







# Development of molecular markers associated with tomato fruitworm (Helicoverpa armigera Hübner) resistance components in wild tomato (Solanum pimpinellifolium L.)

# Pishayapa Thongmalai<sup>1</sup>, Mohamed Rakha<sup>2</sup>, Ramasamy Srinivasan<sup>2</sup>, Shaw-Yhi Hwang<sup>1</sup>

<sup>1</sup>Department of Entomology, National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung, 402 Taiwan <sup>2</sup>World Vegetable Center, PO Box 42, Shanhua, Tainan, 74199 Taiwan Corresponding author: srini.ramasamy@worldveg.org

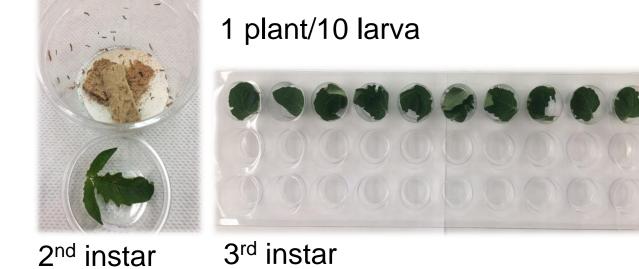
### Abstract

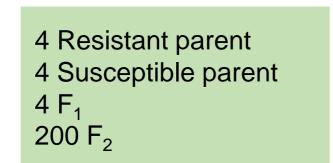
Tomato fruitworm (Helicoverpa armigera Hübner) is a major production constraint to cultivated tomato (Solanum lycopersicum L.) in the tropics and subtropics. Developing pest-resistant cultivars would be an alternative control approach, and contribute to a reduction in the misuse of chemical pesticides in tomato production. Here, molecular markers, glandular trichomes and acylsugars associated with tomato fruitworm resistance were investigated. A total of 200 F<sub>2</sub> plants derived from the interspecific hybridization between WorldVeg breeding lines S. lycopersicum CLN3682C and S. pimpinellifolium VI030462 were genotyped using 8 putative resistance loci previously identified for whitefly resistance on chromosomes 3, 5, 6, 7, 9 and 11. The same plants, along with resistant and susceptible parents, their F<sub>1</sub> and susceptible check tomato line, were bioassayed for larval mortality, larval weight, pupal duration, and egg number using a no-choice test at 7 and 13 weeks after sowing. The results show that the mortality rate of larvae feeding on F<sub>2</sub> populations for 10 days positively correlated with density of type IV trichomes in 7-week-old plants. Type IV trichomes and acylsugar production showed recessive gene action, because the F<sub>1</sub> was skewed strongly toward the susceptible parent. A total of 12, 2, 3, 1 and 9 CAPS markers in 4 regions were significantly associated with the density of type IV trichomes, larval mortality, pupal duration, larval weight and acylsugars, respectively. More studies are underway to confirm these markers in F<sub>3</sub> and BC<sub>1</sub>F<sub>2</sub> populations, which would be very useful for marker-assisted selection in our breeding program for insect resistance.

**Keywords:** Cleaved amplified polymorphic sequence, insect resistance, Solanum lycopersicum L., marker-assisted selection

### **Materials & Methods**

### No-choice assay test







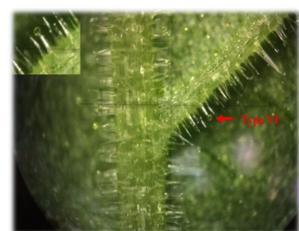


Data recorded: Larval mortality for 10 days Larval weight Pupal duration 7- and 13-week-old-plants

After 10 days, larval weight was recorded and larvae moved to an artificial diet.

#### **Trichome analysis**





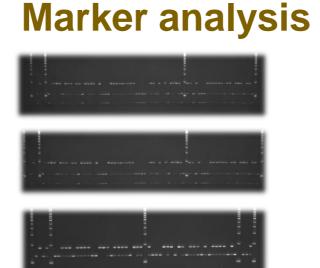
Ranks used: 0 = 01 = <12 = 1-53 = 6-104 = >11

#### **Acylsugar analysis**





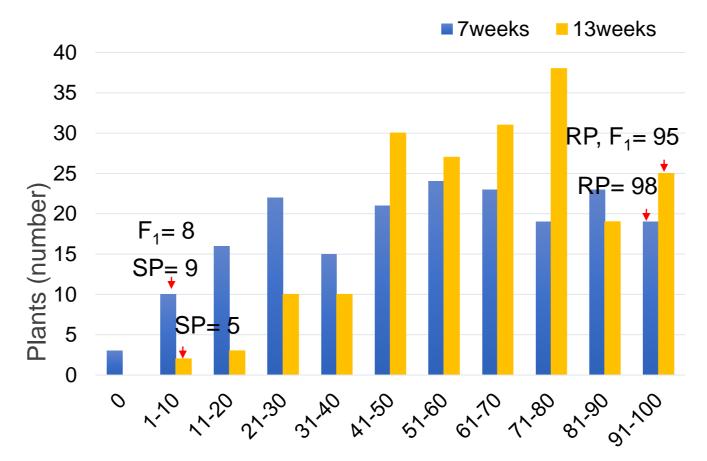




Sixteen CAPS markers associated with whitefly resistance parameters were used to genotype 200 F<sub>2</sub>, resistant parent, susceptible parent, and F₁ plants.

