

Untangling the knot



Identifying a gene for Southern root-knot nematode resistance



1785

The University of Georgia

Shi Li, Peter LaFayette, Wayne Parrott, Zenglu Li

*Dept of Crop and Soil Sciences
Institute for Plant Breeding, Genetics & Genomics*

5th Annual Soybean Precision Genomics Workshop & Mutant Finder Field Day



**August 3-4, 2016,
University of Missouri- Columbia**

- ❏ **Presentations on soybean mutant resources**
- ❏ **Tutorials on cutting edge technologies in soybean**
 - **targeted mutagenesis/gene editing**
 - **gene silencing**
 - **other functional genomics applications**
- ❏ **Mutant populations & seeds**
- ❏ **Funding is available to help defray attendance costs**
 - ❏ **Contact Bing Stacey (staceym@missouri.edu)**



2nd 15 Years

Fine-mapping ▪ Candidates ▪ Identification



❏ 188 F_{5:6} RILs

❏ 17 had recombination between flanking markers

❏ Interval screened with 7 markers

❏ 235-kb with 30 gene models

Theor Appl Genet (2013) 126:1825–1838
DOI 10.1007/s00122-013-2095-8

ORIGINAL PAPER

Fine mapping and identification of candidate genes controlling the resistance to southern root-knot nematode in PI 96354

Anh-Tung Pham · Kaitlin McNally ·
Hussein Abdel-Haleem · H. Roger Boerma ·
Zenglu Li

13 Candidate genes



Based on

- Root-specific expression based on Soyseg
- Upregulated in other spp in presence of nematodes

Sequenced R & S alleles/promoters

- 15 changes in S vs reference
- 93 changes in R vs reference
 - 3 had no polymorphisms
 - 5 had silent mutations or in introns
 - 1 had a deletion not associated with resistance

4 Remaining candidate genes

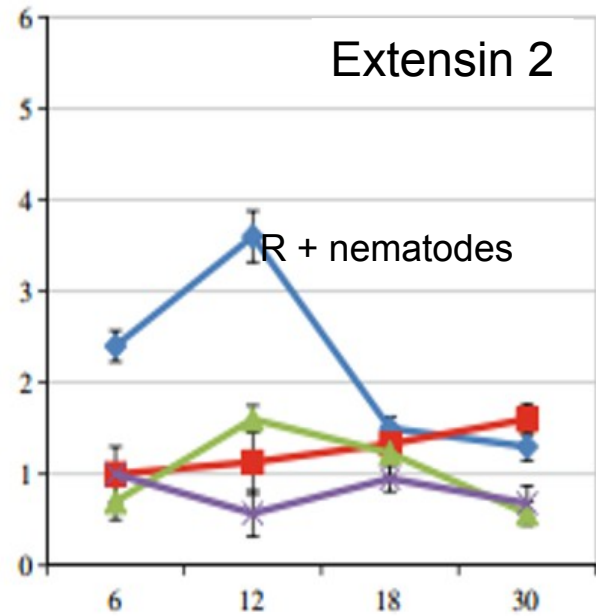
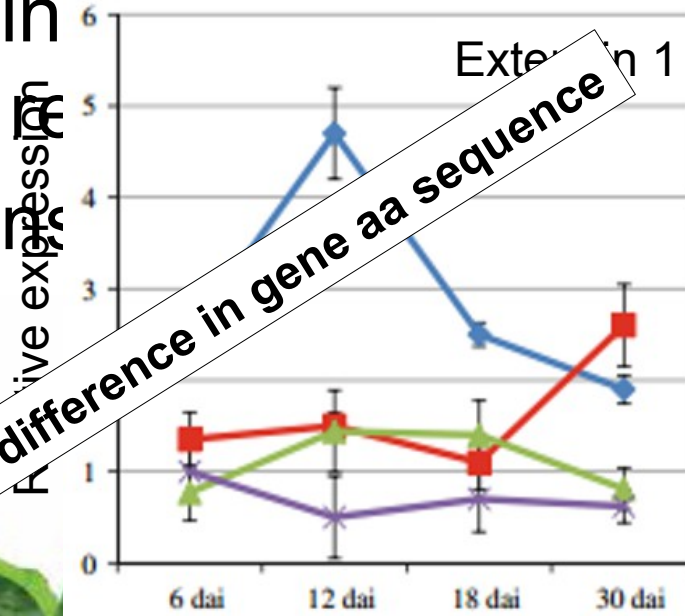


