"An overview of wheat transformation at Kansas State University"

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KANSAS WHEAT COMMISSION

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

Induction CM4

Proliferation

Development MSP

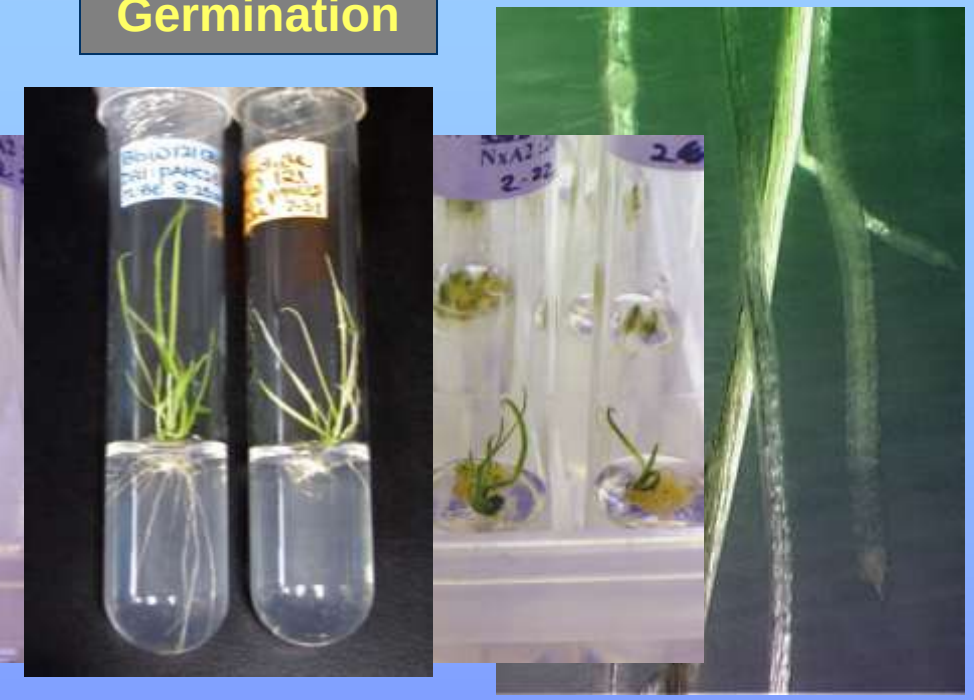
Regeneration MSE

Tissue culture response of select hard winter vs. Bobwhite

Cultivar or germplasm	Callus formation (%)	Plant regeneration (%)
KS920866-B-7	**92.3**	**151.9** ←
KS920709-B-5-2	**96.3**	**124.1** ←
KS85WGRC01	51.0	31.4
KS89WGRC04	**82.7**	**119.2** ←
2137	54.7	39.6
2163	51.0	25.5
Jagger	**70.6**	**152.9** ←
Karl 92	74.0	40.0
Larned	46.3	9.3
Stanof	75.5	26.4
TAM 107	54.6	65.5
Tomahawk	**86.0**	**126.0** ←
Bobwhite	**92.0**	**82.5**

