Screening for Resistance to Curtoviruses in Chile Pepper

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Biological properties of curly top virus

- Infects a wide host range of dicot crops and weeds
- Crops: peppers, tomatoes, sugarbeets, spinach, melons, beans
- Found in vascular system, not seed transmitted
- Young plants most susceptible to infection
- Transmitted by the beet leafhopper

Symptoms of curly top in chile

- Severely stunted plants
- Some plants have chlorotic rolled leaves
- Small rounded fruit
- Stiff plants with brittle leaves
- Symptoms appear 1-2 weeks after infection

Management Options

- Heavy seeding
- Delay thinning
- Weed removal
- Insecticides - systemics to decrease leafhopper numbers
- Predictive model
- Plant resistance in bean, sugarbeet, tomatoes
- Test for resistance in chile

Plant Resistance

- Bean - single gene resistance to virus
- Sugarbeet - multigene tolerance to virus
- Tomato – field resistance/tolerance in Saladmaster, Roza, Rowpak, Columbian
- Chile - field resistance/tolerance in NuMex Las Cruces Cayenne, Tabasco

Methods of Resistance

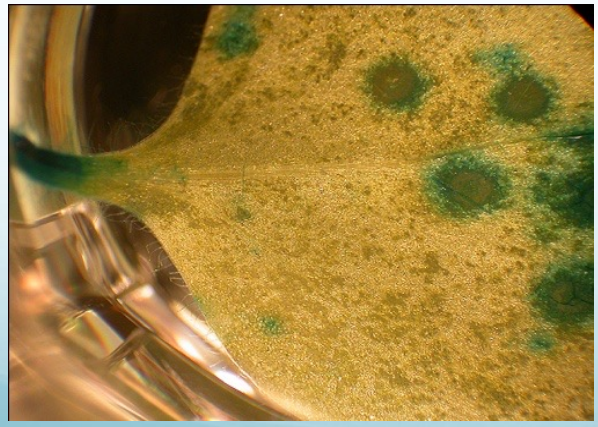
- Resistance prevents virus replication
  - Single gene, no infection
- Resistance allows replication, but prevents virus movement
  - Possible single gene, infection only in inoculated leaf
- Resistance to insect transmission
  - Multigene, tolerance
    - Insect won't land on plant, feed on plants
    - Insect doesn't prefer feeding on the plant

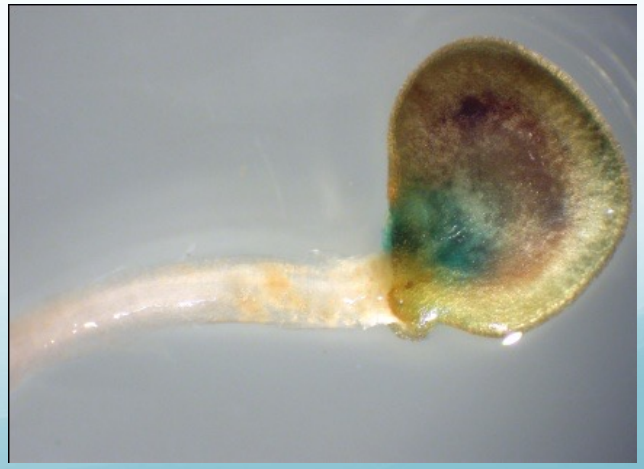
Laboratory screening

- Agroinoculation with BSCTV/BMCTV recombinant infectious clone
  - Tomato seedlings-apical meristem removed, add bacteria with syringe
  - Peppers-germinated seeds inoculated with bacteria using minuten pins
- Leafhopper transmissions using BSCTV
- Plants screened for virus using PCR and ELISA

pGUS Inoculations

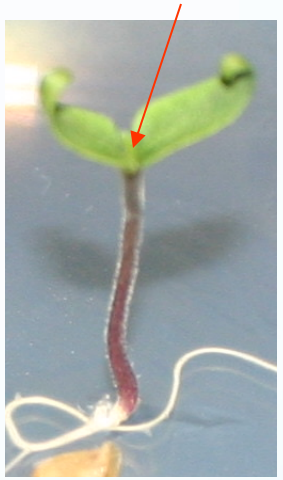
Vascular puncture inoculation of chile with *Agrobacterium* containing pCAMBIA1390-GUS. Chile seedlings were punctured with minuten pins, and GUS activity was visualized using X-gluc as a substrate to provide a blue color.





