Phaseolinone as a new tool to evaluate resistance to charcoal rot

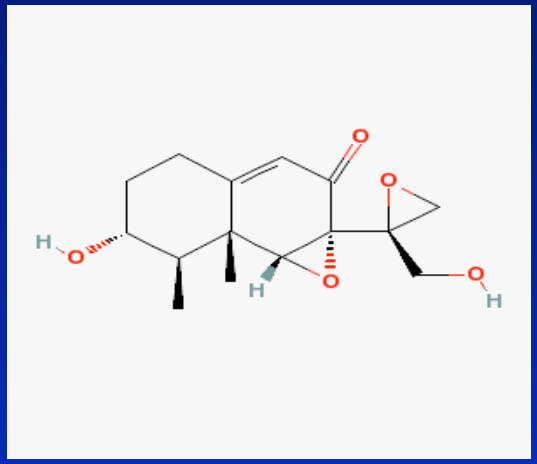
- Available screening methods
- Limitations
- Symptoms development and factors affecting them
- Why phaseolinone?

Objectives

- Develop a phaseolinone-based screening assay
- Develop a QPCR assay to quantify *Macrophomina phaseolina* in infected tissue
- Identify and characterize fungal genes involved in the biosynthesis of phaseolinone

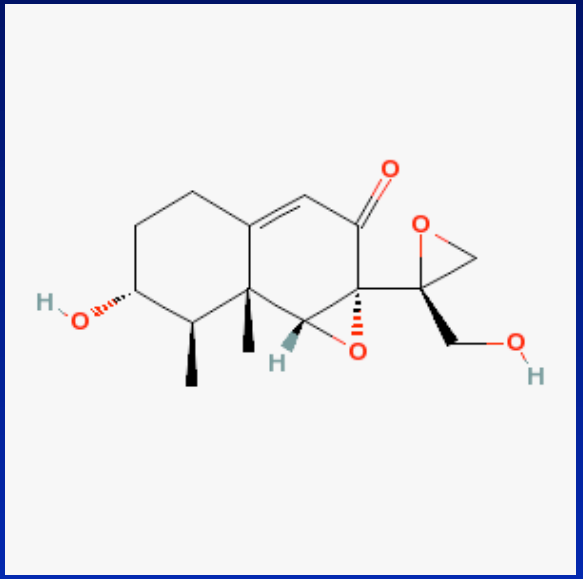
Phaseolinone

- Exotoxin
- Cellulolytic activity
- Heat resistant
- Inhibition of seed germination, wilting of seedling and root rot



Objectives

- Develop a phaseolinone-based screening assay
  - Protocol developed to produce, extract and purify phaseolinone in vitro
  - Culture conditions optimized for the production of phaseolinone in vitro



Fungus grown at 30^0C for 15 days

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Filtration

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Treatment with activated charcoal

