Effect of Transgenic Cotton Plants Transformed with Antimicrobial Synthetic peptide D4E1 on Cotton Seedling Disease and Soil Microbial Diversity

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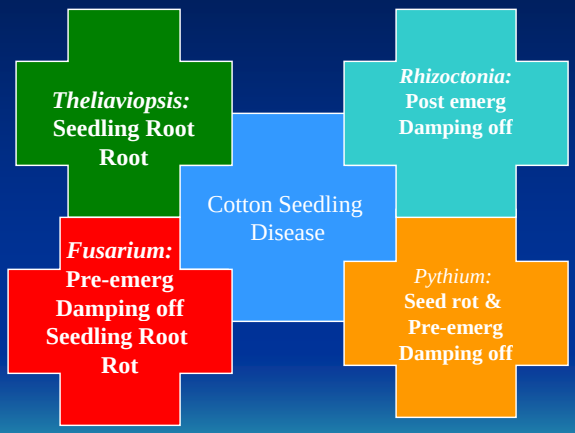
Cotton and Cotton Seedling Disease

- USDA estimates the global cotton crop amount to be approximately 120.3 million bales in 2007.
- USDA projects a '08-09 US cotton crop of 13.77 million bales.
- An average of 2.85% of cotton crop is lost annually as a result of infection of cotton seedling disease
  - translates to over 150 million dollars a year.
- In Alabama specifically, seedling disease is responsible for losses ranging from 3 to 11 percent annually.

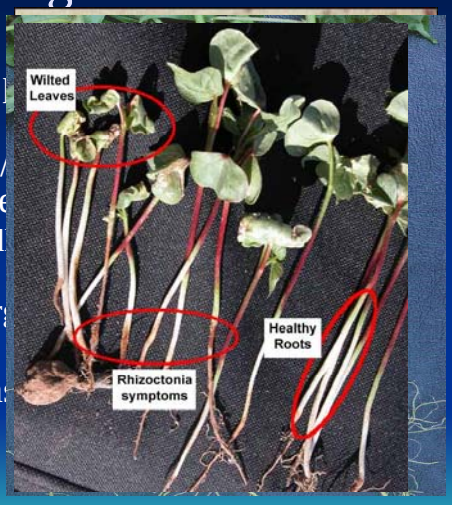
Alabama Cooperative Extension Office **ANR-1011April 1998. William S. Gazaway** (National Cotton Council of America)

Cotton Seedling Disease



Cotton Seedling Disease

- Can occur within hours of
- Can result in death of seed/
  yield and crop performance
  - decay of seed, death of seed
    emergence damping off)
  - death of seedling after emer
- Can effect cotton plants pa
  - Soreshin



Current Management Methods

- Managed by various farm practices
  - Crop Rotation
  - Preparation and Drainage of seedbed
  - Avoid Mechanical Injury
  - Application of Fungicides
- Ineffective
  - No one treatment addresses all pathogens in complex
- **No known lines of cotton are resistant to cotton seedling disease**

Synthetic Antimicrobial Peptides as a Control Method for Phytopathogens

- Jaynes et al. (1993) introduced a gene encoding a designed synthetic peptide in tobacco.
  - Showed resistance to bacterial wilt
- Transgenic potato plants expressing an alfalfa defensin gene, *alfAFP*
  - showed increased field resistance to *Verticillium dahliae* (Arce 1999).
- Expression of the *attacin* gene from the giant silk moth, *Hyalophora cecropia*
  - demonstrated improved bacterial resistance in transgenic potato and pear, respectively

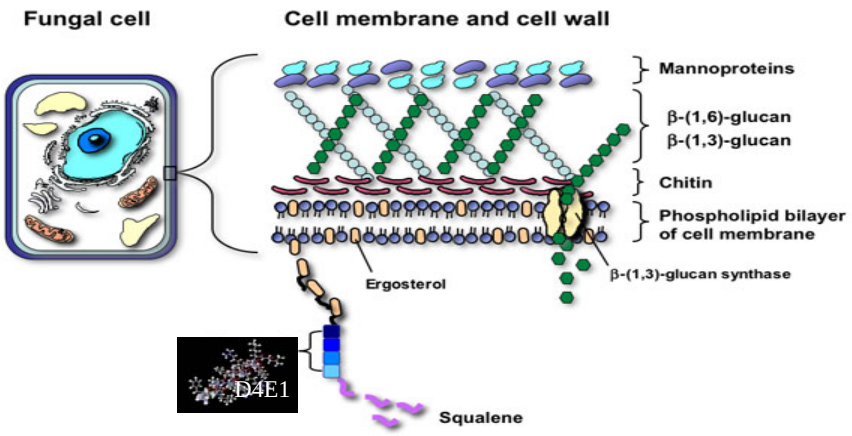
Advantages of Synthetic Antimicrobial Peptides

- More target specificity
- Increased efficacy at lower concentrations.
- Rapid biocontrol ability against a wide range of fungal and bacterial pathogens at low concentrations
- Non-toxic to mammalian and animal cells.

