# California Melon Research Board
# 2012 Annual Report

I. Project title:

Comparative evaluation and breeding of new sources of host plant resistance to CYSDV and development of field-based serological detection of CYSDV.

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V. Location(s) where work was performed

USDA-ARS, U.S. Agricultural Research Station, Salinas, California,

University of California, Plant Pathology Department, Davis, California

University of California, Desert Research and Extension Center (DREC), Holtville

VI. Objectives

A. Evaluate exotic melon germplasm from India for potential new sources of resistance to CYSDV.
B. Characterize host plant resistance to CYSDV and introgress to western U.S. shipping type background adapted to the desert southwest U.S.
    1. Three putative resistant plants identified in 2009 and 2010.
    2. TGR-1937.
    3. Inter-crosses of PI 313970, TGR-1551 and TGR-1937.
    4. Continue to select and introgress resistance to western U.S. shipping type background adapted to the desert southwest U.S.
C. Evaluate lateral flow devices (also known as dip sticks or immunostrips) for detection of CYSDV in the field.

VII. Results and Analysis

Objective A. Evaluate exotic melon germplasm from India for potential new sources of resistance to CYSDV.

One hundred melon accessions from India were planted on 15 August for evaluation of their reactions to CYSDV in a replicated test at DREC. This was a repeat of the group planted that adversely affected in 2011 by gophers, and severe weather damage (hail, rain, winds). Many of the Plant Introductions appeared resistant 7-wks post-planting (WPP), but by 10 WPP CYSDV symptoms were extensive. Cuttings were taken from 18 accessions 10 WPP for self- and cross-pollination in a greenhouse at Salinas.

Objective B. Characterize host plant resistance to CYSDV and introgress to western U.S. shipping type background adapted to the desert southwest U.S.

*Selection for resistance*
Forty-nine selfed and crossed progenies were compared with three susceptible cultivars (Top Mark, Impac and Laredo) and previously reports sources of resistance to CYSDV (PI 313970, TGR-1551, TGR-1937) were compared with nine putative sources of resistance to CYSDV, and 40 selfed and crossed progenies in a naturally infected, un-replicated field test, Holtville, CA, 2012, 10 weeks post-planting. The test was planted on 16 August. Symptoms, which were rated using a 1 (<10% symptomatic foliage) to 10 (100% symptomatic foliage) visual scale, were clearly evident 7 WPP (data not shown) and by 10 WPP CYSDV symptom severity ratings ranged from 4 to 10 on a plot basis (Table 1). 'Laredo' could not be rated for CYSDV because of nearly complete collapse of the plants. Symptoms on the three resistance sources were more severe than anticipated. The three resistant sources did, however, have healthy terminal buds with many open and immature flowers 10 WPP, in contrast to the susceptible cultivars that had few open or immature flowers at that time.

Self-pollinated progenies obtained from six of the 12 single plant selections in the 2011 Plant Introduction test varied in their reactions to CYSDV, and ranged from 4 to 7 (Table 1). Two $F_1$ progenies of PI 145594 with 'Top Mark' and 'Impac' were rated 10 and 8, respectively, which indicates resistance in this line is recessive in nature. PI 123689 and PI 123496 were rated 4 and 5, respectively, and so are of interest for further studies in replicated tests and in crosses with susceptible melons.

One (PI 614479) of three Plant Introductions selected in 2009 or 2010 was rated 3 for CYSDV symptom severity and so is of interest for further research and crossing. The other two were rated 7 for CYSDV symptom severity, and of less interest.

One goal is to transfer resistance from PI 313970 to western shipper type melon (WSTM). An $F_4$ selection from the cross of 'Top Mark' with PI 313970 was rated 6, the same as PI 313970 (Table 1). As expected, the five $BC_1$ progenies to either 'Top Mark' or 'Impac' were susceptible; these will be selfed to create a segregating generation for selection of resistant segregants more like WSTM. The five $S_1BC_1$ progenies exhibited uniformly high symptom, i.e., they did not appear to segregate for symptom expression.