Wheat Transformation

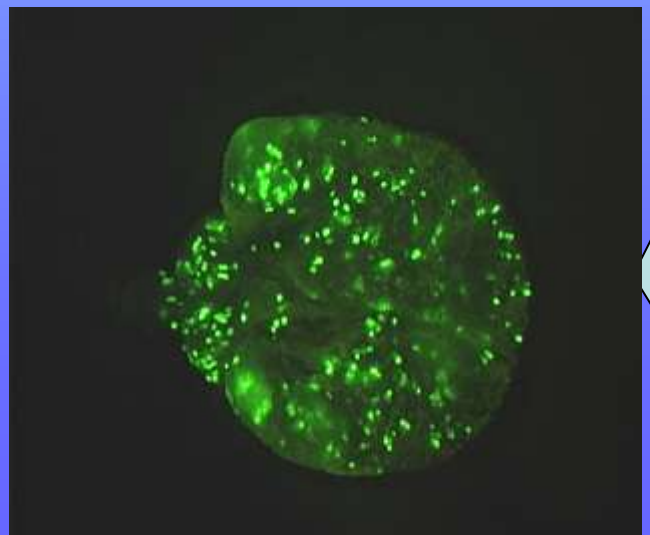
Germination



Development

Proliferation

5 mg/L and 10 mg/L
ammonium glufosinate



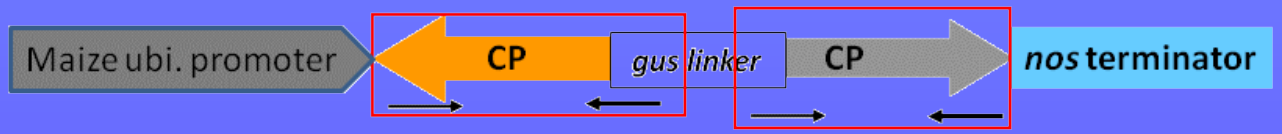
T_0 Plant Preliminary screening

Liberty painting

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

PCR gene detection

bar gene PCR



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

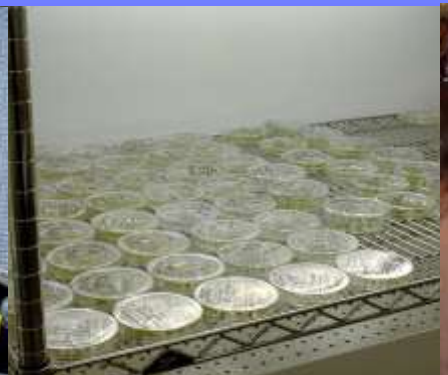
- **Day 10-12:** Transfer to CM4 + 5 mg/ml glufosinate (G) for 2 wks

- **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)

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 T_1 seed

Total time from bombardment:

5- 7 months (Spring wheat)

6-9 months (Winter wheat)

Transformation capacity

Year	Constructs	Events
2008	47	327
2009	23	167
2010	22	75
2011	16	126

Spring

Bobwhite
Fielder
Lalbahadur
Giza 164
Chinese spring

Durham

Ofanto
Belzer
Ben
Maier

Winter

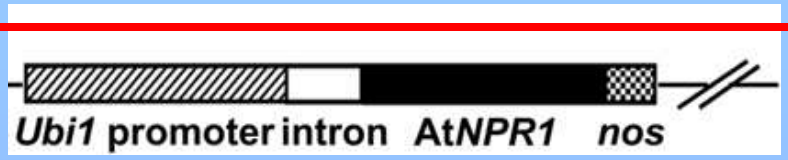
Jagger
Fuller
WGRC42
Molly
Overly
Heyne
Karl92
LR34

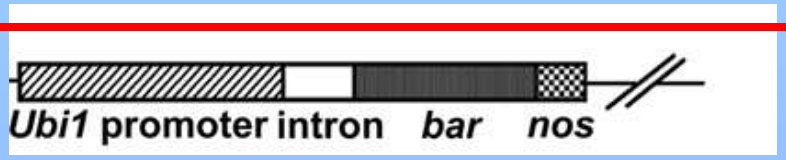
Past and current wheat transformation projects

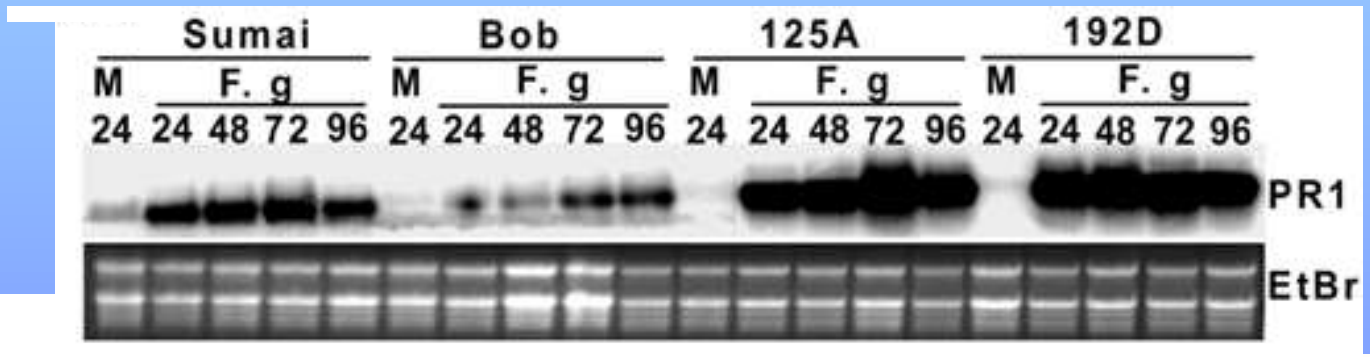
- FHB[1,2]
- WSMV resistance[1]
- TriMV resistance[1]
- Leaf rust resistance[1,2]
- Stem rust[1]
- Hessian Fly resistance[1,2]
- Greenbug tolerance[1,2]
- Lesion nematode resistance[1]
- Al tolerance[2]
- Heat stress[1]
- Value-added projects
  - cellulosic ethanol[1]
  - zein protein expr.[1]
- Gene validations[1,2]