↓

Extraction with chloroform

↓

Defat with Diethyl ether

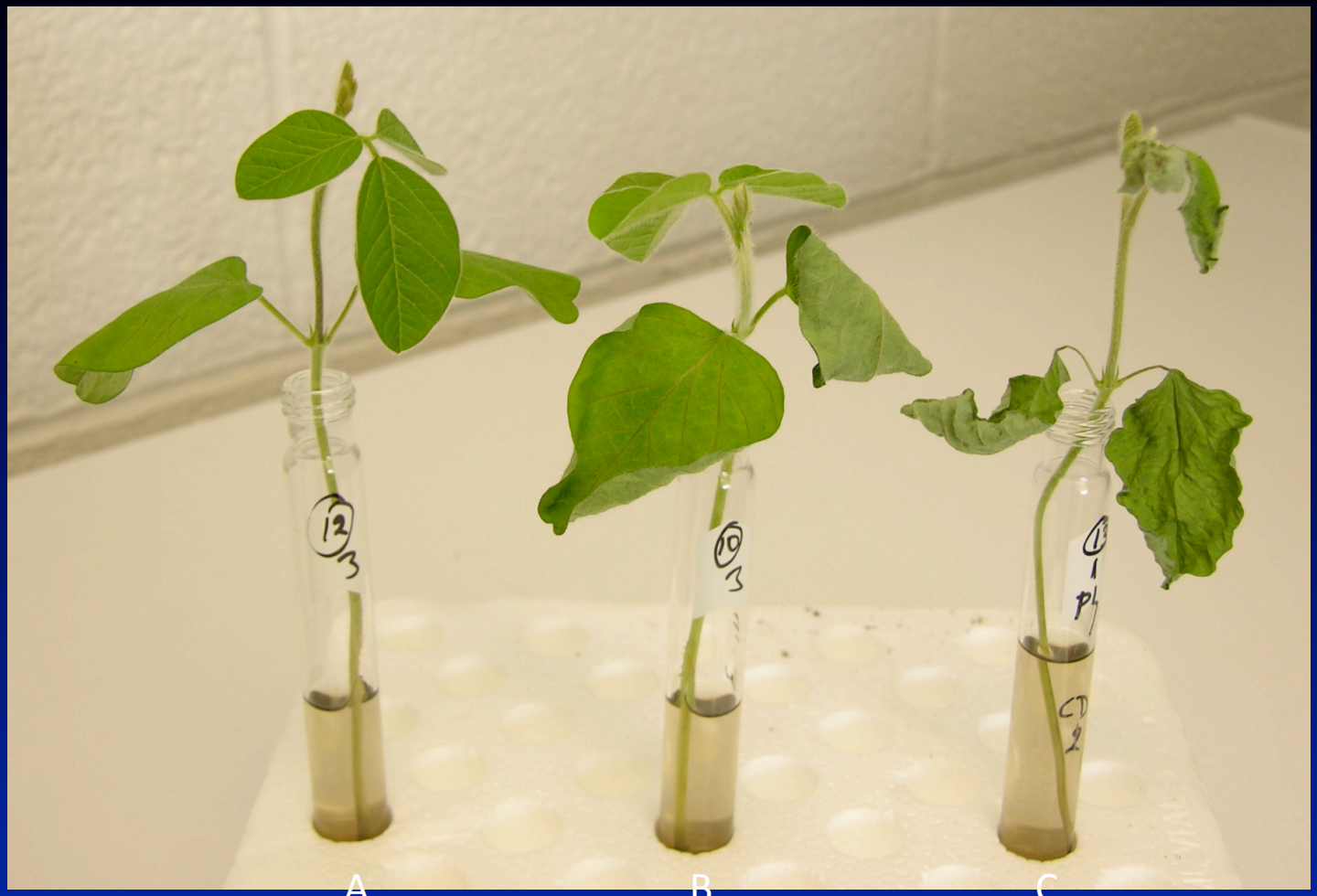
↓

Purification by HPLC

Objectives

- Develop a phaseolinone-based screening assay

- Ten days old seedlings were used
- Cut ends were immersed in vials containing phaseolinone
- The symptoms occurred within 5 hours of exposure to the toxin in seedlings of susceptible soybean lines



Effect of Phaseolinone on different PI's lines Tolerant (A), moderately tolerant (B) and Susceptible (C) after 24 hrs of treatment

Variety	Time (appearance of symptoms)	Time (dessication)	Tolerance/susceptibility
LS92-1088	72 hrs	>4 days	Tolerant
LS98-2574	72 hrs	>4 days	Tolerant
DT99-16864	72 hrs	>4 days	Tolerant
DT99-17483	72 hrs	>4 days	Tolerant
Croton	5 hrs	48 hrs	Moderate tolerance
DK4866	5 hrs	48 hrs	Moderate tolerance
DP4546	5 hrs	48 hrs	Moderate tolerance
DP5806	5 hrs	48 hrs	Moderate tolerance
R01-581F	5 hrs	48 hrs	Moderate tolerance
LS98-3257	5 hrs	48 hrs	Moderate tolerance
LS98-1430	5 hrs	48 hrs	Moderate tolerance
DP3478	5 hrs	24 hrs	Susceptible
Pharoah	5 hrs	24 hrs	Susceptible
LS98-0719	5 hrs	24 hrs	Susceptible
LS98-0358	5 hr	24 hrs	Susceptible