84/93 polymorphisms in these genes

Involved in cell walls

Pectin
of R
Extensin

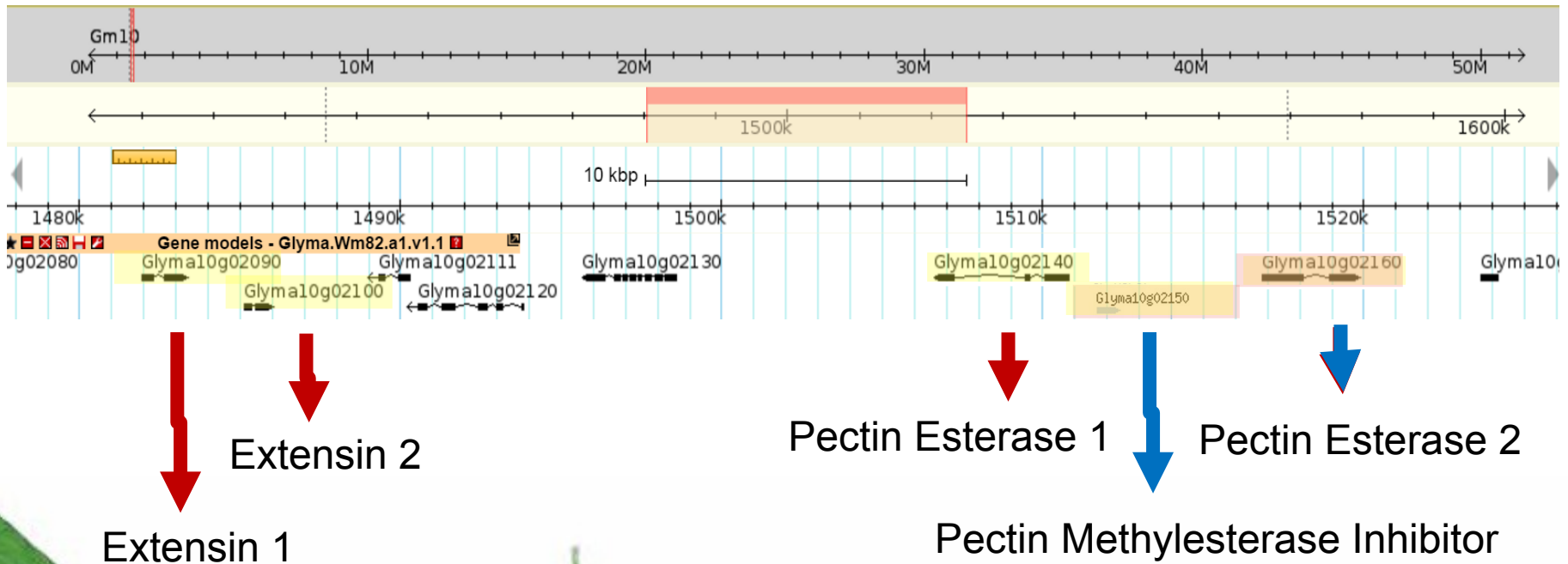
No difference in gene aa sequence



Response
to nematode

Legend:
R - nem (red square)
S - nem (purple triangle)
S + nem (green circle)

