Plant Defense against Pathogens & Pests Through Phytoalexins

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Plant Science Department
Jan 7, 2014
**pericarp color1** – a marker gene to study gene expression mechanisms & Epigenetics

✅ Red pigmentation in pericarp and cob glumes
✅ More than 100 alleles
✅ Unique phenotypes

Phenylalanine → PAL
Cinnamic Acid → C4H
P-Coumaric Acid → 4CL
4-Coumaroyl-CoA + 3-Malonyl-CoA → C2
Chalcone → CHI1
Naringenin → A1
Flavan-4-ols → PHLOBAPHENES (3-DEOXYFLAVONOIDS)

*P1* variants:
- **P1-rr**
- **P1-wr**
- **P1-rw**
- **P1-vv**
- **P1-ovov**
- **P1-ww**
Defense Compounds

Flavonoids and their Importance

➢ As Nutraceuticals
  • Antioxidant activity of flavonoids
  • Anticarcinogens

➢ Role in plant growth and development.
  ✓ Protection from UV damage
  ✓ Pollinator attractant
  ✓ Auxin transport
  ✓ Signaling during nodulation
OUTLINE

• **Sorghum-Colletotrichum Interactions**
  • Induction of phytoalexins

• Transposon Tagging: Isolation of functional and null alleles to develop near-isogenic lines

• **Metabolic profiling of antifungal compounds in sorghum**

• Induction of Phytoalexins in transgenic maize

• Development of Sorghum Resources
  • Isolation of tagged mutants in the anthracnose pathway
  • Transposon Tagging in grain, forage and sweet sorghum
  • GFP tagged fungal strains
  • Project- NIFA Bioenergy Program

• **Sorghum-Insect Interaction**
Anthracnose in Sorghum

Foliar symptoms of anthracnose

- Anthracnose = like coal
- Caused by *Colletotrichum sublineolium*
- Lesions containing *Phytoalexins* or *Antifungal* compounds
- Work on identifying these compounds was pioneered by the late Dr. Ralph Nicholson, Purdue University

*Sorghum phytoalexins have been classified as 3-Deoxyanthocyanidins (3-DAs)*

Flavonoid Biosynthesis-Maize and Sorghum

Phenylalanine $\xrightarrow{\text{PAL} \ C4H}$ 4-Coumarate $\xrightarrow{4CL}$ Chlorogenic acid

4-Coumaroyl CoA $+ 3$ Malonyl-CoA $\xrightarrow{c2}$ CHS

Chalcone $\xrightarrow{\text{Chl}1}$ CHI

Naringenin $\xrightarrow{\text{Y1}} \text{PI}$

$Y1$ $\xrightarrow{\text{PR1}}$ Apiferol $\xrightarrow{\text{DFR}} F3'H$

Luteofofol $\xrightarrow{\text{PR1}} F3'H$

Apigeninidin $\xrightarrow{\text{PR1}} F3'H$

Luteolinidin $\xrightarrow{\text{PR1}} F3'H$

Phlobaphenes (PERICARP)

$\xrightarrow{\text{Polymerization}}$

$\xrightarrow{\text{Polymerization}}$

Apigeninidin $R = H$
Luteolinidin $R = OH$
Apiferol $R = H$
Luteofofol $R = OH$
Apimaysin $R = H$
Maysin $R = OH$
Pelargonidin $R = H$
Cyanidin $R = OH$

Sharma et al., 2012. BMC Plant Biology
Phlobaphenes biosynthesis - Sorghum

4-coumaroyl-CoA + 3 malonyl-CoA

- This branch produces flavan-4-ols
- Structure similar to 3-DAs
- Are 3-DA’s produced by a sub-branch?