Screening for BCTV resistant plants by using *Agrobacterium*

- Inoculated plants with BCTV clone containing tandem of replication region in *Agrobacterium*



➢ Method

-Prick small holes in the meristem of young plants

-Drop 2-3 µl of *Agrobacterium* solution into hole

- Incubate for 3 days

- Transfer plants from culture plate to pots

- Test for BCTV by PCR, 4 wks after inoculation.

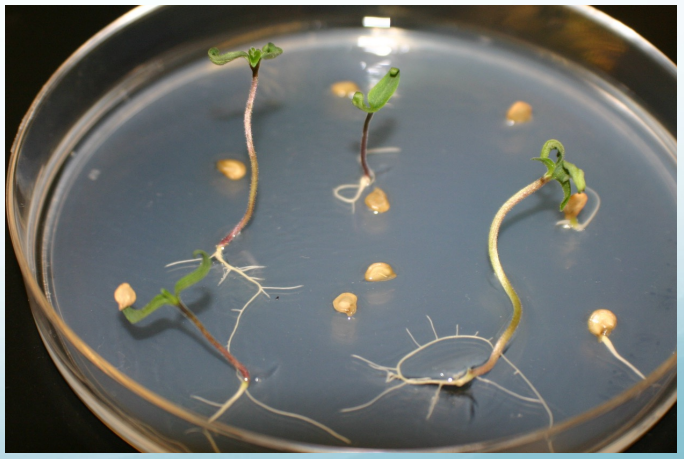
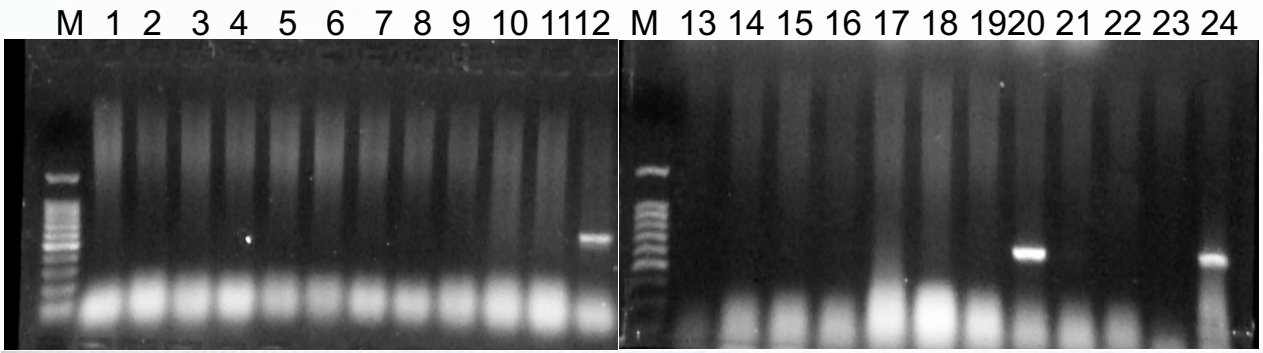


Table 2. Inoculation of tomato varieties using vascular puncture after meristem removal.

Variety	No. plants survived/ no. inoculated with pGD	No. plants infected/no. surviving with pGD (%)	No. plants survived/ no. inoculated with pCAMBIA1390-GUS	No. GUS plants/no. surviving (%)
Lauro 12	21/45	9/21 (42.9%)	7/15	4/7 (57.1%)
CTR 05-01	30/45	18/30 (60.0%)	12/15	7/12 (58.3%)
CTR 05-03	43/45	33/43 (76.7%)	13/15	9/13 (69.2%)
CVF-11	41/45	22/41 (53.7%)	14/15	7/14 (50.0%)
Roza	28/45	23/28 (82.1%)	11/15	3/11 (27.3%)
Columbian	24/45	22/24 (91.7%)	7/15	3/7 (42.9%)
Saladmaster	39/45	22/39 (56.4%)	10/15	6/10 (60.0%)
Rutgers	29/45	20/29 (69.0%)	9/15	6/9 (66.7%)

Results are totals from at least three trials.