Objectives

- Develop a phaseolinone-based screening assay (Ongoing)
  - Developing a numerical scheme to rate the symptoms to assure reproducibility
  - Ready to be used large scale pre-screening

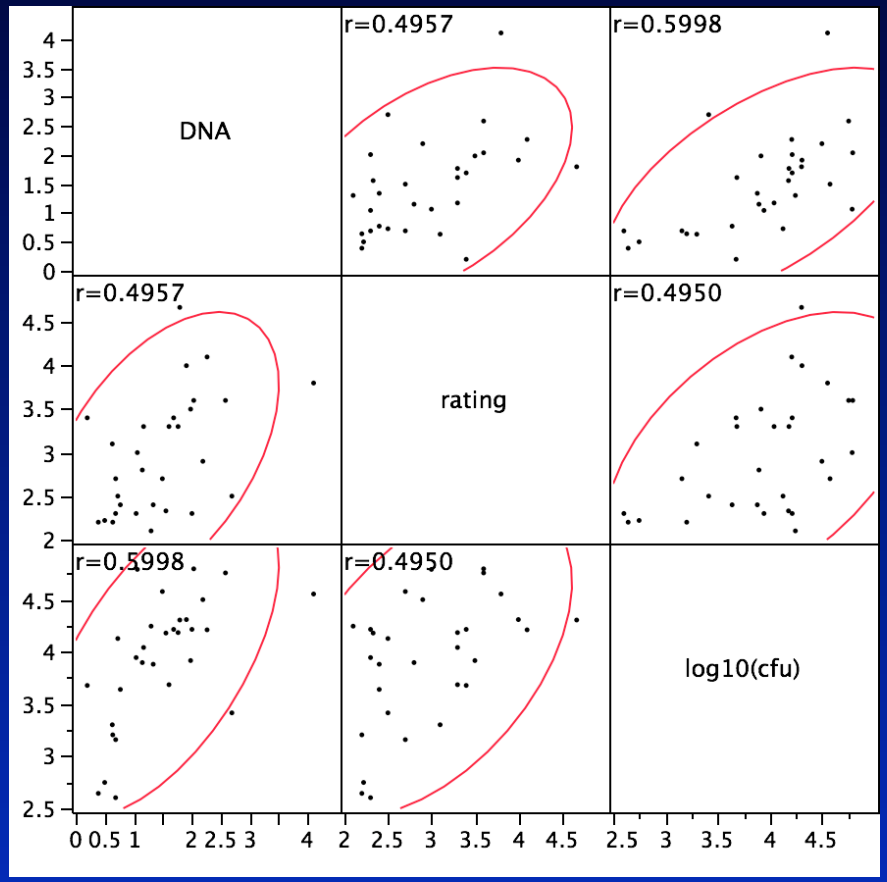
Objectives

- Develop a QPCR assay to quantify *Macrophomina phaseolina*
  - *A* SYBR Green based protocol was developed
  - The following *M. phaseolina* specific primers were used:
    - MpKFI 5'-CCGCCAGAGGACTATCAAAC-3'
    - MpKRI 5'-CGTCCGAAGCGAGGTGTATT-3'

(KISHORE BABU et al. (2007) Mycologia, 99(6) 797–803).