D4E1

- Synthetic peptide
- Dr. Jesse Jaynes
- Linear peptide with 17 amino acids
- Beta sheet configuration.

Action of D4E1



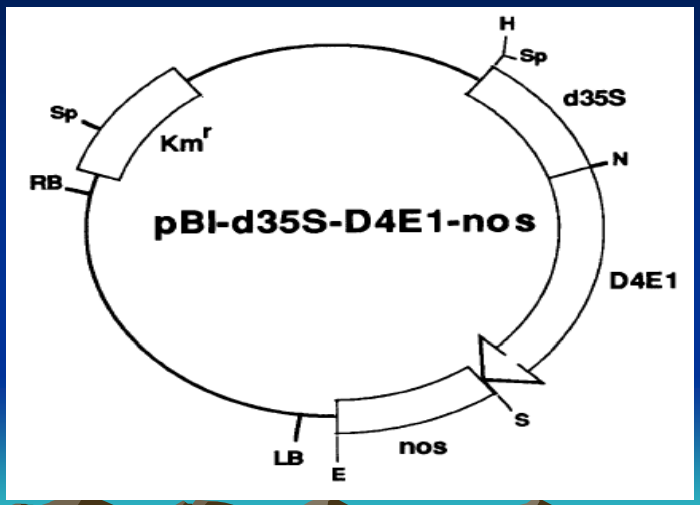
D4E1's effects of Plant Pathogens

- development of transgenic cotton lines with enhanced resistance to several fungal pathogens
  - [*Fusarium verticillioides, Aspergillus flavus,* , *Thielaviopsis basicola]*
- In *in vitro* bioassays and *in planta* assays, broad-spectrum antimicrobial action was also demonstrated
- Active against fungi as well as bacterial pathogens

Details about D4E1 Construct

- Construct: pBI121
- Mode of transformation
  - disarmed Agrobacterium tumafaciens
- Promoter: 35S 5'
  - cauliflower mosaic virus- (double promoter)
- Terminator:
  - nopaline synthase (nos) 3' from Agrobacterium tumafaciens T-DNA
- Selectable Marker
  - Kanomycin Resistance

Illustration of Construct



Cary JW, Rajasekaran K, Jaynes JM, Cleveland, TE. (2000). Plant Science. 154: 171-181.

Specific Objectives of Research

1. Evaluate the effectiveness of *D4E1* on controlling cotton seedling disease in a field trial setting.
   - D4E1' s effects on *Pythium* and *Rhizoctonia* have not tested
2. Determine whether *D4E1* transgenic cotton plants develop comparable agronomic characteristics as non-transgenic varieties.
3. Evaluate whether D4E1 transformed cotton crop expressing a synthetic antimicrobial peptide has any effect on the soil microbial community

Overview of Experiment

Two components:

- Field Trial-conducted in conjunction with USDA
- Controlled Greenhouse Experiment

Overview of Field Experiment and Layout

Lines

Three lines (Coker 312):

- C357(line 1)- two integration sites
- C358 (line 2) –three integration sites
- C373 (line 3) – one integration site
- Control with a GUS reporter gene.

- All 4 sets of seeds were transformed and provided by USDA ARS division, New Orleans, LA.

Rajasekaran,K, Cary, J.W., Jaynes J.E., and Cleveland, T.E. (2005) .Plant Biotechnology Journal 3 (6), 545–554.

Test Plot 1

Test Plot 2

Collection and Scoring of Seeding

Scoring system was then applied to each cotton seedling that was planted in an effort to quantify the emergence of potential disease symptoms.

- 1 2 plant germinated at time of observation
- 2 1 plants germinated at time of observation
- 3 No germination
- 4 Germinating (emerging from soil, but not fully emerged)
- 5 Both Plants weak
- 6 1 plant strong/1 plant weak
- 7 1 plant germinated but weak
- 8 1 plant germinated but died
- 9 2 plants germinated but died

Examples of Diseased Seedlings

Example of Diseased Seedling radicle

Example of Post-emergence seedling root rot

Example of Post-emergence seedling rot

Example of seed rot

Discussion

- Preliminary Results indicated that in both trial 1 and 2 showed the three D4E1 lines showed fewer disease symptoms than the control.
- Further evaluation of transgenic lines will be conducted.
- Comparison of the evaluation rubrics.
  - Greenhouse experiment
  - Replication of field trial

Overall Objectives and Next Steps

1. Evaluate the effect that *D4E1* will have on cotton seedling disease.
   - Begin Greenhouse Experiment (November)
2. Determine whether *D4E1* transgenic cotton plants develop on a comparable level as non-transgenic varieties.
   - Continue cotton plant characterization

Next Steps cont.

3. Evaluate whether effect D4E1 transgenic crop expressing a synthetic antimicrobial peptide have any effect the soil microbial community
   - Analysis of Soil Samples- DNA Extraction, PCR amplification, and DGGE

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Questions…