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Soy2025: 19th Biennial Conference on Molecular & Cellular Biology of Soybean

July 23 - 26, 2025
University of Wisconsin - Madison

Registration and Abstract Submission are now open!

Top speakers – Beautiful lakeside setting – Inexpensive lodging option
Emphasis on extended poster sessions
Nine speakers (any career level) chosen from submitted poster abstracts; eight 5-minute "lightning talk" early-career speakers chosen from poster abstracts.

See: https://conferences.union.wisc.edu/soy2025/

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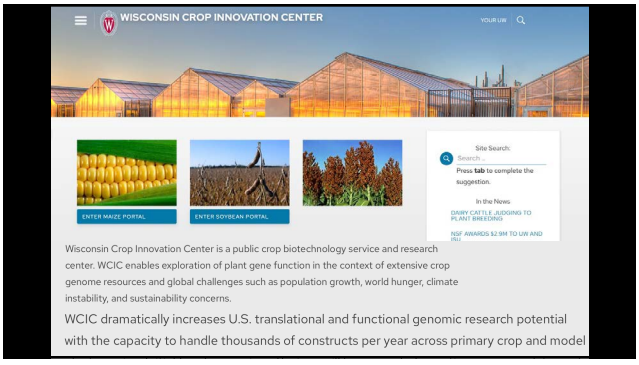

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WCIC transforms dicots via direct meristem approach
Embryonic axes extracted from seeds

Soybean mature meristem explants

- Genotype-independent
- No tissue culture/regeneration
- Quality events
- Low Cost

Soybean transformation efficiency ranges from 5-35% (cargo dependent) for majority of simple constructs

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WCIC transforms dicots via direct meristem approach
Embryonic axes extracted from seeds

Soybean mature meristem explants

But:

- CRISPR germline editing with 35S:Cas9 constructs often failed
- Transformation efficiency with gene editing constructs for all dicots has underperformed, at times catastrophically

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Today's Topics:

1) Development of high-efficiency, single-generation germline gene editing for soybeans transformed using WCIC transformation protocols
2) Development of higher transformation efficiencies with problematic (large, unfavorable) T-DNAs
3) Proof of concept for cisgenic improvement of soybean cyst nematode resistance

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1) CRISPR germline editing with WCIC transgenic soybeans

The Problem:
35S:Cas9 constructs generally failed to give germline edits when using the otherwise-desirable meristematic transformation method of WCIC

1a) Test promoters from soybean homologs of Arabidopsis egg-cell-specific gene

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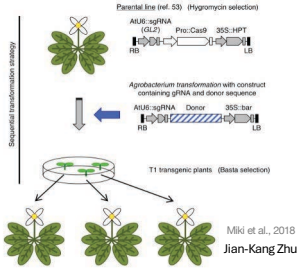
A starting point: Soybean Egg Cell-Specific Promoters, driving Cas9 expression, for gene replacement



Miki et al., 2018
Jian-Kang Zhu

Glycine max DD45-like genes

- Glyma.20G168300.1
- Glyma.09G195200.1 (EC1.1)
- Glyma.06G199900.1 (EC1.2)

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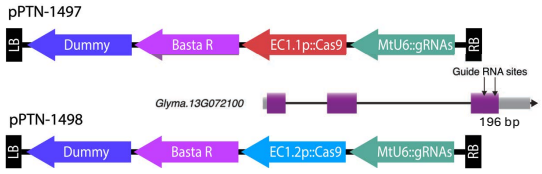
ECp-Cas9: To obtain stable, efficient-editing Cas9 soybean lines that can receive other gRNAs



Basta R: Selection marker (*note that WCIC uses Spec R*)
EC1.1/EC1.2::Cas9: promoters of DD45-like genes used to drive the expression of Cas9
gRNAs: Sequences targeting *W1* locus

Collaboration with Tom Clemente

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Plants transformed with EC1.2p:Cas9 show higher mutation efficiency in T2 plants