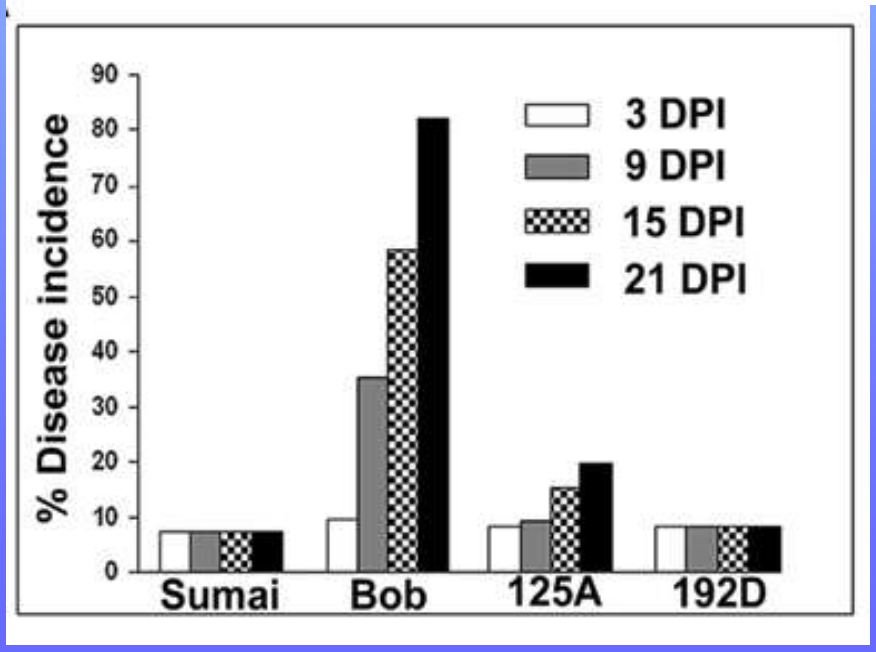
[1]On-campus collaboration; [2]Off-campus collaboration

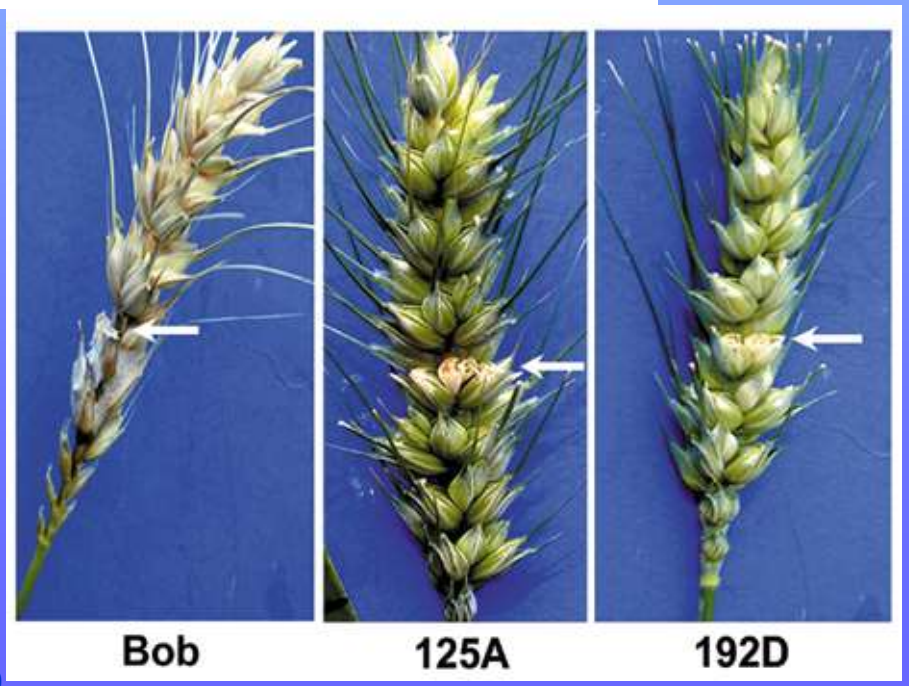
FHB resistance is enhanced in transgenic wheat expressing the *Arabidopsis thaliana* defense regulatory *NPR1* gene





