California Melon Research Board
2011 Annual Report

I. Project title: Comparative evaluation of new resistance sources and development of field-based serological detection of CYSDV.

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IV. Work was performed at the USDA-ARS, U.S. Agricultural Research Station, Salinas, California, University of California, Plant Pathology Department, Davis, California, and the University of California, Desert Research and Extension Center (DREC), Holtville

V. Objectives

1. Evaluate exotic melon germplasm from India for potential new sources of resistance to CYSDV . (McCreight, Wintermantel)
2. Characterize host plant resistance to CYSDV in PI 313970 and TGR-1551, and select and introgress resistance to western U.S. shipping type background adapted to the desert southwest U.S. (McCreight, Wintermantel).
3. Evaluate virus content in PI 313970, TGR-1551 and lines derived from these sources to determine ability of lines to suppress CYSDV accumulation (McCreight, Wintermantel).
4. Develop antiserum for field diagnosis of CYSDV using an immunostrip format (Gilbertson).

VI. Results and Analysis

Objective 1. Evaluate exotic melon germplasm from India for potential new sources of resistance to CYSDV.

Two putative resistant plants in two of the Indian plant introductions evaluated in Fall 2010 were self-pollinated in a greenhouse.

100 accessions of previously untested Indian plant introductions were planted in a replicated test at DREC on August 18, 2011; however, many plants were extensively damaged by ground squirrels at emergence, hail on September 13, and heavy rain and hail on October 2. These accessions were not evaluated for resistance to CYSDV infection and will, therefore, be replanted in 2012 for evaluation.

Despite the extensive damage described above, vegetative cuttings were taken from 12 putative resistant plants in nine of the accessions for self- and cross-pollination at Salinas for confirmation of their reactions to CYSDV in subsequent tests.

Objective 2. Characterize host plant resistance to CYSDV in PI 313970 and TGR-1551, and select and introgress resistance to western U.S. shipping type background adapted to the desert southwest U.S.

Sixty-one progenies from self- and cross-pollinations of CYSDV-resistant or susceptible selections were planted along with resistant and susceptible controls at DREC on August 18, 2011. As in the test of melon accessions (Objectisve 1), the test suffered extensive ground squirrel damage at emergence and subsequent hail damage on September 13 survived 1.5 inches of rainfall in a 45 minute period accompanied by high winds, and more hail on October 2. The primary goal of the trial was not yield, but rather virus resistance

With 100% emergence, there could have been as many as 24 plants of each entry in the three replications; the observed number of plants per entry ranged from 1 to12. Mean CYSDV symptom severity ratings of four surviving plants total of three susceptible cultivars was 6.8 at 78 days post-planting, and the individual ratings ranged from 5 to 9; individual summary data are presented in Table 1. Despite the extraordinary environmental stresses on the test, the three previously reported sources of resistance exhibited resistance to CYSDV and were consistent with previous results (Table 1). An F_3 Top Mark x PI 313970 had a mean rating of 3.8 *vs.* 2.0 and 3.8 for selected and unselected stocks of PI 313970. Mean CYSDV ratings of eleven F_3 progenies and one F_4 progeny from crosses of PI 313970 x TGR-1551 ranged from 1.0 to 5.5 (Table 1). The highly variable numbers of plants preclude meaningful statistical analyses. Despite the messy data set, vegetative cuttings were taken from 39 single plant selections in this test for self- and cross-pollination in the greenhouse at Salinas.

Table 1. Mean and range of CYSDV symptom severity ratings on three susceptible cultivars, three previously reported sources of resistance, two putative new sources of resistance in a naturally infected field test, 78 days post-planting, Holtville. Rated using a 1 (0 to 10%) to 10 (91-100%) scale that estimated the proportion of foliage that exhibited symptoms.

Pedigree	Progeny	n	Mean	Range
Susceptible cultivars				
Impac		1	5.0	-
Sol Real		2	7.0	5–9
Top Mark		1	8.0	-
TGR-1551 lines				
TGR-1551	36090	5	2.0	0–3
TGR-1551 Ames	36886	5	3.2	2–7
TGR-1551 Ames S1	36884	4	3.3	2–6
TGR-1551 MGG bulk sibs	36511	4	1.8	1–3
TGR-1937 lines				
TGR-1937 C	36893	4	2.3	2–3
TGR-1937 C bulk sibs	36512	6	2.7	1–4
PI 313970 lines and selected F_3 and F_4 progenies				
PI 313970 selected	36367	2	2.0	1–3
PI 313970 not selected	36046	6	4.5	2–8
F_3 Top Mark x PI 313970	36894	9	3.8	1–7
F_3 PI 313970 x TGR-1551	36862	6	1.0	0–3
F_3 PI 313970 x TGR-1551	36863	4	1.5	1–2
F_3 PI 313970 x TGR-1551	36865	8	2.5	1–4
F_3 PI 313970 x TGR-1551	36866	5	3.2	2–4
F_3 PI 313970 x TGR-1551	36870	6	2.3	1–3
F_3 PI 313970 x TGR-1551	36871	5	3.0	2–4
F_3 PI 313970 x TGR-1551	36872	4	2.5	2–3
F_3 PI 313970 x TGR-1551	36873	6	2.0	0–4
F_3 PI 313970 x TGR-1551	36876	5	5.4	3–8
F_3 PI 313970 x TGR-1551	36877	6	5.5	3–9
F_3 PI 313970 x TGR-1551	36878	6	4.8	2–6
F_4 PI 313970 x TGR-1551	36889	12	1.3	1–3
Putative new sources of resistance				
PI 614479	36509	1	2.0	-
F_1 PI 614479 x Impac	21178	1	2.0	-
PI 614486	36881	3	1.7	1–2
PI 614486	36882	2	3.0	3–3