Results of screening for BCTV resistant plants



1-20: virus inoculated Tabasco plants

21-22: uninoculated tabasco plant

23: PCR negative control

24: PCR positive control

Pepper-Vascular Puncture

Pepper Variety	Infected/Total Tested	% Infection
NM 6-4	19/28	67.9%
Tabasco	4/24	16.7%
NuMex Las Cruces cayenne	8/28	28.6%

Testing for Leafhopper Resistance

- Beet leafhopper
  - Reared on BCTV-infected sugarbeets
  - 1-10 leafhoppers/plant for 18 hrs
  - Stained leaves for salivary sheaths

Leafhopper Transmission

Pepper Variety	1 LH/plant # infected/plants tested		3 LH/plant # infected / tested	
NM 6-4	4/6			
Tabasco	0/4	0/20	6/36	
PI 205167	0/6			
PI 205174	0/4			
PI 533 10383	0/5	6/9		
PI 312 10335	4/4			
Grif 9303	0/3			
NuMex Bailey Piquin	1/6	4/4		

Leaf Staining

- Stain used is acid fuschin
- Stylet tracks and p
  - Puncture = limited feeding
  - stylet track = extensive feeding

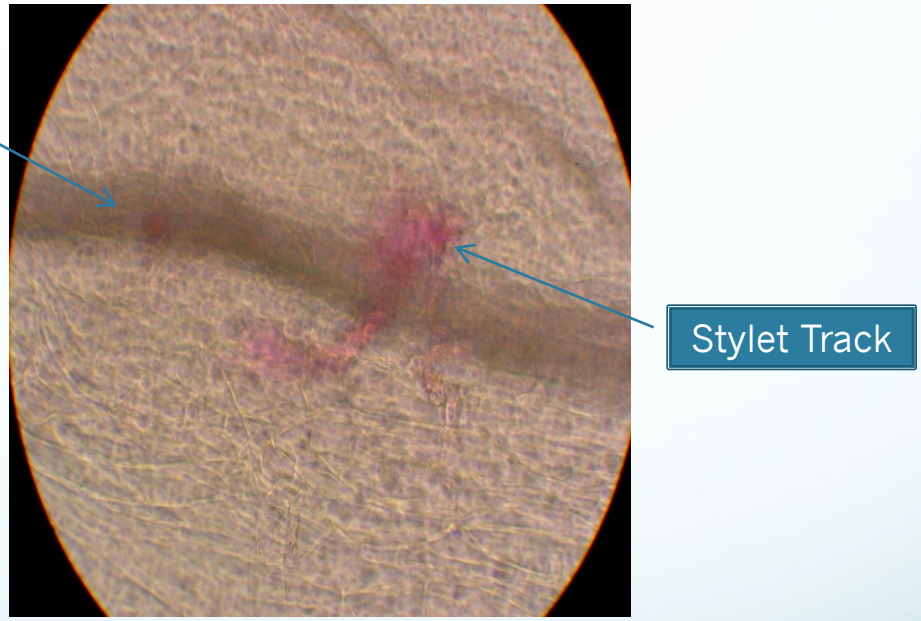


Table 4. Stylet sheath staining of beet leafhopper feeding on peppers.

		Punctures		Stylet Sheaths	
Pepper Variety	**No. plants tested**	**Total**	**Average/plant**	**Total**	**Average/plant**
NuMex Las Cruces Cayenne	30	106	3.5	0	0
Tabasco	20	21	1.0	0	0
New Mexico 6-4	30	1246	41.5	2	0.06

Results are total from two trials, each using 5 leafhoppers/plant.

Conclusions

- Vascular puncture effective inoculation method for rapid screening for R gene resistance
- Leafhopper transmission/stylet sheath staining can be used to screen for other types of resistance
- Tabasco and NuMex Las Cruces cayenne are field resistant/tolerant to curly top virus infection
- Field resistance/tolerance in several tomato varieties
- Mechanism of resistance has not been established, but is effective in both field and greenhouse and includes leafhopper non-preference for feeding