RNAi Mediated Silencing of Endogenous Wheat Genes elf4(iso)-E-2 and elf4G Induces Resistance to Potyviruses Wheat streak mosaic virus and Triticum mosaic virus

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The Importance of Wheat

• Global staple crop
  − Accounts for 1/5 of humanity’s food (Singh et al., 2011)
• Grown on ~590 million acres globally each year
• Global wheat production ~24 billion bushels (FAO 2012)
• Kansas produces ~ 328 million bushels! (KS Dept. of Ag)

Yield Losses Due to Viruses

• Great Plains average yield loss is between 5%-10% annually (GPDN 2011)
• In Kansas, losses due to Wheat Streak Complex average ~2% (K-State Extension 2011)

Two Viruses Infecting Wheat

• Wheat streak mosaic virus
• Triticum mosaic virus

Potyviruses: WSMV and TriMV

• Wsm1, (Mace) Thinopyrum intermedium
• Wsm2, (C0960293-2, ‘Ronl’ and ‘Snowmass’) unknown source
• Wsm3, (Thinopyrum intermedium translocation)

Available Resistance
**Eukaryotic Initiation Factors**

- Eukaryotic initiation factors participate in recruitment of mRNA to the ribosome.
- Most eukaryotic organisms express multiple elf4 family members that recognize the 5'cap structure of mRNA.
- Many plant infecting RNA viruses mimic the 5'cap of mRNA to recruit the host machinery with or without their own capsid.
- Potyviruses, such as WSMV and TriMV, do not require recognition of the 5'cap structure of mRNA.

**Tissue Culture and Transformation**

- Co-bombardment with pAHC20 containing bar and gus.
- Glufosinate selection.
- RNAi hairpin.
- Co-infection Bioassays:
  - Co-infection of T1, elf4(iso)E2 with WSMV and TriMV.
- Results based on PCR detection of transgene, phenotypic score, and ELISA results.

**Event Recovery, Hairpin Presence and Expression**

- 3 events were obtained from elf4(iso)E2.
- 4 events were obtained from elf4G.
- All plants were tested for glufosinate resistance, PCR presence of the transgene/Bar and RT-PCR for expression of the transgene.

**Co-infection Bioassays T2 Generation**

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**Crossing into Adapted Cultivars**

- WSMV Challenged BC2F1's

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Real-time PCR

Summary of eIF Lines

- Knockdown of host genes involved in viral replication is possible without altering plant phenotype.
- T6 generation plants remain resistant to both WSMV and TriMV
- BC$_1$F$_1$’s remain resistant to both WSMV and TriMV independently and during co-infection

Questions?

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USDA
CGAHR