Y1

• Chalcone (c2)
• Flavanone (chi)
• Flavan-4-ols (dfr)

Phlobaphenes

3-Deoxyanthocyanidins

Y1-rr

Sangar 2003, Penn State U
Induction of Phytoalexin compounds

1. Sorghum seeds
2. Germinate in dark
3. Etiolated mesocotyls
4. Inoculate with *C. heterostrophus*
5. Symptomatic mesocotyl

- Difficult to extract compounds from the leaves - interference from chlorophyll
- Developed mesocotyl assay - etiolated seedlings
- Symptom development is rapid and intense with *C. heterostrophus*
Transposon Mutagenesis:
Functional & null alleles of \( y1 \)

Response to fungal inoculation

• Use mesocotyl assay to induce 3-DAs in *Y1-rr3* and *y1-ww1* lines

• Infect mesocotyls with *C. heterostrophus*

• Observe pigment development with time

• *Y1-rr3* produces pigments in 24 h

• *y1-ww1* produces very little pigment even after 36 h
Phytoalexins Produced in Y1 Revertant and y1 Null Lines

HPLC analysis

LC-MS analysis

Disease Severity & Cytology
Resistant vs. Susceptible Lines

Fungal infection - Severity

Disease Severity L4, 11 dpi

Ibraheem, Gaffoor, Chopra. 2010. Genetics
Evaluation And Mitigation Of Anthracnose Disease Pressure Due To The Introduction Of Sorghum For Feedstock Production

Forage, Grain and Sweet Sorghum Genetic Stocks and Cultivar development

Project Team
Dr. Surinder Chopra, Penn State University
Dr. Iffa Gaffoor, Penn State University
Dr. Greg Roth, Penn State University
Dr. Lisa Vaillancourt, University of Kentucky
Dr. Srinivasa Rao, ICRISAT, India.
Dr. Rajan Sharma, ICRISAT, India.

Survival of Fungus in Debris

- Infected midrib
- Infection spreads through stalk
- Acervuli survive over winter and develop on debris under moist conditions

Tim Godfrey doing selections
S Rao, S Deshpande, I Gaffoor
Iffa Gaffoor Greg Roth Sorghum grower
New Project (NIFA, Bioenergy)
NIFA Award Number: 2011-67009-30017

1. Several isolates of *Colletotrichum sublineolum* from the debris collected from different fields in PA and KY.

2. Sweet sorghum lines (Dale, Della, N100, Simon, Sugar Drip and Umbrella) have been screened using 11 isolates of *C. sublineolum*.

3. Field trials for 18 commercial and 24 experimental lines of sorghum.

4. NAM diversity parents for association mapping screened in response to *C. sublineolum*. RNA-seq of selected sorghum lines and identification of candidate genes.

5. At ICRISAT center, India, five hundred sorghum lines and accessions were evaluated and 28 lines were sent to Penn State for further testing in PA.

6. Identification of transposon induced lines of sorghum that produce red seeded phenotype but show susceptibility towards *C. sublineolum*.

7. Breeding sorghum cultivars that inhibit or retard development of the anthracnose in the stalk debris.
## Disease Response & Allelic Diversity of y1 in Sorghum

