera* and *S. litura* against organophosphates, pyrethroids, and carbamates in cotton crops India (Kranthi et al. 2002).

Crop domestication and selective breeding in tomato to favor yield and quality traits has led to the loss of important plant defenses characteristics (Paudel et al. 2019). Genetic resistance to insect pests is absent in cultivated tomato (*Solanum lycopersicum*) but has been identified in some wild tomato species, notably *S. habrochaites, S. pennellii*, *S. cheesmanii*, *S. pimpinellifolium*, and *S. galapagense* (Kennedy 2003; Rakha et al. 2017a,b,c; Vosman et al., 2018). Introgression of insect resistance from *S. habrochaites* and *S. pennellii* has been thwarted by complex inheritance and linkage drag. Insect resistance in *S. pimpinellifolium* and *S. galapagense* has attracted much recent interest because these species are close relatives of cultivated tomato and inheritance of resistance may be relatively simple. Insect resistance in *S. galapagense* is mainly based on accumulation of acylsugars, metabolites produced and secreted by type IV glandular trichomes found on leaf and stem surfaces of solanaceous plants (Vosman et al., 2018). Abundant scientific literature offers strong evidence that acyl sugars offer resistance to a broad range of insects, including whiteflies, thrips, aphids, spider mites, armyworm, among others (Maluf et al. 2010; Vosman et al. 2018; Ben-Mahmoud et al. 2018; de Oliveira et al. 2018; Bac-Molenaar et al. 2019; Paudel et al. 2019; Rodríguez-López et al. 2020). Acylsugars released by glandular trichomes hinder insect movement, but also, they act as feeding and oviposition deterrents, and may cause toxicity to different insect pests (Liedl et al. 1995, Mandal et al. 2020).

**Summary of previous work**

In 2014, the World Vegetable Center (WorldVeg) initiated an insect resistance breeding program targeting whiteflies to achieve sustainable TYLCD control by combining vector and virus resistance. Whitefly resistance offers a second line of defense against whitefly-transmitted viruses and helps "protect" TYLCD (Ty) genes and thus makes resistance more durable. Targeted insect pests were broadened to include *Tuta absoluta*, spider mites, thrips, and tomato fruitworm. Through systematic screening of selected WorldVeg genebank accessions for high densities of glandular type IV trichomes, followed by no-choice assays, six *S. galapagense* accessions were identified demonstrating high levels of resistance to the sweet potato whitefly (*B. tabaci*) based on reduced numbers of eggs and nymphs, and percent adult mortality. Additional resistance was detected in *S. habrochaites* and *S. cheesmanii*.

**Introgression of insect resistance into tomato**

WorldVeg focused on insect resistance from *S. galapagense* because this species is closely related to cultivated tomato and breeding progress is expected to be faster. *S. galapagense* accessions were crossed to the recurrent parent CLN3682C, a WorldVeg tomato line homozygous for *Ty1/3*, *Ty-2*, *Bwr-12*, *I2* and *Mi*. $F_2$ populations were screened for whitefly resistance parameters (egg number and adult mortality) in the greenhouse using a no-choice assay; pollen bulked from highly resistant $F_2$ plants was used for backcrossing to the recurrent parent. The above procedure was repeated for each backcross and by spring 2020, $BC_3F_3$ had been achieved. In the 2021 fall season, 10 BC4F1 developed from crosses between four selected $BC_3F_3$ and four tomato parents are under evaluation in a replicated trial for insect resistance and horticultural and fruit traits in the 2021 Fall season. Based on the results, 3-4 $BC_4F_1$ will be selected and selfed to produce $BC_4F_2$ for further evaluation and generation advance to develop insect resistant lines.

**Selection methods**

Combinations of no-choice assays (% adult mortality, egg numbers), glandular trichome densities, and total acyl sugar content of leaf tissue have been used to select for whitefly resistance (Rakha et al. 2017a, b, c). WorldVeg adopted the total acyl sugar (AS) protocol developed by the Mutschler lab at Cornell University to estimate AS content in leaf samples (Leckie et al. 2012). Acyl sugars account for insect resistance in *S. galapagense* and measurement of total AS content is fast and straightforward; nevertheless, combinations with other tests methods including no-choice assays are important to confirm resistance. Mapping QTLs underlying insect resistance and availability of markers for selecting resistant genotypes will be critical to streamline incorporation of insect resistance into commercial varieties.

**Preliminary mapping of whitefly resistance**

Parents, F1, and $BC_3F_2$ plants were evaluated for type IV trichome density (no./$mm^2$) and whitefly parameters (egg number, adult mortality (%) using a no-choice assay. A major QTL on chromosome 2 associated with type IV trichome density (LOD 5.2), adult mortality (LOD 10.6) and egg number (LOD 9.1) was detected, explaining 14%, 21% and 24% of the variation, respectively. Very likely this is the major QTL previously mapped in a *S. galapagense* accession on chromosome 2 (Andrade et al. 2018) named *Wf-1* (Firdaus et al. 2013). *Wf-1* includes a transcription factor involved in type IV trichome development, production of 76 acyl sugars, and methyl esters of the flavonols myricetin and quercetin (Vosman et al. 2018; Vosman et al. 2019). Besides *Wf-1*, additional insect resistance QTL in *S. galapagense* have been mapped on chromosomes 3 (Andrade et al. 2018), chromosome 9 (Firdaus et al. 2013)

**Field Evaluations**

***2020 spring***: A population of 240 $BC_3F_3$ plants derived from *S. galapagense* and homozygous for *Wf-1* were individually assessed for total AS levels at three dates. Total AS content over all $BC_3F_3$ and the three dates was 6.50 ± 2.74 µmol/g dry leaf tissue. AS means for individual $BC_3F_3$ plants over the three sample dates ranged from 1.65–18.47 µmol/g dry leaf tissue. Fruit weight means ranged from 15.1–45.5 g with an average of 31.4 g. Large variation in AS levels pointed to additional QTLs underlying whitefly resistance besides *Wf-1*.