Previous data suggested that the combined resistances from PI 313970 and TGR-1551 might provide higher, or more stable or uniform expression of resistance to CYSDV. To this end, nine of 12 $F_4$ progenies from the cross of PI 313970 with TGR-1551 exhibited either similar (five progenies) responses as the parents, or more resistant responses than the parents (Table 1). One of five $F_2$ families from the cross of resistant $F_2$ segregants from PI 313970 x TGR-1551 exhibited higher resistance than either parent, and three others were equal to PI 313970.

*Enzyme linked immunosorbent assay (ELISA) for selection of resistant plants.*
Virus titers were determined using ELISA of selected samples 7 WPP. One leaf was taken from two plants each of three susceptible melon cultivars (Top Mark, Impac and Laredo) and the three previously reported sources of resistance to CYSDV. One leaf was taken from each of 15 plants of three $F_2$ families from crosses of 'Impac' with resistant $F_2$ (PI 313970 x TGR-1551) selections. The sampled plants were rated for CYSDV symptoms using the 1 to 10 visual scale with one modification; those that appeared asymptomatic were rated 0. Leaves from the plants rated $\geq$1 exhibited incipient (faint) yellowing; those from plants rated 0 were taken from comparable positions.

Scatter plots of the susceptible and resistant entries clustered accordingly with one exception, where the susceptible plants had CYSDV ratings >4 and ELISA values $\geq$0.178, and the resistance sources had CYSDV ratings that ranged from 1 to 3 and ELISA values that ranged from 0.000 to 0.303. Thus, as in previous years, there was overlap in terms virus titer between susceptible and resistance genotypes (Figure 1). The $F_2$ data revealed less than perfect correlation between visual symptoms and virus content. For example, one individual of progeny 36958 was rated 10 for CYSDV symptoms but had a virus titer comparable to a plant of TGR-1551 that was rated 1 for symptoms.

*Genetic studies.*
Four genetic studies from controlled crosses were planted 15 August and evaluated 10 WPP.

1. *TGR-1937*. Resistance in this accession appeared to be recessive to susceptibility.*
2. *TGR-1551*. Resistance in this accession appeared to be recessive, not dominant as reported from controlled inoculation studies in Spain. Research in Texas suggested recessive or multigenic control.*
3. *TGR-1937 x TGR-1551*. The data were ambiguous, with $F_1$ and $F_2$ data skewed in opposite directions.*
4. *PI 614479*. Resistance in this accession appeared to be recessive to susceptibility.*

* The data may have been adversely affected by *Monosporascus* infection and should be repeated in a *Monosporascus*-free test.

Objective C. Evaluate lateral flow devices (also known as dip sticks or immunostrips) for detection of CYSDV in the field.

We have raised two polyclonal antisera against the *E. coli*-expressed capsid protein of an isolate of CYSDV from the Imperial Valley of California. These antibodies were assessed in two standard detection assays: Western blot and enzyme-linked immunosorbent assay (ELISA) and both were found to effectively detect the virus with relatively little background. One of these antibodies is not being used for ELISA detection of CYSDV in Objective B (3). This year, we conducted preliminary experiments to assess whether this antibody could be used to develop a rapid in-field lateral flow device. We provided a quantity of antisera and purified CYSDV CP to a company (Bioreba) that specializes in this technology. They purified the IgG component of the antisera and used this to make 'agristrips'. The strips were tested with the *E. coli*-expressed CYSDV CP and a sample of CYSDV that was provided from Lebanon. Unfortunately, the strip did not detect either the expressed CP or the virus in the CYSDV field sample. This was considered a preliminary test and the company is interested in continued testing. It is also possible that the antibody is not suitable for the lateral flow technology, and there are examples of antibodies that work in ELISA tests but not in lateral flow tests.