### I. Near Isogenic Lines

<table>
<thead>
<tr>
<th></th>
<th>Y1-rr3</th>
<th>y1-ww1</th>
<th>BTx623</th>
<th>N321</th>
<th>N322</th>
<th>N327</th>
<th>N330</th>
<th>N332</th>
<th>N334</th>
<th>N338</th>
<th>N340</th>
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<tr>
<td><strong>Putative genotype</strong></td>
<td>RRYY ppqq</td>
<td>R Ryy ppqq</td>
<td>RRyy ppQQ</td>
<td>RRyy ppQQ</td>
<td>RRyy ppQQ</td>
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<td>RRyy PPQQ</td>
<td>RRYY PPQQ</td>
<td>RRYY PPQQ</td>
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<tr>
<td><strong>Plant body pigmentation</strong></td>
<td>tan necrotic</td>
<td>tan necrotic</td>
<td>tan necrotic</td>
<td>tan necrotic</td>
<td>tan necrotic</td>
<td>tan necrotic</td>
<td>purple necrotic</td>
<td>purple necrotic</td>
<td>purple necrotic</td>
<td>purple necrotic</td>
<td></td>
</tr>
<tr>
<td><strong>Pericarp color</strong></td>
<td>red</td>
<td>white</td>
<td>white</td>
<td>white</td>
<td>white</td>
<td>red</td>
<td>red</td>
<td>white</td>
<td>white</td>
<td>red</td>
<td>red</td>
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<tr>
<td><strong>C. heterostrophus infection</strong></td>
<td><img src="c_heterostrophus.png" alt="Image" /></td>
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<td><img src="c_heterostrophus.png" alt="Image" /></td>
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<td><strong>C. sublineolum infection</strong></td>
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<td><strong>Water control</strong></td>
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<td><img src="water_control.png" alt="Image" /></td>
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</tr>
<tr>
<td><strong>3-Deoxyanthocyanidins from mesocotyl</strong></td>
<td><img src="3-deoxyanthocyanidins.png" alt="Image" /></td>
<td><img src="3-deoxyanthocyanidins.png" alt="Image" /></td>
<td><img src="3-deoxyanthocyanidins.png" alt="Image" /></td>
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<tr>
<td><strong>ALB (field test)</strong></td>
<td>3.3</td>
<td>2.4</td>
<td>1.9</td>
<td>1.6</td>
<td>1.7</td>
<td>1.2</td>
<td>2.3</td>
<td>1.7</td>
<td>1.9</td>
<td>3.3</td>
<td>NA</td>
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<tr>
<td><strong>ASR (field test)</strong></td>
<td>2.0</td>
<td>1.3</td>
<td>1.6</td>
<td>1.9</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.4</td>
<td>1.8</td>
<td>2.5</td>
<td>NA</td>
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<tr>
<td><strong>Flavan-4-ols in pericarp</strong></td>
<td>√</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>√</td>
<td>√</td>
<td>X</td>
<td>?</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td><strong>Flavan-4-ols in glumes</strong></td>
<td>√</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>√</td>
<td>√</td>
<td>X</td>
<td>X</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Disease Response & Allelic Diversity of \textit{y1} in Sorghum

II. Sorghum Diversity Panel

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SC1345</th>
<th>Segalone</th>
<th>Ajab sido</th>
<th>SC1103</th>
<th>SC971</th>
<th>SC283</th>
<th>SC265</th>
<th>SC35</th>
<th>RTx430</th>
<th>\textit{Y1-rr3}</th>
<th>\textit{y1-ww4}</th>
</tr>
</thead>
</table>

Disease Response & Allelic Diversity of y1 in Sorghum

HPLC Profile of 3-DA Phytoalexins

Resistant

Susceptible

Y1-rr3

N338

SC1345

y1-ww4

N321

SC265

Coordinated Induction of Flavonoid Pathway Genes’ Transcripts

Phytoalexins Synthesis

CELLULAR RESPONSE – Sorghum x Anthracnose

Y1+ AP

Y1

TF

SIGNAL

E

RE
Sorghum Genetic Resources

Development of Transposon Tagged Lines
Transposon Mutagenesis: Anthracnose Resistance

- High throughput screening for sorghum mutant for Anthracnose:
- Resistant Mutants that **over-produce** 3-DA phytoalexins
- Resistant Mutants that **produce yet to identified** phytoalexins
- **Susceptible mutants** that produce 3-DA phytoalexins?
- **Resistant mutants** that do not produce 3-DA phytoalexins?
Identification of Deletion Derivatives of \( y_1 \) and \( cs_1 \) Transposon