Objective 3. Evaluate virus content in PI 313970, TGR-1551 and lines derived from these sources to determine ability of lines to suppress CYSDV accumulation.

Leaf samples from 70 plants were collected 78-days post-planting and assayed in for virus content by ELISA using antiserum specific to CYSDV. The correlation between symptom severity ratings of the samples plants and their virus contents was statistically significant; *r* =

0.54, $P_{<0.0001}$. However, the correlation between CYSDV symptom severity ratings and ELISA absorbance was better in 2010; $r = 0.79$, $P_{<0.0001}$. Nevertheless, 2011 data support the method as a viable tool for evaluating varietal performance.

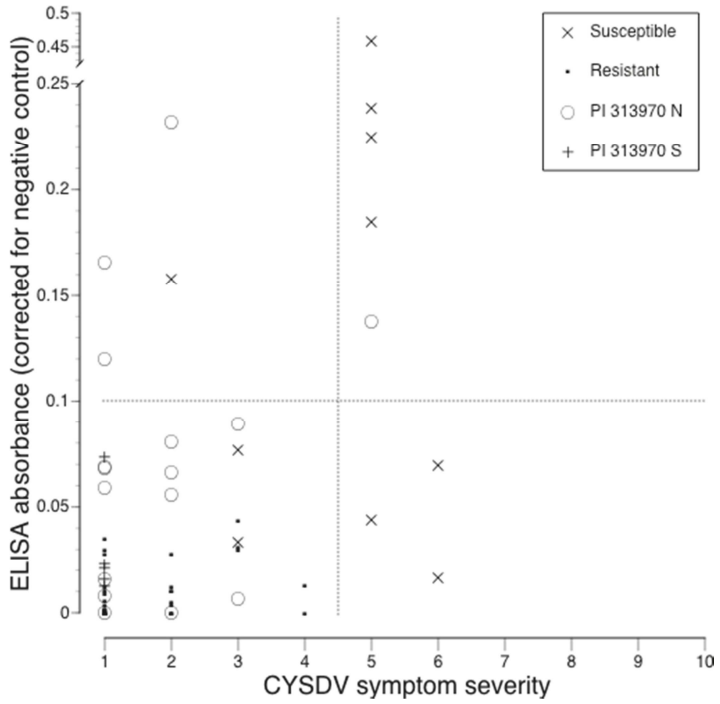

Figure. 1. Scatter plot of CYSDV ELISA absorbance values *vs.* symptom rating 78-days post-planting for leaf samples from four different genotype groups: 1) Susceptible is 'Laredo'; 2) Resistant is TGR-1551 and TGR-1937; 3) PI 313970 N was not selected for uniform reaction to CYSDV (cannot predict correlation between symptom severity and virus titer); and 4) PI 313970 S is a self-pollination of a single resistant plant (anticipate some correlation between virus titer and symptom severity).

Lack of a perfect correlation ($r = 1$) between symptom severity and virus content as measured by ELISA can be compensated in selection for resistance by discarding plants with symptom severity ratings greater than 4 and ELISA absorbance values greater than 0.10. Low virus content (ELISA absorbance less than 0.10) in a plant with high symptom severity rating (Figure 1, lower right quadrant) may be due to sampling error, i.e., the sampled leaf had not accumulated sufficient virus at the time of sampling, and thus did not provide an accurate estimate of plant virus content. In contrast, high virus content (ELISA absorbance greater than 0.10) in a plant with low symptom severity rating (Figure 1, upper left quadrant) may be due to senescence or loss of symptomatic crown leaves from extremely heavy whitefly feeding, typical of Imperial Valley in the Fall season, or hail as happened in Fall 2011 in Imperial Valley.

In addition to confirming phenotypic evaluations of plants in segregating populations, these data suggest the possibility of using an ELISA assay for early identification of

resistant segregants. This would be advantageous for making pollinations in the field, or for propagating resistant plants from vegetative cuttings and subsequent self- and cross-pollination in a greenhouse.

Objective 4. Develop antiserum for field diagnosis of CYSDV using an immunostrip format.

An antibody was developed previously through this project against the bacterially expressed capsid protein (CP) of CYSDV. This antibody was shown to be highly specific for detection of CYSDV in western blot and enzyme-linked immunosorbent assay (ELISA) analyses of the bacterially expressed protein and extracts prepared from CYSDV-infected plants as described in the 2010 annual report.