Objectives

- Develop a QPCR assay to quantify *Macrophomina phaseolina*

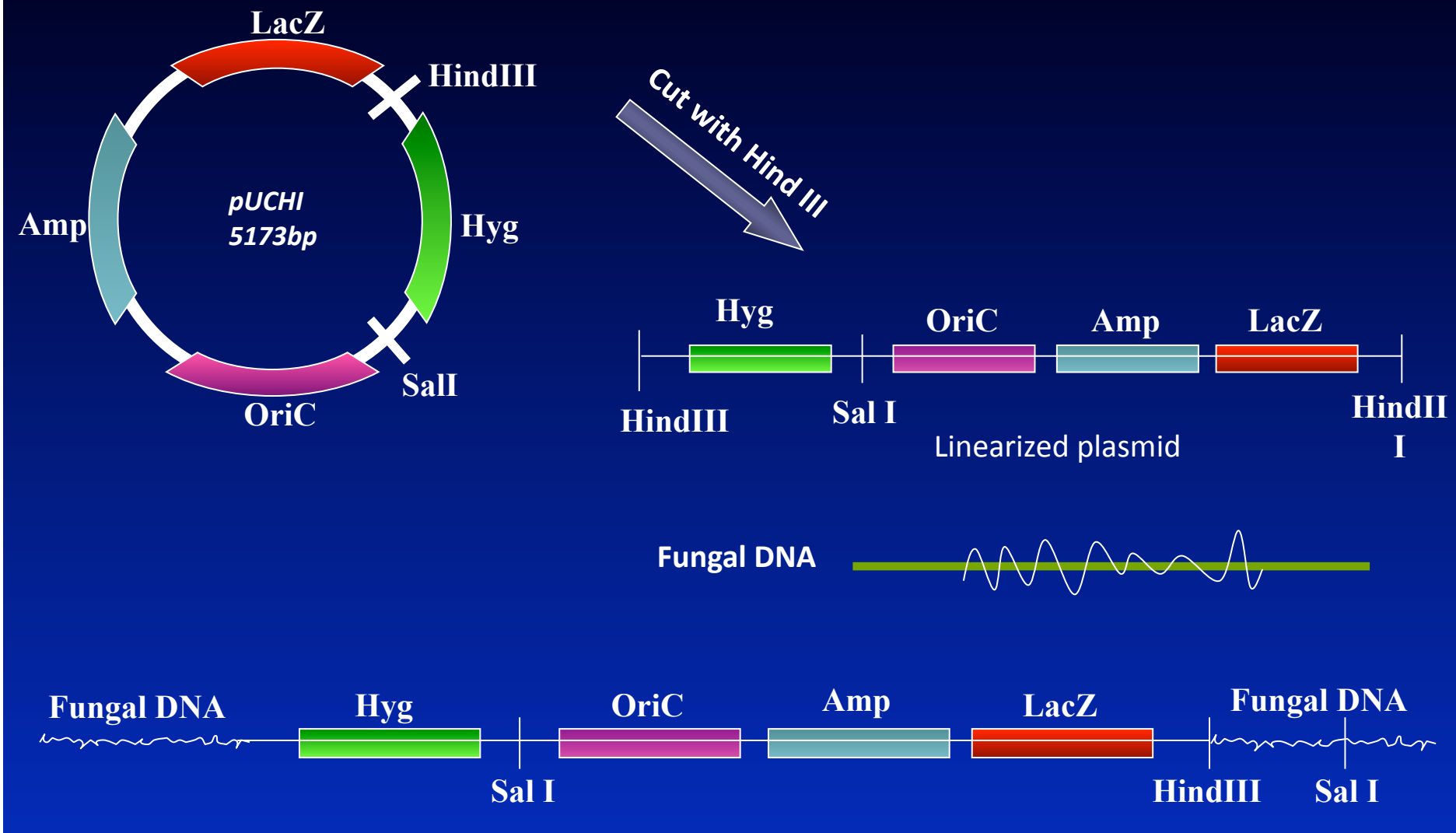


DNA was extracted from 30 ground samples

Objectives

- Identify and characterize fungal genes involved in the biosynthesis of phaseolinone
  - Development of a PEG-based transformation system for the fungus
  - Hygromycin resistance was used as a marker