***2020 Fall trial**:* Twelve $BC_3F_4$ lines were assessed in a replicated trial for total AS content measured at 32, 48, 78, and 94 days after transplanting (DAT) from October 2020-January 2021. Mean entry AS levels over the four dates ranged from 1.88–8.40 µmol/g dry leaf tissue. The highest mean AS level over entries was found at 48 DAT and levels declined to 3.76 and 3.68 µmol/g dry leaf tissue at 78 and 94 DAT, respectively, coinciding with lower average temperatures and increased plant age. Large differences among single plants within entries indicates segregation for AS. AS means of the selected individual plants ranged from 8.66–13.37 µmol/g dry leaf tissue.

**Proposed outputs**

**Output 1. Intermediate stage (non-segregating) insect resistant lines developed, distributed and tested by participants**

6-8 whitefly-resistant, high acyl sugar, $BC_4F_3$ (homozygous for Ty1/3 and Bwr-12) will be multiplied in the 2022-2023 fall season (November 2022-March 2023) at WorldVeg. After seed treatment and passing SHQ and BAPHIQ viroid and virus tests, seed will be available to project partners for insect resistance evaluations and for use in breeding by the beginning or middle of 2023. WorldVeg will coordinate the testing of the lines in field trials and controlled greenhouse trials under different seasons to better understand environmental effects (temperatures, rainfall, etc.) and plant age on acyl sugar levels and whitefly resistance.

**Output 2. Insect resistance workshop conducted for participants**

A tomato insect resistance workshop (two weeks) will be offered to project participants at the WorldVeg South Asia research station in Hyderabad, India, and a second workshop at the WorldVeg Southeast Asia research station in Thailand. Topics to be covered include insect resistance and resistance mechanisms; hands-on training in methods to assess insect resistance (trichome analysis, measurement of acyl sugars; choice and no-choice assays); methods for mass rearing of insects.

**Output 3: Intermediate stage (non-segregating) insect resistant lines evaluated for resistance to sucking pests and caterpillar pests through no-choice assays conducted at WorldVeg**

$BC_3F_5$ lines will be screened in controlled no-choice trials for resistance to resistance to sucking pests (*Myzus persicae*, *Thrips palmi/ Frankliniella occidentalis*), and caterpillar pests *Spodoptera exigua*, *Helicoverpa armigera*, *Tuta absoluta* through no-choice assays conducted at WorldVeg Taiwan, other labs in Taiwan or India. The lines will be grown in the field in Taiwan and in India in dry and wet seasons. Whitefly parameters (eggs, nymphs), and acyl sugar levels will be assessed at different plant growth stages to monitor environmental effects (temperatures, rainfall, etc.) and plant age on acyl sugar levels and insect resistance.

**Output 4: Loci associated with insect resistance validated and fine-mapped, and previously additional undetected loci identified**

Two mapping populations will be developed from crosses between multiple disease resistant *S. lycopersicum* and insect-resistant $BC_3F_4$ lines derived from *S. galapagense* accessions to locate QTLs for pest resistance. Populations will be genotyped and phenotyped for whitefly resistance components including adult mortality, egg number, type IV trichome density and total AS content. The previously mapped QTL on chromosome 2, *Wf-1*, will be fine-mapped, and additional QTLs will be identified. Validated resistance gene alleles will be tagged with molecular markers to support breeding for insect resistance. Marker protocols will be provided to participants for use in breeding.

**Output 5. Near-commercial insect resistant tomato lines developed**

Selected $BC_4F_4$ insect resistant lines will be individually crossed to multiple disease resistant lines representing 3-5 major tomato market segments. Selection within segregating $BC_4$ populations will be carried out using markers developed from Output 4 to incorporate insect resistance QTLs and reduce the size of wild tomato introgressions, and additional markers distributed throughout the genome will be applied to select against *S. galapagense* introgressions not associated with insect resistance. During generation advance, selection will be practiced for multiple disease resistance (bacterial wilt, TYLCD, fusarium wilt, TMV, early blight), plant traits (vegetative vigor, good vine cover) and fruit traits (high fruit set, >80 g, firm, deep red internal and external color).

**Timeline**

| | **Months** | | | | | |
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| **Activity** | **6** | **12** | **18** | **24** | **30** | **36** |
| Inception workshop and insect resistance methods workshop | | | | | | |
| 6-8 insect resistant $BC_4F_3$ lines completed, multiplied and distributed to project participants for use in breeding | | | | | | |
| Evaluation of intermediate-stage lines for resistance to diverse sucking pests and leaf-feeding pests | | | | | | |
| BC4F2 populations created and MAS conducted for insect resistance QTL, disease resistances, plant and fruit traits. | | | | | | |
| Development and delivery of near-commercial insect resistant lines | | | | | | |

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