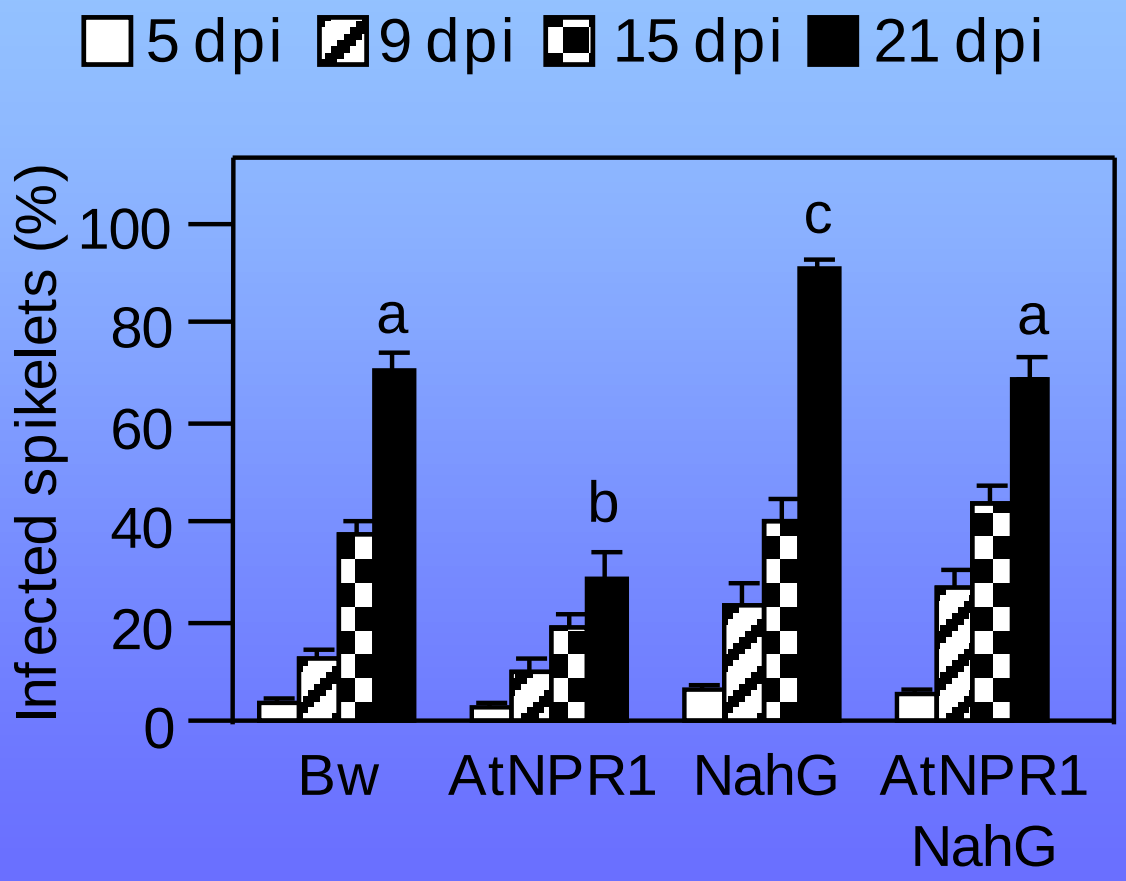






Makandar et al. Mol Plant-Microbe Interact 2006, 19:123–129

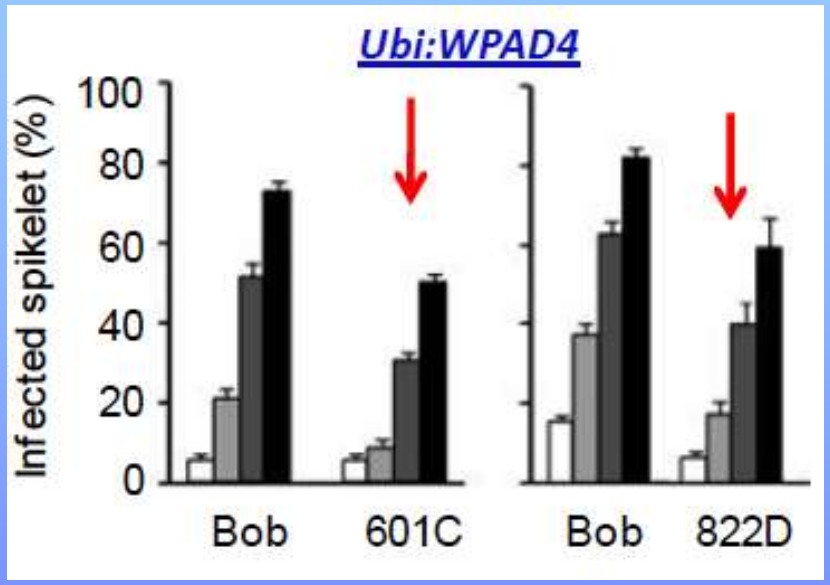
FHB severity is enhanced in plants expressing the *NahG* gene, which encodes a salicylic acid degrading enzyme