Fungal Transformation



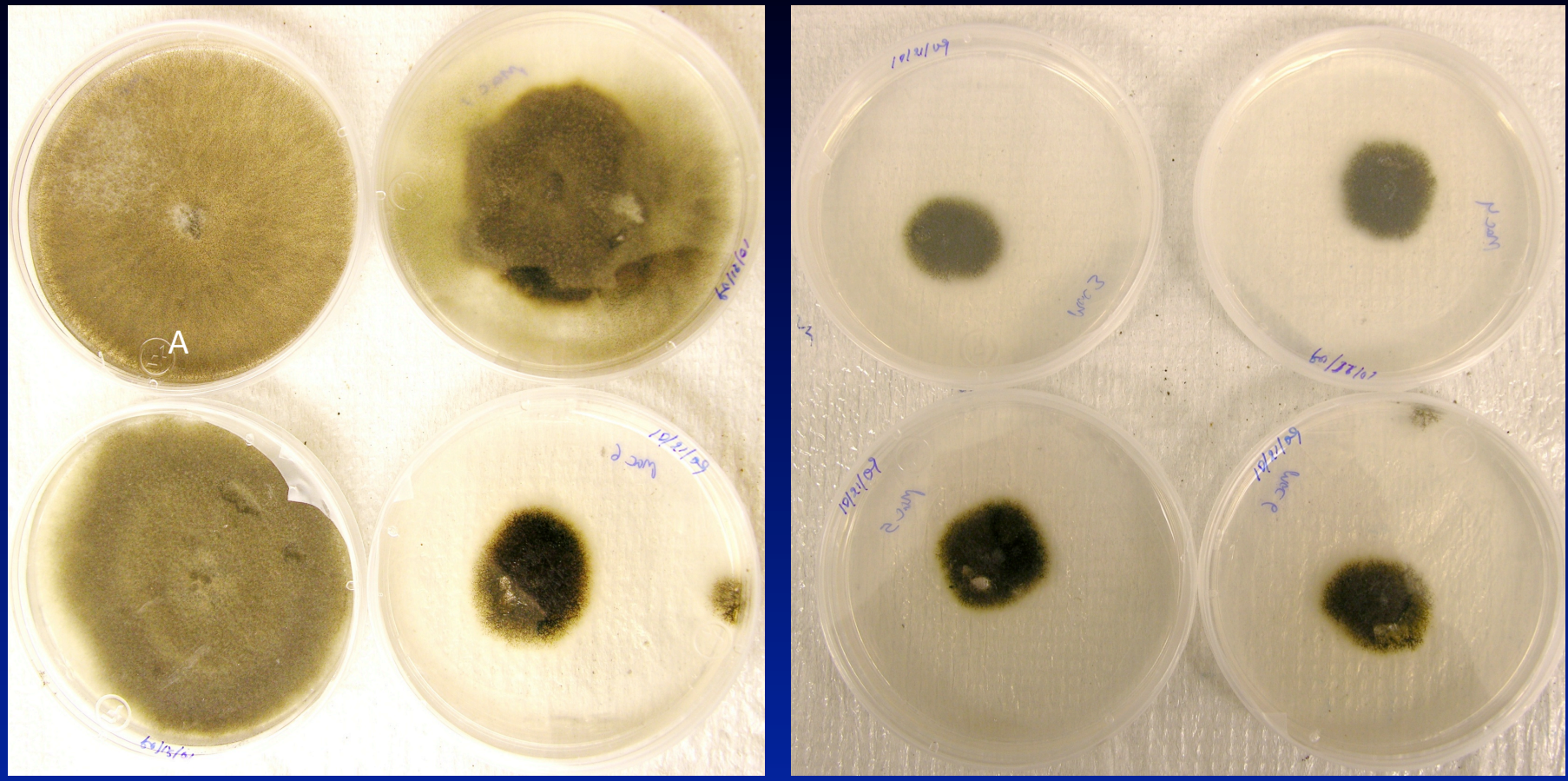


Fig: Different transformant s on Czapek-Dox Agar + Hygromycin , Wild type (A)