Western blot (binding protein on membrane) analysis was also performed with this antibody to confirm its ability to bind virus particles attached to a membrane without cross reactivity with other plant proteins. This is critical to our ability to develop an immunostrip using the antibody. Western blot analysis demonstrated strong binding to both the purified virus coat protein as well as to whole virus in extract from CYSDV-infected melons (Figure 2).

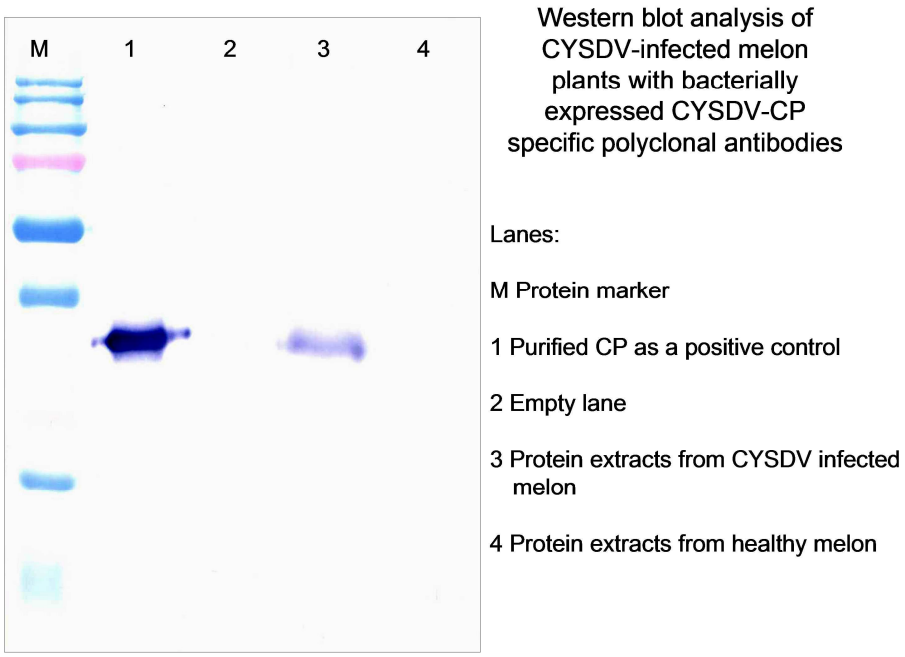

Figure 2. Western blot analysis of CYSDV-infected melon plants with bacterially expressed CYSDV-CP specific polyclonal antibodies. Antibody dilution was 1:5000. 1 ul expressed viral CP (1:1000 dilution) was used as a positive control (Lane 1). Plant extracts were crude proteins extracted from 0.1g tissue. 10 ul of 500 ul total protein extract was loaded.

Further field testing of the ELISA method was performed during evaluation of the fall 2010 and 2011 melon trials (Objectives 1-3). This involved indirect ELISA, and confirmed effectiveness

of the antiserum for ELISA-based detection of CYSDV. Although indirect ELISA works quite well, plans are to conjugate the antibody to an enzyme for use in double antibody sandwich ELISA format as well, since this can improve sensitivity.

We have an agreement with a private company for development of a lateral flow device (immunostrip) for the antibodies we developed against CYSDV, and work is in progress. This collaboration should lead to the development and commercial availability of an immunostrip test for CYSDV within the next year or two.

Additional Accomplishments

The Gilbertson Lab recently established new method for RT-PCR tests of stored tissue extracts of RNA viruses from different plant samples. Initial experiments detecting Potyvirus, Tospovirus and Torradovirus were successful, and samples from various locations successfully tested positive for CYSDV in melons as well. Now we are working on optimization of these tests to eliminate possible false negative results.

The Wintermantel Lab is evaluating previously identified common weed and crop hosts for their significance as reservoirs for CYSDV. Research has identified bean and buffalo gourd as high concentration reservoir hosts. Lettuce also accumulates high levels of CYSDV relative to most weeds and non-cucurbit hosts. This is reflected in the relatively efficient transmission of CYSDV from these hosts to melon. London rocket and Shepherd's purse accumulate very low levels of CYSDV, and transmission of virus from these latter hosts to melon is much less prevalent based on preliminary and ongoing studies. Evaluation of additional hosts is in progress.

Both laboratories have been involved with diagnosis of multiple sets of San Joaquin Valley melon samples exhibiting virus infection this year. Recently, several samples have tested positive for the aphid-transmitted viruses, Watermelon mosaic virus (WMV) and Cucumber mosaic virus (CMV), sometimes occurring together and associated with potentially significant yield loss. A tombusvirus, *Lettuce necrotic stunt virus* (LNSV), was identified in some plants as well, co-infecting with WMV and CMV, although the relationship between this soil-borne virus best known for causing lettuce dieback and disease symptoms on melon is still being determined.