"An overview of wheat transformation at Kansas State University"

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Why Transform Wheat?

- Trait introduction for sexual incompatible sources
- Over-expression of transgene
- Tissue/organ localized trait expression
- Knockout phenotype (gene silencing)
- Gene pyramid (single breeding locus)

Public acceptance is needed before deployment

Validation of candidate genes
Wheat Tissue culture

- **Plant Recovery**
- **Starting Material**
- **Regeneration MSE**
- **Development MSP**
- **Proliferation**
- **Induction CM4**
### Tissue culture response of select hard winter vs. Bobwhite

<table>
<thead>
<tr>
<th>Cultivar or germplasm</th>
<th>Callus formation (%)</th>
<th>Plant regeneration (%)</th>
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<tbody>
<tr>
<td>KS920866-B-7</td>
<td>92.3</td>
<td>151.9</td>
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<tr>
<td>KS920709-B-5-2</td>
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<td>124.1</td>
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<tr>
<td>KS85WGRC01</td>
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<tr>
<td>KS89WGRC04</td>
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<tr>
<td>2137</td>
<td>54.7</td>
<td>39.6</td>
</tr>
<tr>
<td>2163</td>
<td>51.0</td>
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<tr>
<td>Jagger</td>
<td>70.6</td>
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<td>Karl 92</td>
<td>74.0</td>
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<tr>
<td>Larned</td>
<td>46.3</td>
<td>9.3</td>
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<tr>
<td>Stanof</td>
<td>75.5</td>
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<tr>
<td>TAM 107</td>
<td>54.6</td>
<td>65.5</td>
</tr>
<tr>
<td>Tomahawk</td>
<td>86.0</td>
<td>126.0</td>
</tr>
<tr>
<td>Bobwhite</td>
<td>92.0</td>
<td>82.5</td>
</tr>
</tbody>
</table>
Wheat Transformation

Germination

Development

Proliferation

5 mg/L and 10 mg/L ammonium glufosinate
$T_0$ Plant Preliminary screening

Liberty painting

Application of a 0.2% aqueous solution of Liberty: plants evaluated after one week.

PCR gene detection

Maize ubi promoter $\rightarrow$ bar gene $\rightarrow$ nos terminator

bar gene PCR

Maize ubi. promoter $\rightarrow$ CP $\rightarrow$ gus linker $\rightarrow$ CP $\rightarrow$ nos terminator

GOI PCR

Antisense arm Sense arm
Wheat transformation timeline:

- plant seeds for immature embryo production (~ -60 to 120 da.) (plant 5 pots with 3-4 seeds/pot)
- Day 0: Harvest immature embryos (10-14 post anthesis) plate and initiate on CM4 media for 2-7 days
- Day 7: Select for embryogenic calli, bombard, then recover
- Day 10-12: Transfer to CM4 + 5 mg/ml glufosinate (G) for 2 wks
- Week 3: Transfer to CM4 + 10 mg/ml G (2 wks)
- Week 5: Transfer to CM4 + 10 mg/ml G (2wks)
- Week 7: Transfer to MSP + 10 mg/ml G and to light (2wks)
- Weeks 8-16: Transfer to MSE+ 5 G (tubes) for shot elongation and rooting

Dehlie McAfee, research assistant
Wheat transformation timeline (cont.):

- **Weeks 8-16**: Transfer to MSE+ 5 G (tubes) for shot elongation and rooting

- **Weeks 9-20(+)**: Transfer to soil (peat pots) and condition to lower RH

- **Week 12**: Transfer to one gallon pot

- **Week 13-14**: Paint with Liberty (3-5 leaf-stage)

- **Week 14**: DNA sampling for PCR analysis

- **Week 20**: Harvest T1 seed

**Total time from bombardment:**
- 5-7 months (Spring wheat)
- 6-9 months (Winter wheat)
### Transformation capacity

<table>
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<tr>
<th>Year</th>
<th>Constructs</th>
<th>Events</th>
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<tr>
<td>2008</td>
<td>47</td>
<td>327</td>
</tr>
<tr>
<td>2009</td>
<td>23</td>
<td>167</td>
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<td>2010</td>
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<td>75</td>
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<tr>
<td>2011</td>
<td>16</td>
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**Spring**
- Bobwhite
- Fielder
- Lalbahadur
- Giza 164
- Chinese spring

**Durham**
- Ofanto
- Belzer
- Ben
- Maier

**Winter**
- Jagger
- Fuller
- WGRC42
- Molly
- Overly
- Heyne
- Karl92
- LR34
Past and current wheat transformation projects

- FHB$^{1,2}$
- WSMV resistance$^1$
- TriMV resistance$^1$
- Leaf rust resistance$^{1,2}$
- Stem rust$^1$
- Hessian Fly resistance$^{1,2}$
- Greenbug tolerance$^{1,2}$
- Lesion nematode resistance$^1$

- Al tolerance$^2$
- Heat stress$^1$
- Value-added projects
  - cellulosic ethanol$^1$
  - zein protein expr.$^1$
- Gene validations$^{1,2}$

$^1$On-campus collaboration; $^2$Off-campus collaboration
FHB resistance is enhanced in transgenic wheat expressing the *Arabidopsis thaliana* defense regulatory *NPR1* gene.
FHB severity is enhanced in plants expressing the *NahG* gene, which encodes a salicylic acid degrading enzyme.

*NPR1*-conferred FHB resistance is attenuated when *NahG* is co-expressed.
FHB resistance is enhanced in transgenic wheat expressing the *Arabidopsis thaliana* *PAD4* and *WRKY18* genes.
FHB resistance is enhanced in transgenic wheat expressing a RNAi construct for silencing expression of a gene that encodes a lipid oxidizing enzyme.
WSMV/TriMV Resistance

VIRAL COMPONENTS

<table>
<thead>
<tr>
<th></th>
<th>CP</th>
<th>CI</th>
<th>P1</th>
<th>6k2NaI</th>
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<tbody>
<tr>
<td>WSMV</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>TriMV</td>
<td>✓</td>
<td>✓</td>
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</table>
Bioassays on WSMV-CP RNAi T$_1$ Plants

ELISA values

Absorbance (OD 405 nm)

Line - tiller

Jessica Rupp
PhD student
ELISA Results- $T_3$ Generation WSMV-CP

- WSMV resistance is currently being crossed into Overly
Current Team Members:

Hyeonju Lee, research assistant
Dehlia McAfee, research assistant
Jessica Rupp, PhD student
Dr. John P. Fellers, USDA-ARS
Jyoti Shah, UNT

Previous Lab Members:
Dr. Marcy Main, DVM, research assistant
Juliane Essig, research assistant
Sheila Stevens, research assistant
Melissa Wohler, research assistant
Luisa Cruz, MS student