Plasmid	Event	Predominant Mutations	Average ICE Score	Average KO Score	Mutation trend
pPTN-1497 EC1.1p	1268-12	- 6 (49%); + 3 (10%)	81.5	37	No trend
	1268-14	- 11(35%)	73.6	68	Deletion
	1268-3	- 1 (28%); + 1 (28%)	76.67	57	No trend
	1268-5	0 (100%)	0	0	WT
	1269-2	**- 8 (99%)**	99	99	Deletion
	1271-6	- 6 (48%); + 3 (2%)	33	2.67	No trend
pPTN-1498 EC1.2p	1268-10	**- 196 (100%)**	99.6	99.6	Deletion
	1268-7	**- 196 (100%)**	99.8	99.8	Deletion
	1270-2	- 10 (26%); - 8 (25%)*	93.67	77.67	Deletion
	1271-3	- 196 (70%)	75.5	75.5	Deletion
	1271-5	**- 196 (100%)**	93.29	93	Deletion
	1272-1	0 (100%)	0	0	WT

* 1 T2 plant with -8 (100%)

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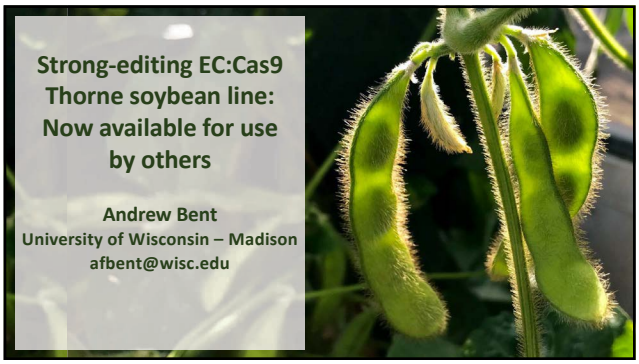

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1) CRISPR germline editing with WCIC transgenic soybeans

The Problem:
35S:Cas9 constructs generally failed to give germline edits when using the otherwise-desirable meristematic transformation method of WCIC

1a) Test promoters from soybean homologs of Arabidopsis egg-cell-specific gene

1b) Compare edit efficiencies with EC promoters and non-35S constitutive promoter

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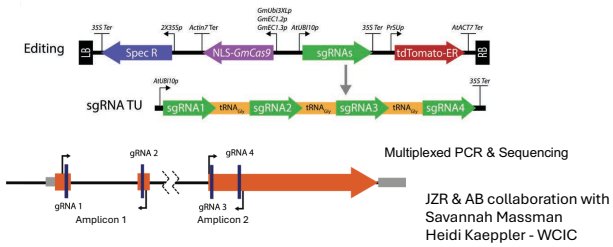
Cas9 expression driven by egg-cell-specific promoters or a constitutive ubiquitin promoter

Wisconsin Crop Innovation Center - Meristem-Based Germline Transformation (Savannah Massman)



JZR & AB collaboration with Savannah Massman Heidi Kaeppler - WCIC

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Experiment Structure: Different promoters driving Cas9

Three different constructs:
Three different promoters driving SpCas9 (egg-cell and ubiquitin promoters)
Four sgRNA in each construct (target 4 sites within '*Glabrous*' gene)

Amplify, bar code and Illumina sequence leaf DNA from 192 T1 plants:
For each construct, 16 T1 plants from each of 4 events (= 4 T0 plants)

(3 Cas9 promoters) x (4 T0 events) x (16 T1 plants) = 192 plants

x 4 sgRNA = 768 sequenced target sites

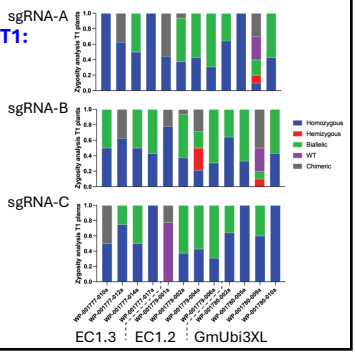
Used CRISPResso2 software package to analyze data

16

Zygosity Analysis of Edits in T1:

Each bar is ~16 T1 progeny plants from one T0 plant

(Data from 4th sgRNA not further analyzed; at repetitive site in gene)