Table 1. Reactions of three susceptible melons, three previously reported sources of resistance to *Cucurbit yellow stunting disorder virus* (CYSDV), nine putative sources of resistance to CYSDV, and 40 selfed and crossed progenies in a naturally infected, un-replicated field test, Holtville, CA, 2012, 10 weeks post-planting.

| Entry | Progeny | CYSDV[z] |
|---|---|---|
| *Susceptible* | | |
| Top Mark | – | 8 |
| Impac | – | 8 |
| Laredo | – | – |
| *Resistant* | | |
| PI 313970 | – | 6 |
| TGR-1551 | – | 5 |
| TGR-1937 | – | 6 |
| *Putative resistance sources* | | |
| PI 145594 ⊗ | 36936 | 7 |
| $F_1$ (PI 145594 x Impac) | 21234 | 10 |
| $F_1$ (PI 145594 x Top Mark) | 21235 | 8 |
| PI 124431 ⊗ | 36937 | 6 |
| PI 124107 ⊗ | 36938 | 6 |
| PI 123689 ⊗ | 36939 | 4 |
| PI 116482 ⊗ | 36940 | 7 |
| PI 123496 ⊗ | 36941 | 5 |
| $F_1$ (PI 123496 x Impac) | 21236 | 7 |
| PI 614479 ⊗ | 36949 | 3 |
| PI 614486 ⊗ | 36960 | 7 |
| PI 614553 ⊗ | 36961 | 7 |
| $F_1$ (PI 614553 x TGR-1551) | 21239 | 10 |
| *Resistance from PI 313970* | | |
| $F_4$ (Top Mark x PI 313970) | 36966 | 6 |
| | 36967 | 6 |
| | 36968 | 6 |
| $BC_1F_3$ Impac (Top Mark x PI 313970) | 21246 | 8 |
| $BC_1F_3$ Top Mark (Top Mark x PI 313970) | 21249 | 9 |
| | 21248 | 8 |
| | 21251 | 8 |
| | 21247 | 8 |
| $S_1BC_1$ Top Mark x $F_3$ (PI 313970 x Top Mark) | 36948 | 8 |
| $S_1BC_1F_2$ Top Mark (Top Mark x PI 313970) | 36947 | 9 |
| $S_1BC_1F_2$ Top Mark (Top Mark x PI 313970) | 36945 | 7 |
| $S_1BC_1F_2$ (Top Mark x PI 313970) Top Mark | 36944 | 7 |
| $S_1BC_1F_3$ Impac (Top Mark x PI 313970) | 36943 | 7 |

(continued)

Table 1 (continued)

| Entry | Progeny | CYSDV[z] |
|---|---|---|
| *Combined resistances from PI 313970 and TGR-1551* | | |
| $F_4$ (PI 313970 x TGR-1551) | 36959 | 7 |
| | 36965 | 7 |
| | 36951 | 5 |
| | 36950 | 4 |
| | 36955 | 5 |
| | 36952 | 6 |
| | 36969 | 6 |
| | 36954 | 7 |
| | 36972 | 3 |
| | 36962 | 4 |
| | 36957 | 4 |
| | 36956 | 5 |
| $F_2$ Top Mark x $F_3$ (PI 313970 x TGR-1551) | 36946 | 8 |
| $F_1F_3$ (PI 313970 x TGR-1551) Impac | 21241 | 9 |
| $F_1F_3$ Impac (PI 313970 x TGR1551) | 21242 | 8 |
| $F_1F_4$ (PI 313970 x TGR-1551) Impac | 21243 | 9 |
| $F_1F_4$ Laredo (PI 313970 x TGR-1551) | 21245 | 8 |
| $F_2BC_1F_2$ [(PI 313970 x TGR-1551) Impac)] Top Mark | 36942 | 7 |
| $F_2F_2$ (PI 313970 x TGR-1551) Impac | 36958 | 6 |
| | 36963 | 6 |
| | 36964 | 4 |
| | 36970 | 7 |
| | 36971 | 6 |

[z]rated on a 1 (<10% symptomatic foliage) to 10 (100% symptomatic foliage) visual scale.