Ibraheem, Gaffoor, Chopra. 2010. Genetics
<table>
<thead>
<tr>
<th>Allele Name</th>
<th>Seed Pericarp and Leaf phenotypes</th>
<th>Molecular Structure of ( y_1 ) and ( C_s_1 )</th>
<th>Reference/ Isolated by</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutable Alleles of ( y_1 ).</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( Y_1-c_s_1 ) (stock: CS8110419) (From Late Prof. J. Axtell).</td>
<td>Red-White stripes (variegated) on pericarps and leaves of mature plant</td>
<td>Known: ( C_s_1 ) inserted in intron II of ( y_1 )</td>
<td>Chopra et al., 1999.</td>
</tr>
<tr>
<td>( Y_1-c_s 30 )</td>
<td>Abundant large and small red stripes on pericarp and leaves.</td>
<td>Known: Hyper-mutable allele that has very high frequency of somatic and germinal excisions.</td>
<td>Chopra et al., 2002; Sangar, 2003.</td>
</tr>
<tr>
<td>( Y_1-c_s 2 )</td>
<td>Early and late excisions of ( C_s_1 ), showing large and small stripes on pericarp and leaves.</td>
<td>Fully characterized. Derived from ( Y_1-c_s 4 ) and has ( d_c s_2 ) insertion. The ( d_c s_2 ) element is a deletion derivative (see Figure 4).</td>
<td>J. Boddu, P. Wang, and S. Chopra, unpublished</td>
</tr>
<tr>
<td>( Y_1-c_s 4 )</td>
<td>Occasional stripes. ( C_s_1 ) excisions can be early (seedling leaf) or late (pericarp).</td>
<td>Derived from a ( Y_1-c_s 30 ) x ( Y_1-r_r 30 ) test cross. It has a deletion derivative ( d_c s_2 ) at a nearby location along with the original ( C_s_1 ) element.</td>
<td>Chopra et al., 2002.</td>
</tr>
<tr>
<td><strong>Revertants Alleles of ( y_1 ).</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( Y_1-r_r 1-1 )</td>
<td>Red. Stable inheritance</td>
<td>Known: Functional ( y_1 ); ( C_s_1 ) from ( Y_1-c_s 30 ) excised and left ‘tt’ footprint</td>
<td>Ibraheem et al., 2010.</td>
</tr>
<tr>
<td>( Y_1-r_r 2, ) ( Y_1-r_r 3 )</td>
<td>Red. Stable inheritance</td>
<td>Known: Functional ( y_1 ); ( C_s_1 ) excised and left ‘tcttt’ footprint</td>
<td>Carvalho et al., 2005</td>
</tr>
<tr>
<td>( Y_1-r_r 30 )</td>
<td>Red. Stable inheritance</td>
<td>Known: Functional ( y_1 ); ( C_s_1 ) excised and left ‘tt’ footprint</td>
<td>Chopra et al., 2002.</td>
</tr>
<tr>
<td><strong>Loss of Function Alleles of ( y_1 ).</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( y_1-w_w 623 )</td>
<td>White pericarp, glume, leaf and all other tissues where ( y_1 ) is functional</td>
<td>From inbred BTx623; tested and allelic to ( Y_1-w_w 4 ); Partial deletions in the exon1 and exon 2 of ( y_1 ).</td>
<td>Boddu et al., 2005.</td>
</tr>
<tr>
<td>( y_1-w_w 1, ) ( y_1-w_w 4 )</td>
<td>-ditto-</td>
<td>Derived from ( Y_1-c_s 30 ). ( C_s_1 ) has large deletion of the 5’ region leaving behind a small 3’ region of the transposase protein.</td>
<td>Ibraheem et al., 2010.</td>
</tr>
<tr>
<td>( y_1-w_w 971, ) ( y_1-w_w 972, ) ( y_1-w_w 914 )</td>
<td>Abundant stripes/red sectors in seed pericarp.</td>
<td>Derived from ( Y_1-c_s 30 ) x ( Y_1-r_r 30 ) cross. Complete structure not known. Gel blots show partial deletions of ( C_s_1 ). ( Y_1 ) gene intact.</td>
<td>Gaffoor, I and S. Chopra, Unpublished</td>
</tr>
</tbody>
</table>
Phenolic Compounds & Disease Tolerance (GC/MS Profile)
Candystripe1 tagged putative sorghum mutants.
Genetic Engineering of Anthracnose Resistance in Maize?

• Why 3-DA phytoalexins are NOT induced in maize leaves?
• Can phytoalexins be engineered in maize and other cereals?
• Can we develop maize and sorghum lines with constitutive levels of compounds that provide tolerance against diseases?
Induction of Phytoalexins in Maize

ZmP1

SbY1
**SbY1 Transgene in Maize**

**C. Graminicola on 4 W old leaves**

<table>
<thead>
<tr>
<th>Ear</th>
<th>Y1-rr</th>
<th>Y1-pr</th>
<th>Y1-wr</th>
<th>NS</th>
<th>P1-rr</th>
<th>P1-wr</th>
<th>Hl II</th>
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<tbody>
<tr>
<td>Husk</td>
<td></td>
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<td>Tassel</td>
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<td></td>
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</tr>
<tr>
<td>Mid-rib</td>
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<tr>
<td>Silk</td>
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</tbody>
</table>