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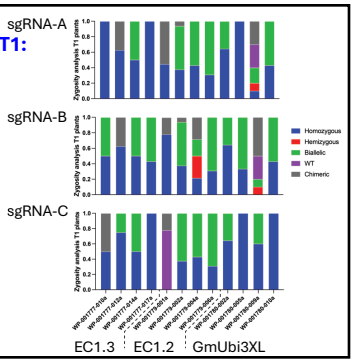
Zygosity Analysis of Edits in T1:

Each bar is ~16 T1 progeny plants from one T0 plant

(Data from 4th sgRNA not further analyzed; at repetitive site in gene)



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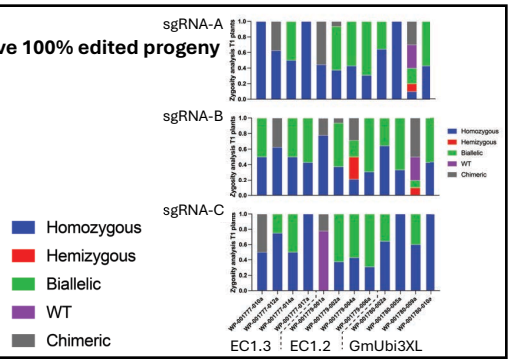
2/3 of T0 gave 100% edited progeny

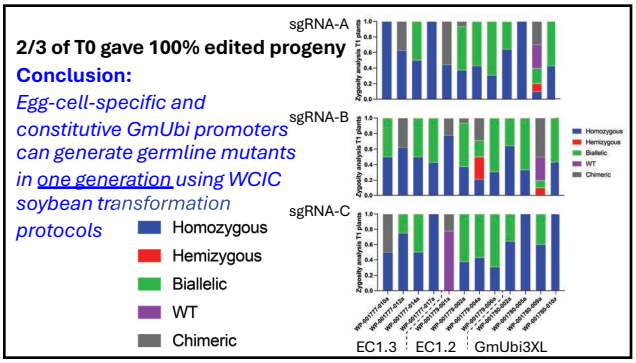


19

2/3 of T0 gave 100% edited progeny

Conclusion:

Egg-cell-specific and constitutive GmUbi promoters can generate germline mutants in one generation using WCIC soybean transformation protocols



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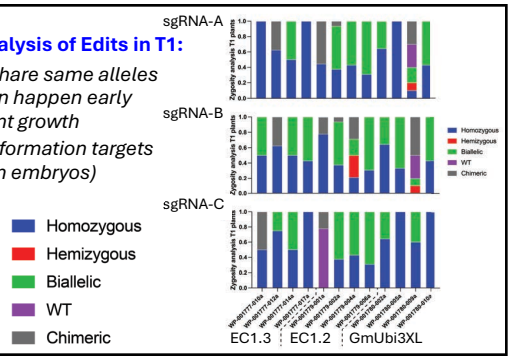
Zygosity Analysis of Edits in T1:

T1 siblings share same alleles

- *Edits often happen early in T0 plant growth*

(WCIC transformation targets meristems in embryos)



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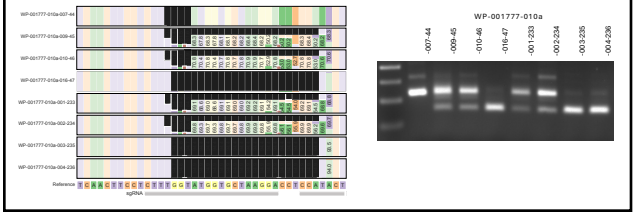
Larger (few hundred bp) deletions between guides were observed but infrequent

None observed with EC promoter, a few events had deletions with GmUbi promoter driving Cas9

Example:
Segregation of 4bp indel and 0.6kb deletion in progeny from one biallelic GmUbi:Cas9 event