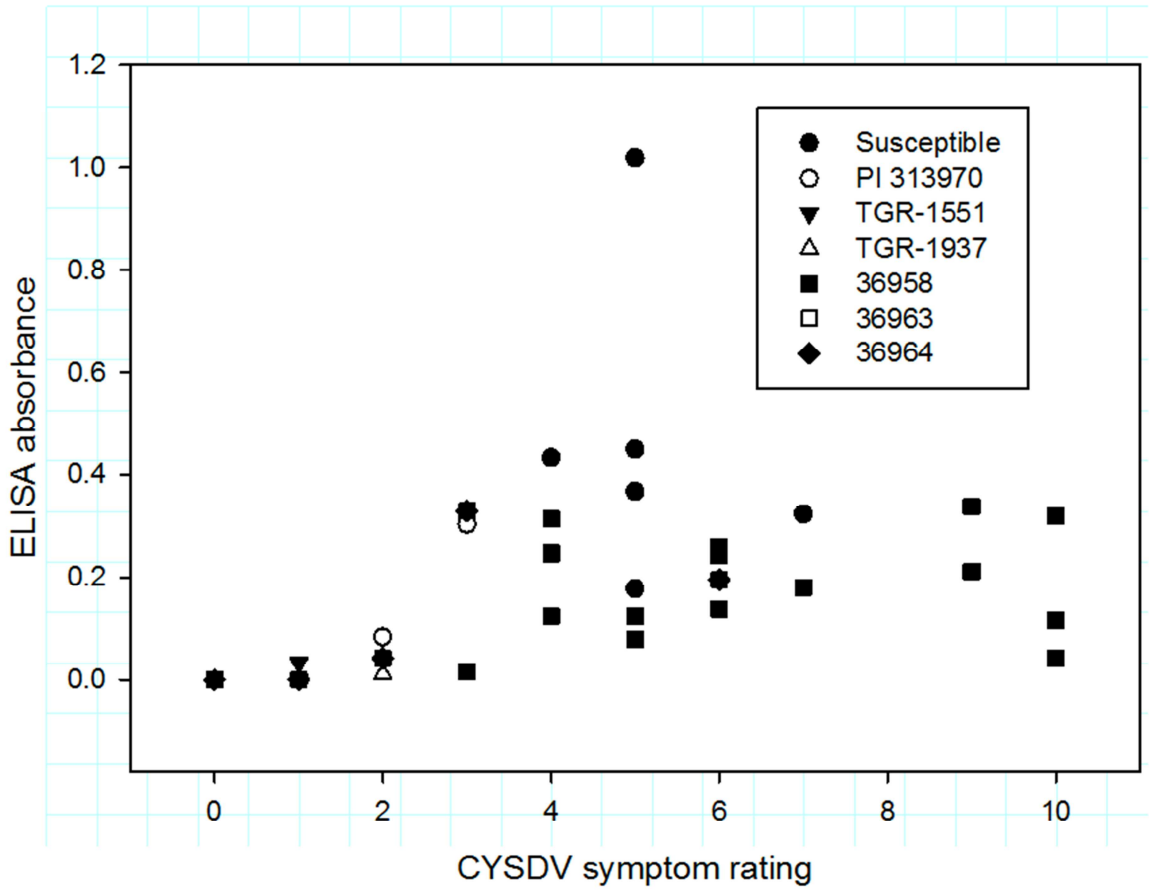


Figure 1. Scatter plot of *Cucurbit yellow stunting disorder virus* (CYSDV) symptom ratings versus ELISA absorbance values for a composite of three susceptible melon cultivars (Top Mark, Impac. Laredo), three previously reported sources of resistance to *Cucurbit yellow stunting disorder virus* (CYSDV), and three $F_2$ families from crosses of 'Impac' with resistant $F_2$ (PI 313970 x TGR-1551) selections in a naturally infected, un-replicated field test, Holtville, CA, 2012, 7 weeks post-planting. CYSDV symptoms rated using a 1 (<10% symptomatic foliage) to 10 (100% symptomatic foliage) visual scale.