‘Variation in the p25 region of resistance breaking *Beet necrotic yellow vein virus* from infected plants in Minnesota’

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Texas AgriLife Research, Amarillo
• Tolerance to BNYVV conferred by the Rz1 loci (inherited as a single dominant gene)

• *Rz1* has been widely deployed in resistant cultivars from the mid 1980s onwards

• In 2002-2003 BNYVV tolerant cultivars showed severe rhizomania symptoms in Minnesota
Symptoms of BNYVV

Lateral root proliferation and tap root constriction

Healthy

Diseased
BNYVV Symptoms - field
• How and why is BNYVV able to overcome this resistance?

• Is there an underlying reason for the epidemiological differences seen in field distribution?
Beet necrotic yellow vein virus (BNYVV)

**Polyprotein (helicase-polymerase)**

- RNA 1
  - 237K
  - (A)n 6746 nt
  - Coat Proteins
  - Movement Proteins

- RNA 2
  - 21K, 54K, 42K, 13K, 15K, 14K
  - (A)n 4612 nt

- RNA 3
  - 25K
  - (A)n 1773-4 nt

- RNA 4
  - 31K
  - (A)n 1465-7 nt

- RNA 5
  - 26K
  - (A)n 1342-7 nt

**Symptom development in roots**

**Vector transmission and root specific silencing**

**Symptom severity**

**• replication**

**• Assembly**

**• cell to cell movement**
Amino Acid Motifs In the p25 of BNYVV

- Reverse genetics approach by Koenig et al. (1991) showed this region is important for pathogenicity of BNYVV.

- In addition p67 and p68 in the p25 protein of BNYVV are correlated with resistance breaking (Tamada et al. 2011, Koenig et al. 2009).
**BNYVV RNA 3**

**Amplicon:**

- **RNA 3**
- **25k**
- **4.6k**
- **N**
- **(A)n 1773-4 nt**

Features:

- **Nuclear localization signal (57-62 aa)**
- **Hypervariable tetrad (67-70 aa)**
- **Nuclear export signal (169-178 aa)**

**Dimensions:**

- **0.0** to **0.3**
- **0.6**
- **0.9**
- **1.2**
- **1.5**
- **1.8 kb**
Methodology

Sampled roots from symptomatic and asymptomatic plants

Blinkers and spots

Amplify and sequence the p25 region of BNYVV
Methodology

• Important to sample plants rather than soil

• Important to sample symptomatic and asymptomatic plants

• Test for the presence of the \( Rz \) gene
Consensus sequencing

<table>
<thead>
<tr>
<th>Isolate</th>
<th>A. A. Motif p67</th>
<th>A. A. Motif p68</th>
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<tbody>
<tr>
<td>Minnesota WT</td>
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<td>C</td>
</tr>
<tr>
<td>Minnesota RB</td>
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<tr>
<td>California RB</td>
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R. Acosta – Leal and Rush 2007
# Amino Acids at p 67 and 68 in symptomatic plants from Minnesota spots

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<tr>
<th>Plant Cond.</th>
<th>Location</th>
<th>R loci</th>
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<th>p68</th>
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<td>C</td>
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<td>A</td>
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<td>C</td>
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<tr>
<td>yellow spot</td>
<td>Rz1</td>
<td>V &amp; A</td>
<td></td>
<td>C</td>
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<tr>
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### Amino Acids at p 67 and 68 in symptomatic plants from Minnesota

#### Blinkers

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Amino acid motifs in *Rz1* Cultivars in Minnesota

**Diagram:**

- **BNYVV infected plants from diseased spots in *Rz1* Cultivars**
  - VC: 78%
  - AC: 17%
  - mixed (AC+VC): 5%
  - mixed other: 0%
Amino acid motifs in Rz1 Cultivars in Minnesota

BNYVV infected Rz1 blinker plants

- VC: 39%
- AC: 28%
- mixed (AC+VC): 11%
- mixed other: 17%
- A*: 5%
Conclusions

• Predominant motif in Rz1 diseased spots is $V_{67}C_{68}$

• This is not the case in Rz1 blinker plants where there is much more variation in the haplotypes present
Hypothesis

- Fitness of viral haplotypes

- In time, blinkers will initiate spots of disease in the field as the $V_{67}C_{68}$ haplotype becomes fixed in the population

- The $V_{67}C_{68}$ haplotype ‘wins’ out as the fittest viral strain overcoming $Rz1$ resistance

- Not all haplotypes are equally fit and therefore do not initiate diseased patches
Final thoughts!
Final Thoughts

• Other mutations present in other parts of the genome? (Tamada et al. 2011)

• Influence of minor effect QTL loci in different cultivars on R phenotype
• Is this pattern true of cultivars containing other R sources?

• ‘Industry cooperation is key’!
Acknowledgements

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Thank you for listening!
Real-time PCR

- PCR products are detected as they accumulate
  - DNA binding dyes e.g. SYBR green or specific fluorescent probes
- Amount of DNA amplified is measured after each cycle
- Initial amount of target can be related to cycle threshold (Ct)
- Target DNA quantified using calibration curve relating Ct to known template amounts
Real-time PCR

Polymerization

Strand displacement

Cleavage

Polymerization completed

R = Reporter
Q = Quencher
What is Polymyxa?

• Obligate intracellular root-infecting organism

• *Polymyxa* is one of 10 genera in the *Plasmodiophoraceae*

• Two Species: *Polymyxa graminis*
  *Polymyxa betae*
The Life Cycle of *Polymyxa*

- Resting spore cluster
- Synaptonemal complexes formed suggesting meiosis
- Sporogenic plasmodium
- Primary zoospore
- Encystment and injection of zoospore contents
- Rounds of mitotic, cruciform nuclear division
- Sporogenic plasmodium
- Secondary zoospore
- Zoosporangium
- Rounds of mitotic non-cruciform nuclear division
The Tree of Life