22

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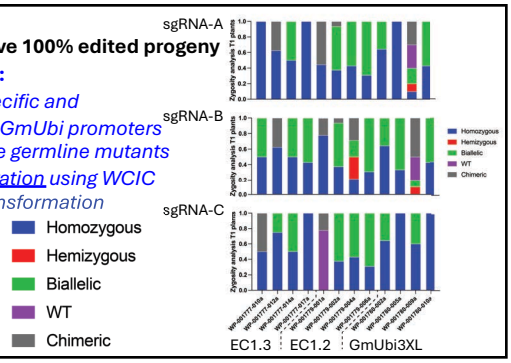

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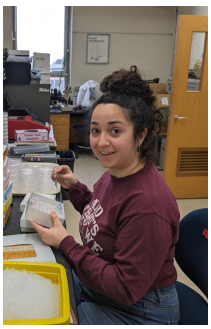
SoyEdit Team:
Jeysika Zayas-Rivera
Savannah Massman
Shaojie Han
Ray Collier
Heidi Kaeppler
Andrew Bent



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3) Proof of concept for cisgenic improvement of soybean cyst nematode resistance

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2) Significantly elevated WCIC transformation efficiency with difficult T-DNAs, via GA*A*NTRY methods

The Problem:
Abysmal transformation efficiencies with large or Cas9-containing constructs, using the otherwise-desirable meristematic transformation method of WCIC.

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The Plant Journal (2018) **95**, 573–583 doi: 10.1111/tpj.13992

TECHNICAL ADVANCE

A versatile and robust Agrobacterium-based gene stacking system generates high-quality transgenic Arabidopsis plants

Ray Collier†, James G. Thomson* and Roger Thilmony*
United States Department of Agriculture-Agriculture Research Service, Western Regional Research Center, Crop Improvement and Genetics Research Unit, Albany, CA 94710, USA

Received 9 April 2018; revised 15 May 2018; accepted 18 May 2018; published online 14 June 2018.
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SUMMARY

Biotechnology provides a means for the rapid genetic improvement of plants. Although single genes have been important in engineering herbicide and pest tolerance traits in crops, future improvements of complex traits like yield and nutritional quality will likely require the introduction of multiple genes. This research reports a system (GA*A*NTRY; Gene Assembly in *Agrobacterium* by Nucleic acid Transfer using Recombinase technologY) for the flexible, *in vivo* stacking of multiple genes within an Agrobacterium virulence plasmid Transfer-DNA (T-DNA). The GA*A*NTRY system utilizes *in vivo* transient expression of unidirectional site-spe-

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Previous Methods:

In addition, these systems all utilize either a binary vector plasmid or a binary bacterial artificial chromosome plasmid vector as the transformation construct. These platforms have been shown to be unstable in Agrobacterium without maintenance of antibiotic selection and/or when they carry large cargoes (McBride and Summerfelt, 1990; Hellens *et al.*, 2000; Song *et al.*, 2003; Murai, 2013). Thus, plant synthetic biology and genetic engineering efforts would significantly benefit from the development of a simple, efficient and highly stable approach for transgene assembly and effective plant transformation with large stacked constructs.

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The Solution (one part of their system):

The GA*A*NTRY recipient bacteria (ArPORT1) is a disarmed *Agrobacterium rhizogenes* strain NCPPB 2659 modified to contain an *Agrobacterium tumefaciens* strain C58 Left Border (LB) sequence, an A118 *attP* site, a kanamycin resistance marker and a ParA *MRS* site integrated in place of the native T-DNA of the pRi virulence plasmid (Figure S2). In addition, this strain was rendered *recA*- as previously described (Valdes Franco *et al.*, 2016), improving

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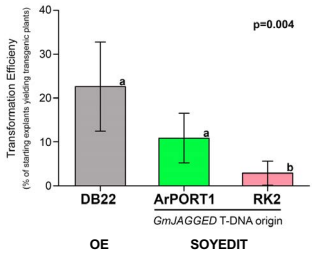
GA*A*NTRY improves soybean transformation efficiency (5X!) with gene editing constructs



Dr. Guifen Li and her amazing *GmJAGGED* edited soybeans!