Screening of transformants by PCR

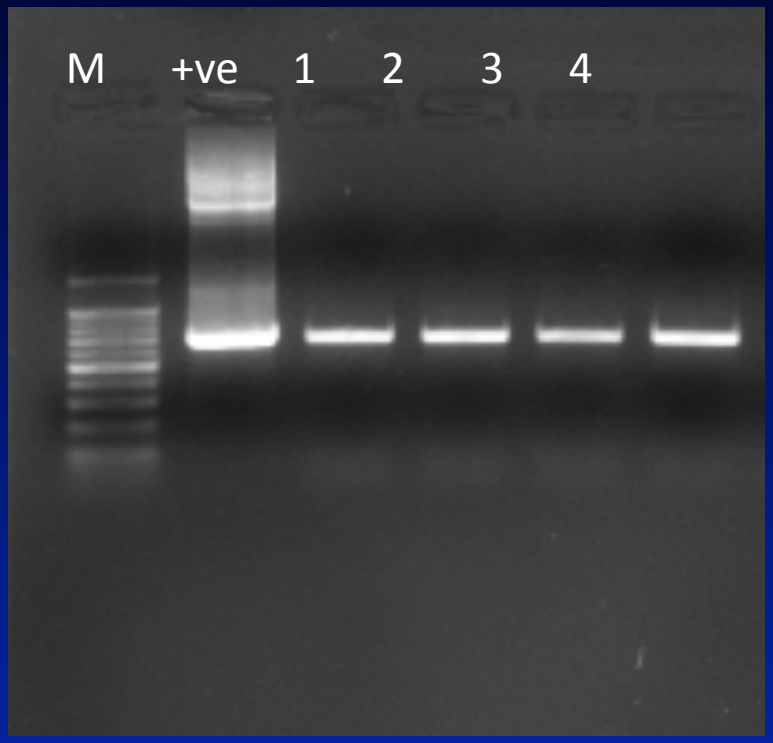