**SbY1 Phenocopy’s ZmP1**

Gaffoor et al., 2013

HPLC - Phytoalexin profile
Maize Transgenics: Phytoalexin Profiling

M = maysin; aM = apimaysin; CGA = chlorogenic acid; L = luteolinidin * = luteolinidin glycosides
Sorghum- Aphid interactions

*Sorghum bicolor* - *Rhopalosiphum maidis*
Experimental Design

- 4 genotypes, 20 plants each
  - 3 NILS: y1 mutant and wild types
  - 1 wild type line BTx623
- Clip cages placed on each plant
- 2 on alternate younger leaves
- 2 on older leaves
- Each cage had 2 mature aphids
- Replicated three times
Aphid count after 48 hrs

Adult aphids were removed

5 immature aphids/ cage left to develop

Each plant had a total of 8 adults
Data on aphid numbers collected at 5, 10 days after..

**Mean number of aphids after 5 days**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aphid Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>y1-ww4</td>
<td>a</td>
</tr>
<tr>
<td>y1-rr3</td>
<td>a</td>
</tr>
<tr>
<td>BT-623</td>
<td>a</td>
</tr>
</tbody>
</table>

**Mean number of aphids after 10 days**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aphid Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>y1-ww4</td>
<td>a</td>
</tr>
<tr>
<td>y1-rr3</td>
<td>b</td>
</tr>
<tr>
<td>BT-623</td>
<td>a</td>
</tr>
</tbody>
</table>
Aphids only seen on mutants (\textit{y1-ww})

\textit{y1-ww}  \quad \textit{Y1-rr}  \quad \text{BTx623}
Preliminary Results

1. Aphids in cages reproduced significantly higher on y1-ww and BTx623 seedlings and adult plants with heads.

2. In greenhouse, aphid incidence was higher on y1-wws than Y1-rr3 plants when aphids moved from a source (other sorghum plants).

3. When Y1-rr3 and y1-ww plants were subjected to EPG analyses, no difference observed in the feeding behavior of aphids.

4. When artificial aphid diet was enriched with a dilution factor of 10 of Y1-rr3 and y1-ww extracts, there was significantly higher mortality on Y1-rr3 compared to control and y1-ww plant diet.
Future Experiments of Plant-Aphid interactions

(Collaborative: ICRISAT - Dr. HC Sharma)

- Aphid population assay in lab
- Emigration assay
- Choice tests
- Volatile organic compounds
- Flavonoid quantification
- Phytohormone analyses
Thank you....

Grads and Post-docs
- Iffa Gaffoor, Post-doc.
- Rupesh Kariyat, Post-Doc
- Michael Robbins, Post-doc.
- Weiya Xue, Post-doc.
- Dinakaran Elango
- Kameron Wittmeyer, Grad St.
- Jay Boddu, Post-doc. (Monsanto, St. Luis)
- Nur Suhada Abu Baker, Grad St. (SIMPLOT)
- Vineet Sangar, Grad St. (Ins Systems Biol)
- Farag Ibraheem, Grad St. (U of Monsoura)
- Rajandeep Sekhon, Grad St. (UW, Madison)
- Mandeep Sharma, Grad St. (AZU, Phoenix)
- PoHao Wang, Grad St. (DAS, Indianapolis)

Undergrad students
- Tierney Dincher
- Shereen Elmaghrabi
- Laura Reese
- Fatimah Audu

Collaborators
- Blake Meyers, Tzuu-fen Lee, UDEL
- Jay Rohila, Ansuman Rao, SDSU
- Don Auger, SDSU
- Marcia Buanafina, PSU
- Seogchan Kang, PSU
- Greg Roth, PSU
- Lisa Vailancourt, UKY
- Srinivasa Rao, ICRISAT, India
- Rajan Sharma, ICRISAT, India
- Hari Sharma, ICRISAT, India
- Jeff Pedersen, USDA/ARS for N lines
- Scott Settler, USDA/ARS
- Jianming Yu, ISU (NAM lines)
- Agnes Ricroch, Agro Paris Tech
- Robert Thierry, Univ of Paris-Sud

Visiting Scientists
- Rashmee Thakare, U of Wagningen
- Sanjeev Deshpande, UAS, Dharward

PSU Agronomy Farm Staff