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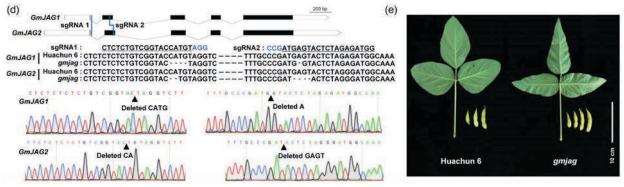
GmJAGGED1 successfully targeted with CRISPR/Cas9 to produce low latitude soybean varieties with narrow leaves and increased number of seeds per pod



Adapted from Figure 1 from Plant Biotechnology Journal (2021) v19: 1898-1900

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A better gene editing target than _PDS_: _Ln_ or _In_ exhibit different leaf phenotypes

Figure 1. Phenotypic expression of narrow (left), intermediate (middle), and ovate (right) leaflet types in soybean.

Adapted from Figure 1 from Journal of Heredity (2002) v93: 346-351

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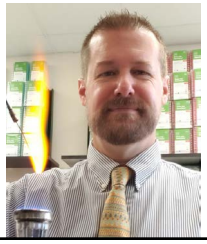
Part II: Summary

The GAANTRY/*A. rhizogenes* system is apparently solving low soybean transformation efficiency issues that WCIC encountered with certain T-DNA constructs.



Ray Collier, UW-Madison WCIC

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Today's Topics:

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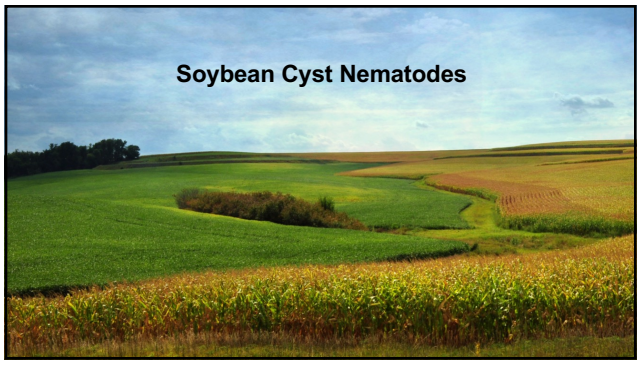

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Soybean Cyst Nematode (SCN) is the most economically damaging pathogen of soybean

TABLE 4
Ten most destructive diseases and associated estimated soybean yield losses (bushels in thousands) by disease or type of disease in the northern United States[w] and Ontario, Canada, from 2010 to 2014

	2010		2011		2012		2013		2014	
Rank	Disease	Loss	Disease	Loss	Disease	Loss	Disease	Loss	Disease	Loss
1	Soybean cyst nematode	110,325	Soybean cyst nematode	90,525	Soybean cyst nematode	118,697	Soybean cyst nematode	112,394	Soybean cyst nematode	108,008
2	Sudden death syndrome	70,658	Seedling diseases	46,847	Charcoal rot	59,481	Seedling diseases	43,672	Seedling diseases	60,305
3	Seedling diseases[x]	55,000	Phytophthora root and stem rot	33,180	Phytophthora root and stem rot	23,950	Charcoal rot	31,865	Sudden death syndrome	46,815
4	Phytophthora root and stem rot	35,967	Charcoal rot	29,403	Seedling diseases	23,642	Phytophthora root and stem rot	29,134	Sclerotinia stem rot (White mold)	40,709
5	Charcoal rot	25,935	Sudden death syndrome	22,835	Sudden death syndrome	21,831	Sudden death syndrome	20,391	Phytophthora root and stem rot	32,864
6	Septoria brown spot	25,306	Septoria brown spot	17,954	Fusarium wilt and root rot	14,636	Septoria brown spot	20,209	Septoria brown spot	24,030
7	Sclerotinia stem rot (White mold)	24,520	Fusarium wilt and root rot	16,492	Brown stem rot	12,532	Sclerotinia stem rot (White mold)	17,663	Charcoal rot	18,347
8	Brown stem rot	13,465	Brown stem rot	14,064	Viruses[y]	11,661	Brown stem rot	10,385	Brown stem rot	13,686
9	Fusarium wilt and root rot	10,689	Sclerotinia stem rot (White mold)	12,770	Septoria brown spot	6,379	Viruses[z]	7,543	Stem canker	11,871
10	Pod and stem blight	9,514	Pod and stem blight	8,404	Sclerotinia stem rot (White mold)	5,530	Stem canker	6,052	Pod and stem blight	10,540

Allen et al. 2017 Plant Health Progress 18:19-27

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SCN yield loss >$1 billion every year in U.S.