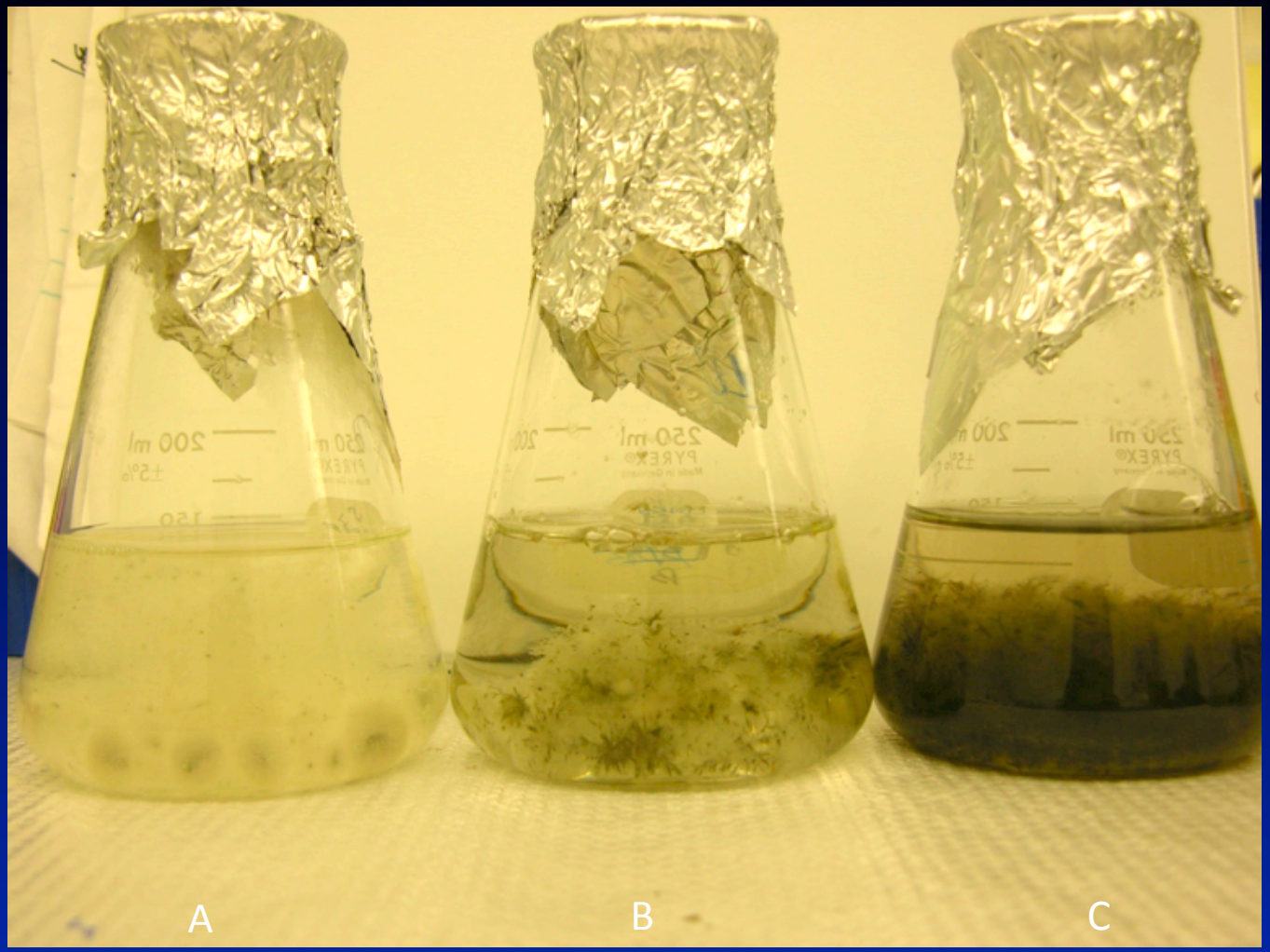