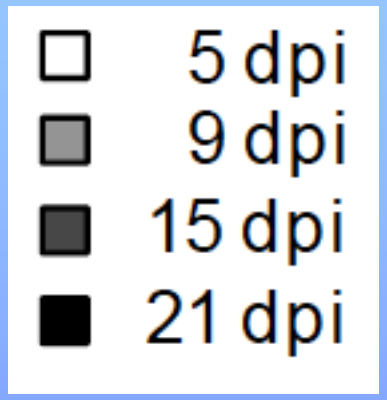


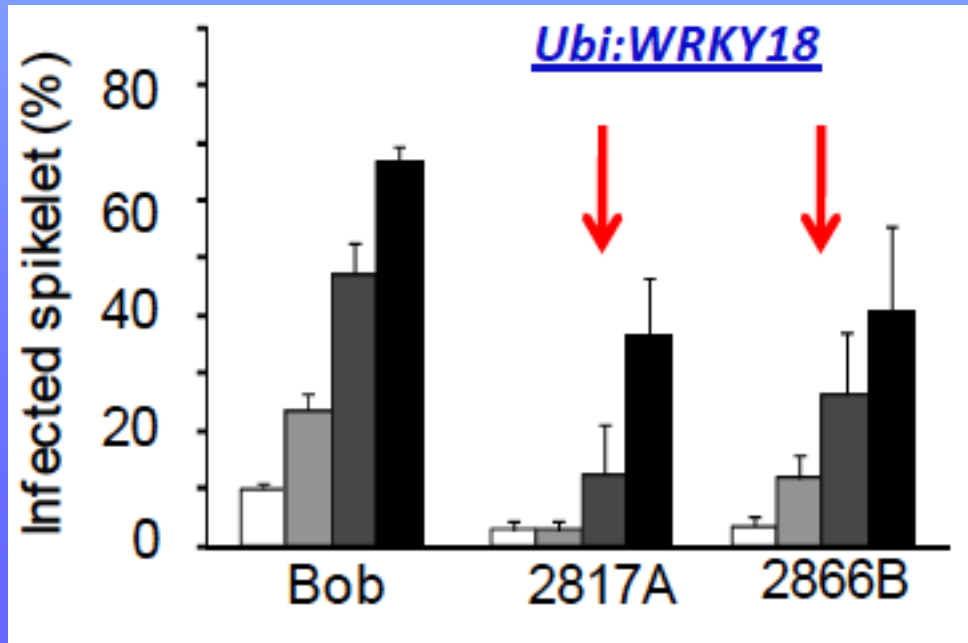
NPR1-conferred FHB resistance is attenuated when *NahG* is co-expressed

Makandar et al. Mol Plant-Microbe Interact. in press

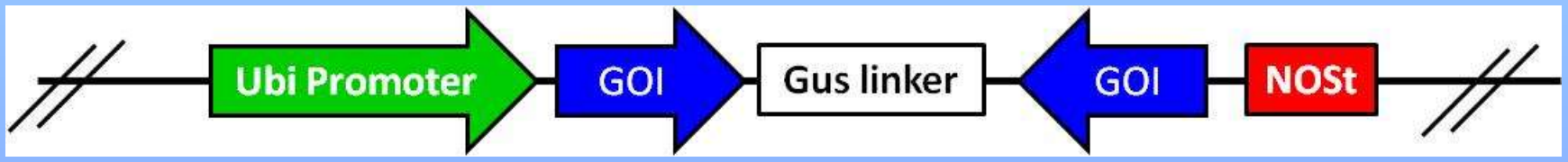
FHB resistance is enhanced in transgenic wheat expressing the *Arabidopsis thaliana PAD4* and *WRKY18* genes