TABLE 6
The 10 most yield reducing diseases based on estimates of loss and associated estimated soybean yield losses (bushels in thousands) by disease or disease type from 13 soybean producing states in the northern United States[a] and Ontario, Canada, from 2015 to 2019

	2015		2016		2017		2018		2019	
Rank	Disease	Loss	Disease	Loss	Disease	Loss	Disease	Loss	Disease	Loss
1	Soybean cyst nematode	100,679	Soybean cyst nematode	87,963	Soybean cyst nematode	102,453	Soybean cyst nematode	108,899	Soybean cyst nematode	69,564
2	Seedling diseases[b]	56,690	Seedling diseases	47,460	Sclerotinia stem rot (white mold)	61,086	Diaporthe (Phomopsis) seed decay[c]	63,457	Sclerotinia stem rot (white mold)	31,066
3	Sclerotinia stem rot (white mold)	45,360	Sudden death syndrome	45,448	Seedling diseases	45,778	Frogeye leaf spot	47,187	Seedling diseases	24,617
4	Sudden death syndrome	44,243	Sclerotinia stem rot (white mold)	39,551	Sudden death syndrome	29,004	Sudden death syndrome	36,944	Sudden death syndrome	19,138
5	Phytophthora root and stem rot	25,148	Phytophthora root and stem rot	26,959	Charcoal rot	23,612	Seedling diseases	32,612	Phytophthora root and stem rot	11,832
6	Septoria brown spot	24,267	Brown stem rot	14,510	Phytophthora root and stem rot	20,949	Phytophthora root and stem rot	26,565	Frogeye leaf spot	9,625
7	Brown stem rot	19,451	Septoria brown spot	14,416	Brown stem rot	13,534	Pod and stem blight[d]	25,967	Pod and stem blight	6,577
8	Stem canker	11,434	Pod and stem blight	9,445	Stem canker	11,585	Sclerotinia stem rot (white mold)	24,172	Brown stem rot	4,433
9	Charcoal rot	11,382	Frogeye leaf spot	8,378	Septoria brown spot	9,733	Charcoal rot	19,244	Septoria brown spot	3,601
10	Pod and stem blight	9,444	Stem canker	8,159	Pod and stem blight	9,212	Stem canker	16,872	Other nematodes[e]	3,264

Bradley et al. 2021 Plant Health Progress 22:48

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Soybean *Rhg1* locus: The primary control measure against SCN for decades

- "PI 88788" *rhg1-b* used very successfully on millions of acres/year for last few decades
- "HG 2.5.7" SCN with partial virulence on *rhg1-b* are getting more common and more virulent... Growers need improved SCN resistance

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Soybean *Rhg1* locus: The primary control measure against SCN for decades

- "PI 88788" *rhg1-b* used very successfully on millions of acres/year for last few decades
- SCN with partial virulence on *rhg1-b* are getting more common and more virulent... Growers need high-yielding *rhg1-a* (+) varieties

SCN resistance is a quantitative trait. 20-30% more resistance can mean $25/acre for a grower, higher seed sales for company

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Improving SCN Resistance in Soybean

- Identify novel QTL to stack with *rhg1* haplotypes
- Improve *rhg1* resistance functions

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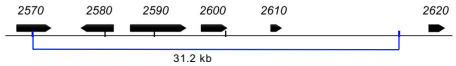
Soybean *Rhg1* locus: The primary control measure against SCN for decades

Copy Number Variation of Multiple Genes at *Rhg1* Mediates Nematode Resistance in Soybean

David E. Cook,[1*] Tong Geon Lee,[2*] Xiaoli Guo,[3*†] Sara Melito,[1†‡] Kai Wang,[3] Adam M. Bayless,[1] Jianping Wang,[2§] Teresa J. Hughes,[4||] David K. Willis,[4] Thomas E. Clemente,[5] Brian W. Diers,[2] Jiming Jiang,[3] Matthew E. Hudson,[2,4¶] Andrew F. Bent[1¶#]