Fig: PCR with Hyg primers

Objectives

- Identify and characterize fungal genes involved in the biosynthesis of phaseolinone
  - Grow fungus under conditions conducive/non conducive to the production of phaseolinone
  - Test fungal filtrate for the presence of phaseolinone by HPLC
  - Confirm toxicity of fungal filtrate using our screening bioassay
  - Extract mRNA
  - Analyze gene expression using cDNA AFLP

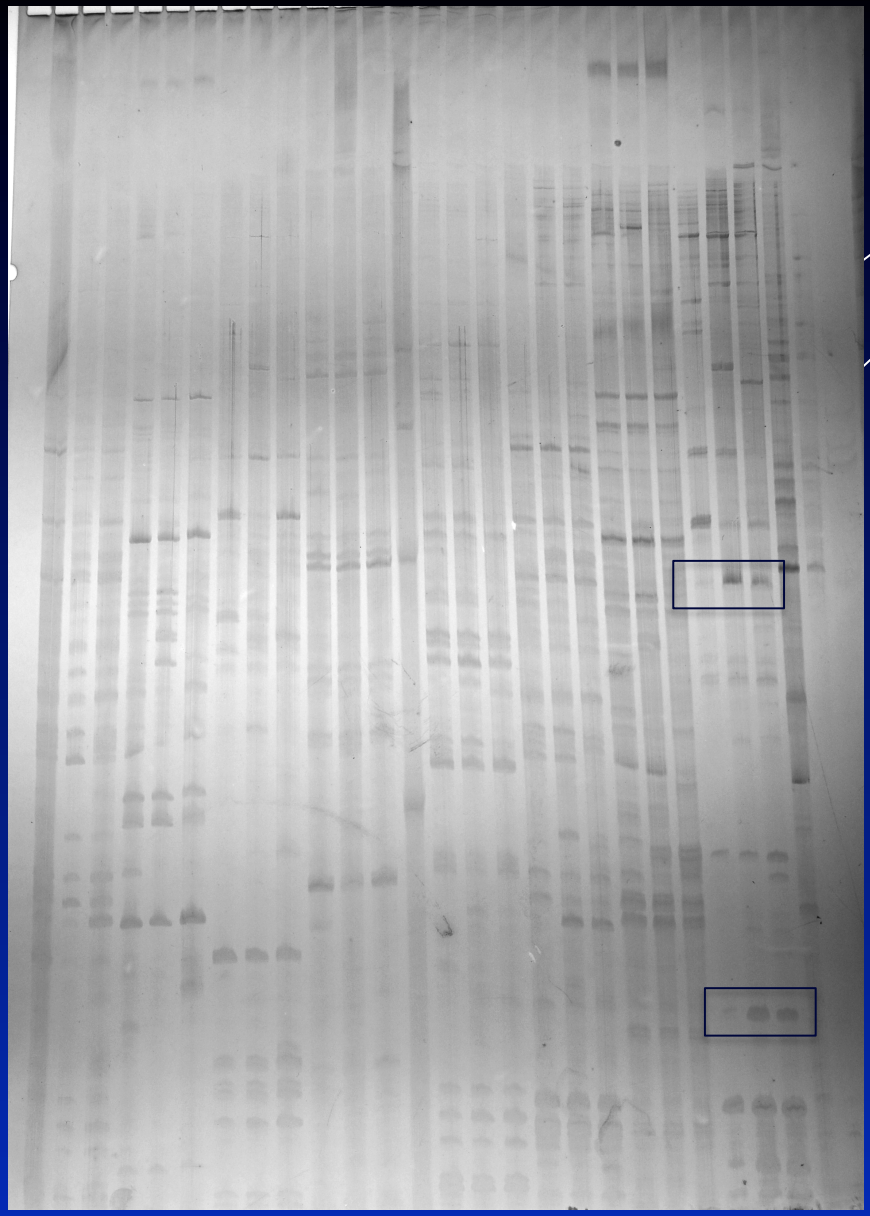


Non- conducive

Conducive

Effect of temperature (A) and Sodium nitrate (B) on pigmentation of *M. phaseolina*. Control (C)

cDNA AFLP



Primers	TDFs	SP	EP
15	950	14	50

- **Cloned**
- **Sequenced**

1- Tox^{-} 2- Tox^{+} 3- Tox^{+}

Transcripts up-regulated under Tox^+ conditions

Tentative annotation
DNA polymerase
No significant similarity
Sybindin-like family protein
No significant match
TIP41-like family; pfam04176
Hypothetical protein MPER_04295
KLTH0B04598p, sugar transporter
Glyoxylase I:Glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily
FHA domain protein SNIP1
Hypothetical protein An11g00230
Nonribosomal peptide synthetase

Transcripts down-regulated under Tox^+ conditions

Tentative annotation
NWD2
30S ribosomal protein S14
L-PSP endoribonuclease family protein
50S ribosomal subunit L7
Ttranscription factor
L-PSP endoribonuclease family protein
aldehyde reductase II
No significant similarity
hypothetical protein NCU09165
protein transport protein sec22
Sfi1 spindle body protein
asparagine synthetase B
hypothetical protein CNE00840
binuclear zinc transcription factor
No significant match
hypothetical protein SS1G_05579
No significant match
Sfi1 spindle body protein; pfam08457
translation initiation factor SUI1
TPA: RNA polymerase II Elongator subunit,WD40 domain
transposase-like protein

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