# Results

Frequency distribution of 10 days mortality in a 195 plant F<sub>2</sub> population



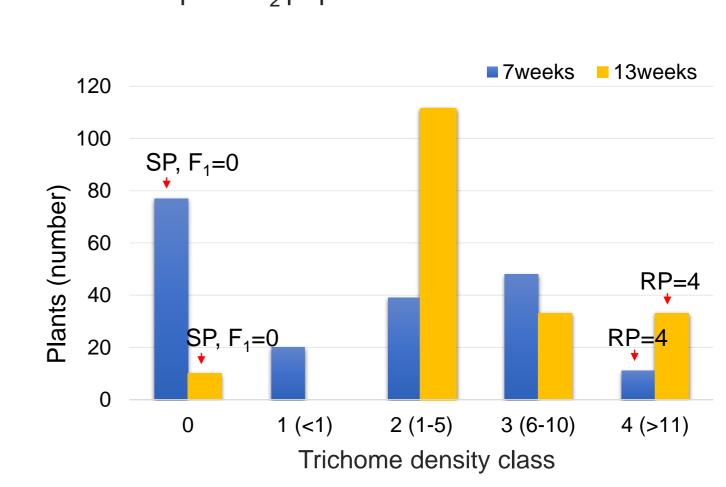
Tomato fruitworm 10days mortality (%)

Linear correlations between tomato fruitworm resistance parameters, trichome types, and acylsugars of F<sub>2</sub> population evaluated in no-choice bioassays

Parameters	Trichome type				Acylsugars
raiameters	IV	Spherical VI	Intermediate VI	Lobe VI	
7 weeks					
10 days mortality	0.30**	0.02 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.23**	0.22**
Larval weight	-0.10 <sup>ns</sup>	0.07 <sup>ns</sup>	0.23 <sup>ns</sup>	-0.16 <sup>ns</sup>	-0.12 <sup>ns</sup>
Pupal duration	-0.02 <sup>ns</sup>	0.29*	-	-0.05 <sup>ns</sup>	-0.16 <sup>ns</sup>
10 weeks					
Acylsugars	0.45**	0.6 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.12 <sup>ns</sup>	
13 weeks					
10 days mortality	0.20*	0.07 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.07 <sup>ns</sup>	0.14*
Larval weight	-0.25**	-0.11 <sup>ns</sup>	-0.09 <sup>ns</sup>	0.05 <sup>ns</sup>	-0.20*
Pupal duration	0.23 <sup>ns</sup>	0.17 <sup>ns</sup>	0.02 <sup>ns</sup>	0.22 <sup>ns</sup>	0.11 <sup>ns</sup>

\* and \*\* indicate significance at P < 0.05 and P < 0.001, respectively; ns indicates not significant

Frequency distribution of type IV trichome density in a 195 plant F<sub>2</sub> population



Stepwise multiple regression analysis of putative QTL linked to tomato fruitworm resistance in an F<sub>2</sub> population

			pupal	
	IV trichome	Mortality	duration	Acylsugars
Model	Model R <sup>2</sup>	Model R <sup>2</sup>	Model R <sup>2</sup>	Model R <sup>2</sup>
Single marker analysis- Lo	cus R <sup>2</sup>			
(single QTL)				
Marker 2 (58.87/3)	0.07**	-	-	0.05**
Marker 4 (58.42/3)	0.07**	-	-	0.05**
Marker 6 (63.24/5)	0.11**	-	-	0.07**
Marker 9 (64.17/5)	0.09**	-	0.11*	0.05*
Marker 10 (43.07/6)	-	0.09**	-	-
Marker 11 (45.13/6)	0.03*	0.03*	-	-
Marker 14 (5.17/9)	-	-	0.13*	-
Significant marker model- N	flodel R <sup>2</sup>			
(QTLxQTL effects)				
2+6	-	-	-	0.10*
6+4	0.17**	-	-	-
6+4+11	0.20*	-	-	-

\* and \*\* indicate significance at P < 0.05 and P < 0.001, respectively; ns indicates not significant

# **Major findings**

- Type IV trichomes and acylsugars were significantly associated with tomato fruitworm resistance parameters based on nochoice assay in 7- and 13-week-old plants.
- Overall, 13-week-old plants were more resistant to tomato fruitworm than 7-week-old plants.
- Type IV trichome and acylsugars production showed recessive gene action because the F<sub>1</sub> was skewed strongly toward the susceptible parent in 7-week-old plants.
- A total of 12, 2, 3, 1 and 9 CAPS markers in 4 regions were significantly associated with density of type IV trichomes, larval mortality, pupal duration, larval weight and acylsugars, respectively.

#### References

- ➤ Rakha, M., N. Bouba, S. Ramasamy, J.-L. Regnard, and P. Hanson. 2016. Evaluation of wild tomato accessions (Solanum spp.) for resistance to two-spotted spider mite (Tetranychus urticae Koch) based on trichome type and acylsugar content. Genet. Resour. Crop Evol. 64(5): 1011-1022.
- ➤ Rakha, M., P. Hanson, and S. Ramasamy. 2015. Identification of resistance to Bemisia tabaci Genn. in closely related wild relatives of cultivated tomato based on trichome type analysis and choice and no-choice assays. Genet. Resour. Crop Evol. 64(2): 247–260.

# Acknowledgments

The authors thank the World Vegetable Center and Ministry of Science and Technology (Taiwan) for financial support, and the Taiwan Agricultural Research Institute for providing the tomato fruitworm colony. Core funding to support WorldVeg activities worldwide is provided by the Republic of China (ROC), UK aid, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea and Japan.