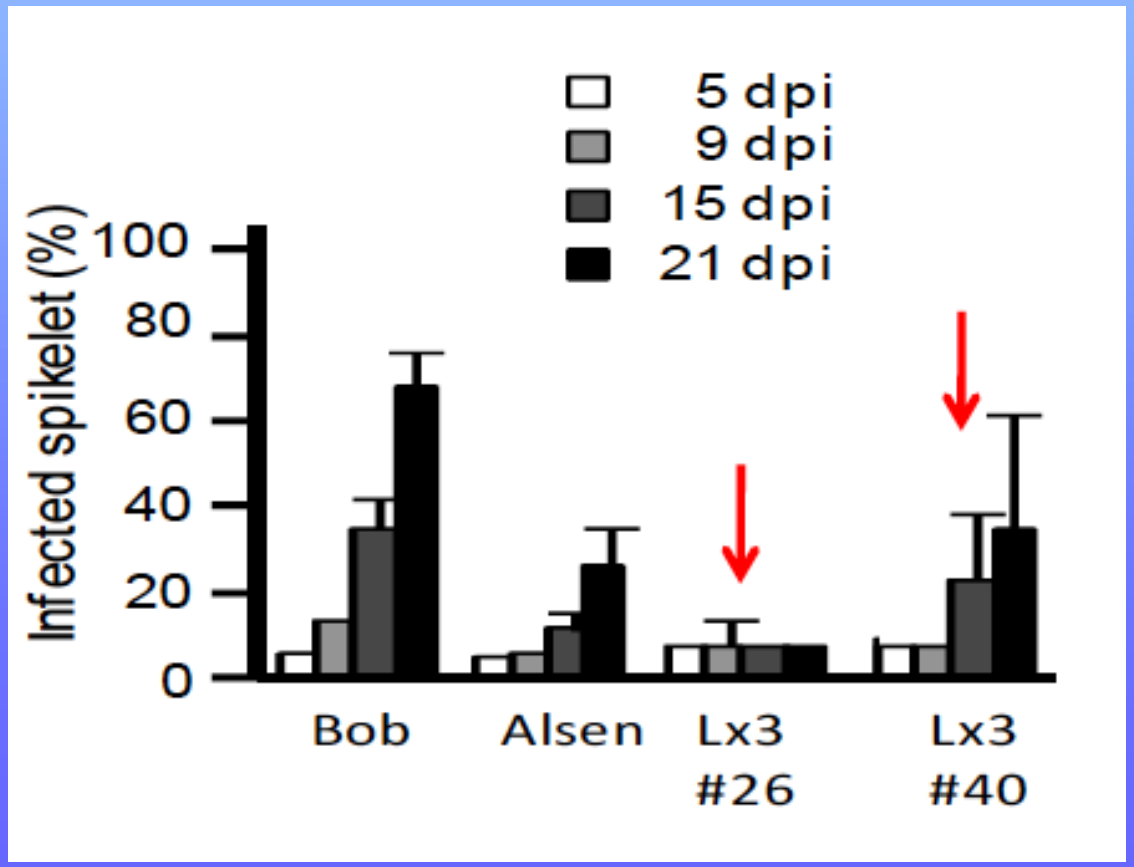




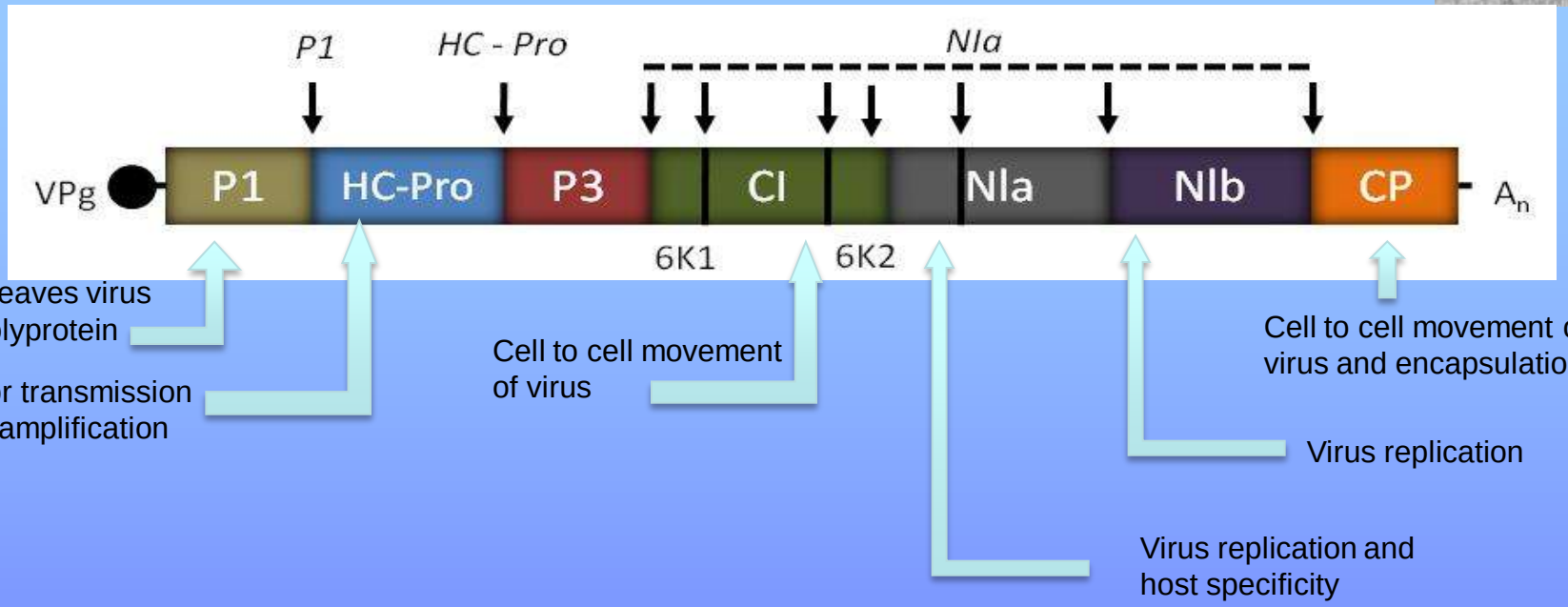


FHB resistance is enhanced in transgenic wheat expressing a RNAi construct for silencing expression of a gene that encodes a lipid oxidizing enzyme