Rhg1 is a ~31 kb block containing 3 genes that contribute to SCN resistance



α-SNAP$_{Rhg1}$ (= "GmSNAP18") is the best-studied *Rhg1* gene product

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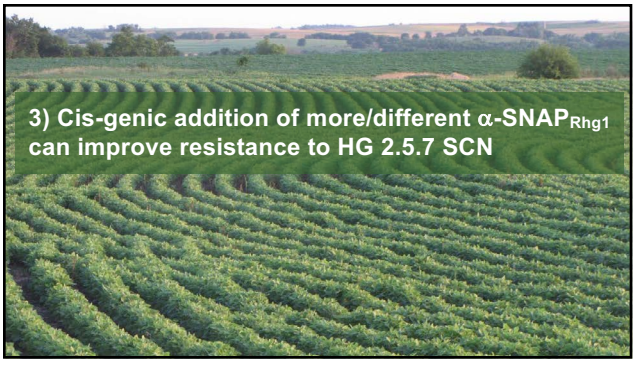

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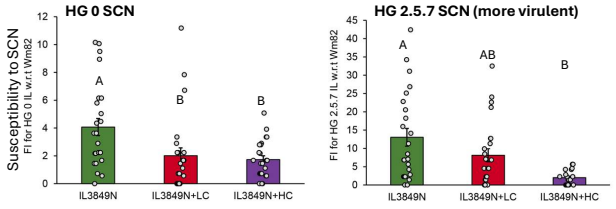
Over-expressing LC or HC α-SNAP$_{Rhg1}$ in a Peking background can incrementally improve SCN resistance



IL3849N = *rhg1-a, Rhg4*
Letters: ANOVA Tukey HSD p < 0.05
Whole-plant cup assays done ***TWICE*** with each SCN population, with 12 replicates each time

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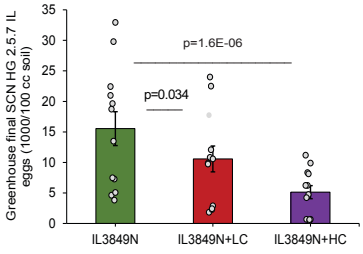
Over-expressing LC or HC α-SNAP$_{Rhg1}$ in a Peking background can incrementally improve SCN resistance

Also observed:
Fewer end-of-season SCN eggs in soil



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α-SNAP$_{Rhg1}$ Overexpression Soybeans: No detected impact on soybean yield

Two year field trial:
RCB design, four blocks/year
Four-row 3m plots with borders
Two α-SNAP$_{Rhg1}$ HC lines
Two α-SNAP$_{Rhg1}$ LC lines
One non-transgenic parent control

Seven of the eight genotype-site OE-α-SNAP$_{Rhg1}$ entries had yield not distinguishable from control

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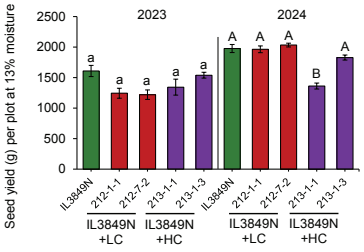
Field Trials: Over-expressing LC or HC α-SNAP$_{Rhg1}$ caused no significant decreases in yield

Nebraska field trials
Low SCN pressure


Deepak Haarith



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Part III: Summary
Enhanced expression of α-SNAP$_{Rhg1}$ incrementally improved the SCN resistance of soybean lines that already have some *Rhg1*-mediated resistance.

Offers a future cisgenic approach to enhanced efficacy and durability of soybean resistance to SCN.

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Thank You!

Plant Pathology at the University of Wisconsin-Madison

Jeysika Zayas-Rivera
Savannah Massman
Heidi Kaeppler
Ray Collier
Deepak Haarith

Tom Clemente
Shaojie Han
Alvar Carlson

USB United Soybean Board
USDA
Wisconsin Soybean Program

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