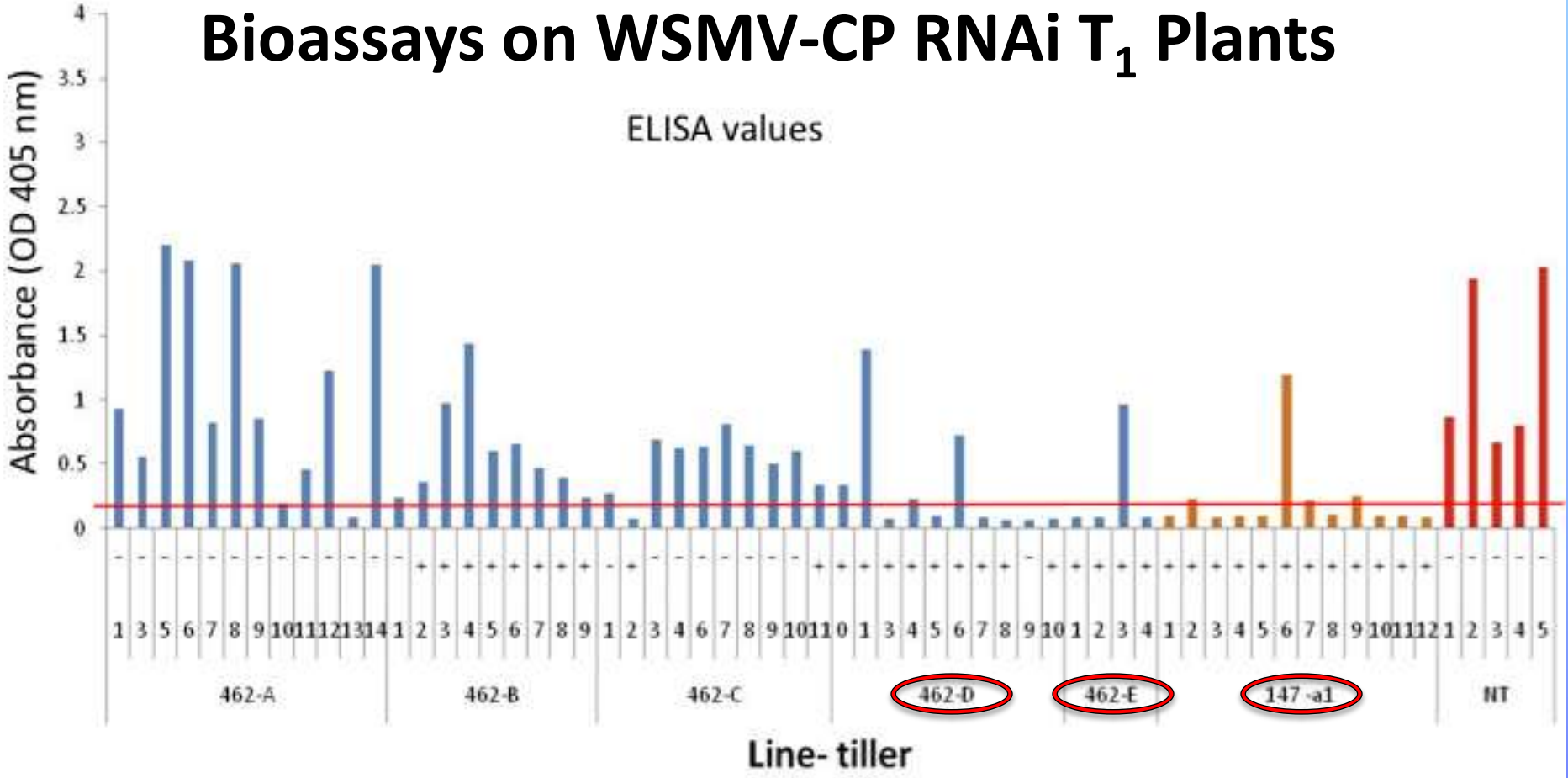


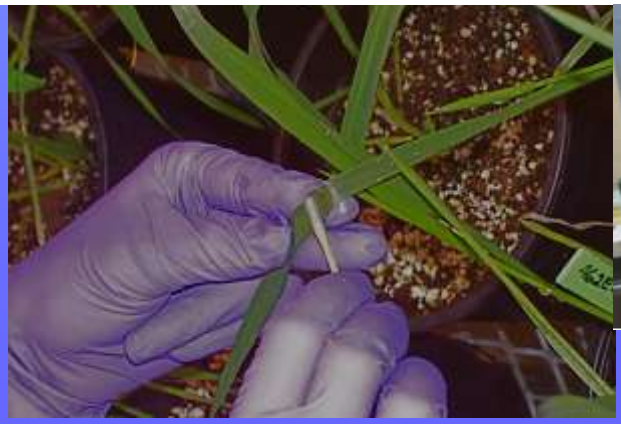
WSMV/TriMV Resistance



VIRAL COMPONENTS				
	CP	CI	P1	6k2Nal
WSMV	✓	✓	✓	✓
TriMV	✓			

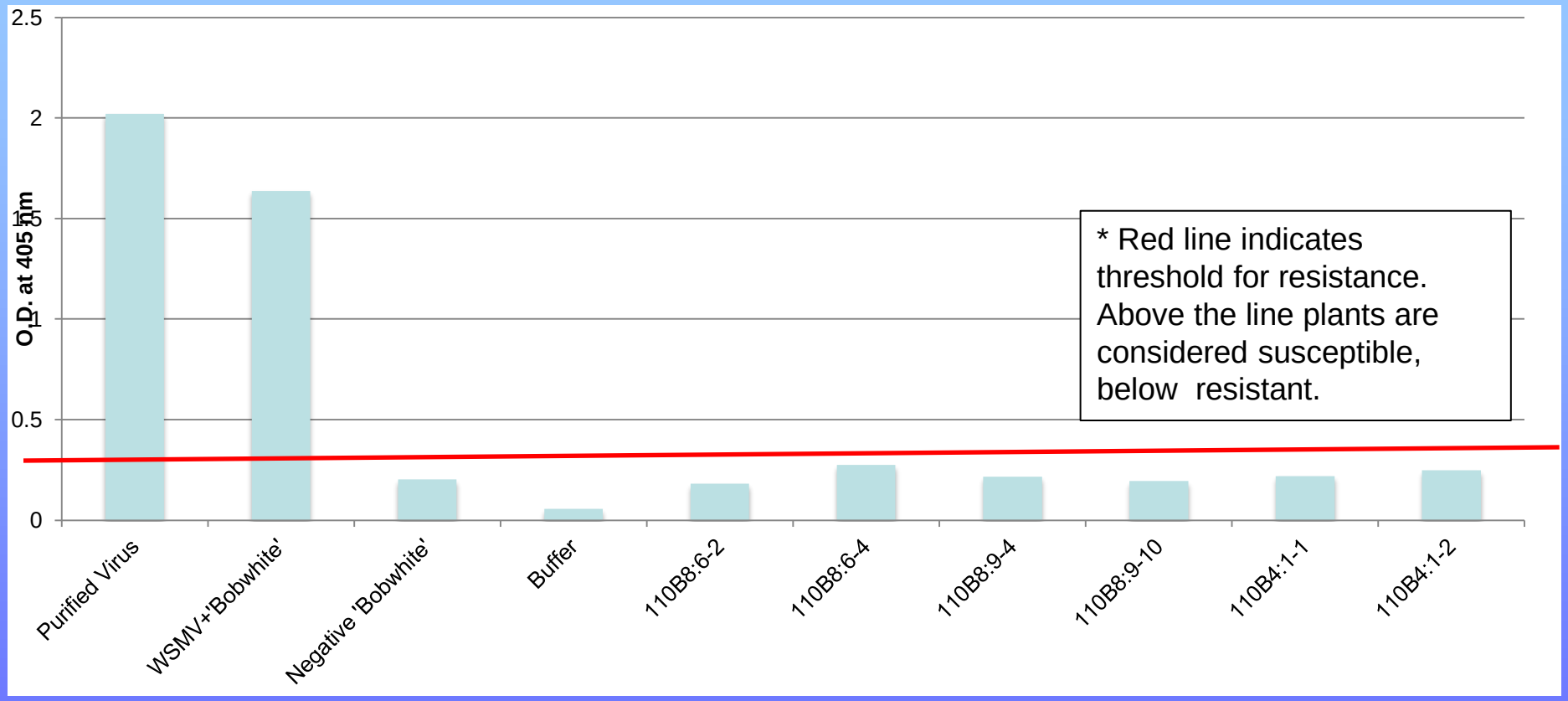
Bioassays on WSMV-CP RNAi T_1 Plants





Jessica Rupp
PhD student

ELISA Results- T_3 Generation WSMV-CP



- WSMV resistance is currently being crossed into Overly

KANSAS WHEAT COMMISSION

Current Team Members:

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Jessica Rupp, PhD student
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Sheila Stevens, research assistant
Melissa Wohler, research assistant
Luisa Cruz, MS student

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