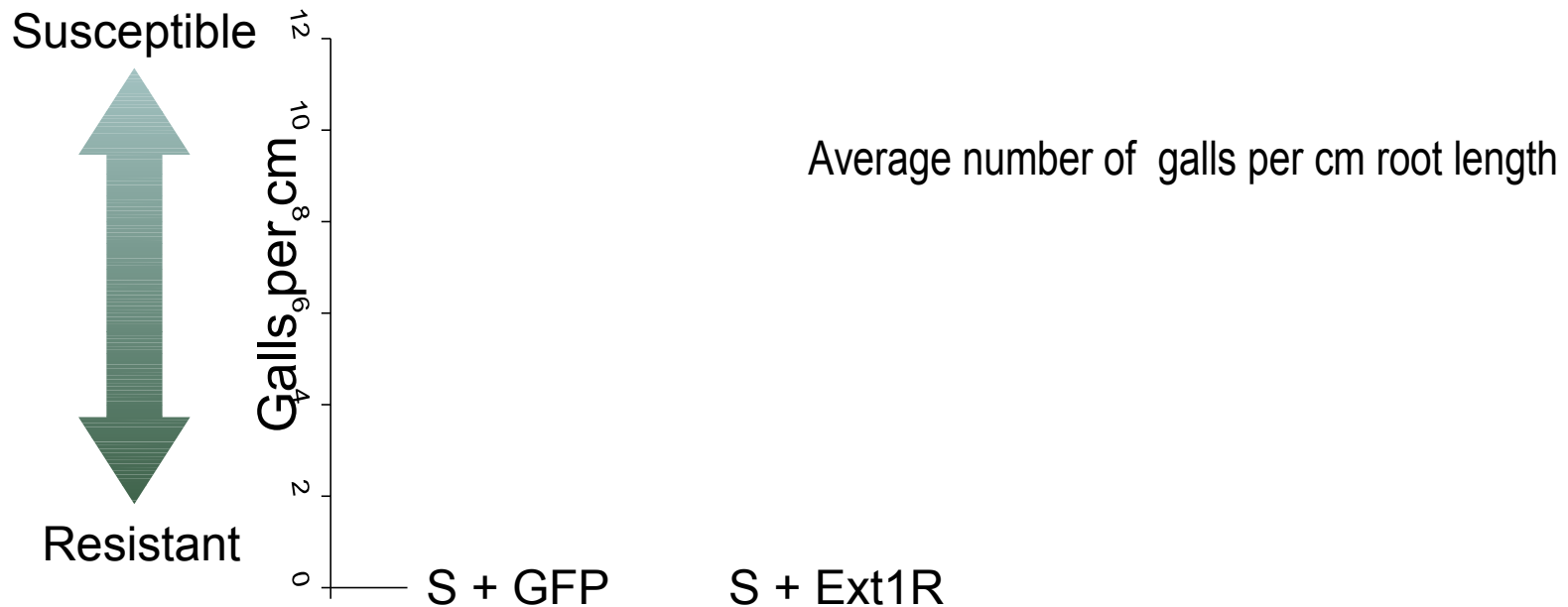
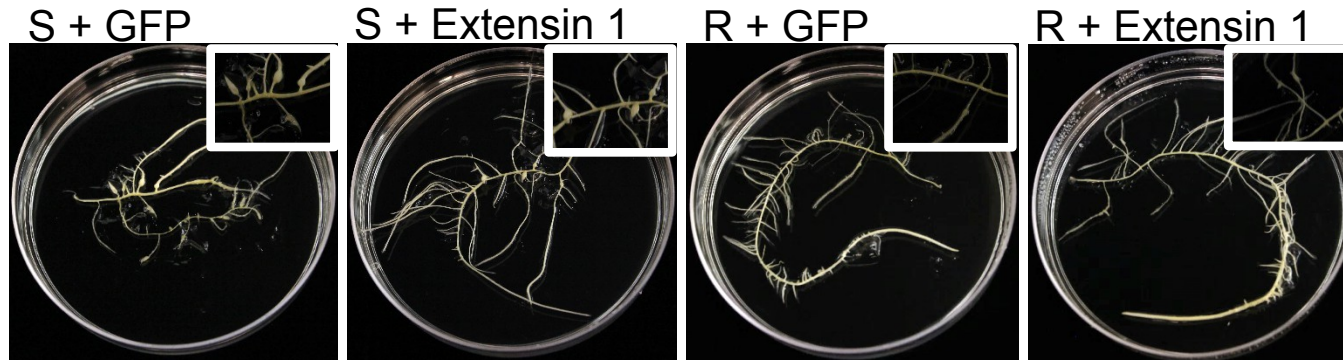
4 Candidate genes



Overexpression of Extensin 1R



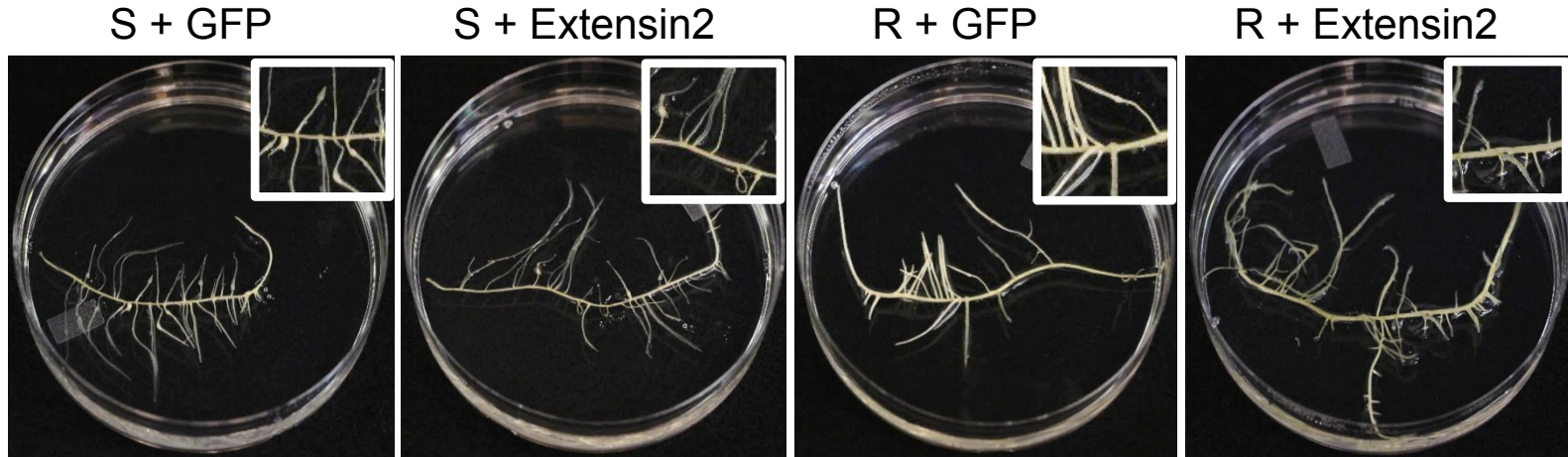
Enhanced resistance in both genotypes



Overexpression of Extensin 2R



Enhanced resistance in both genotypes



Susceptible



Resistant

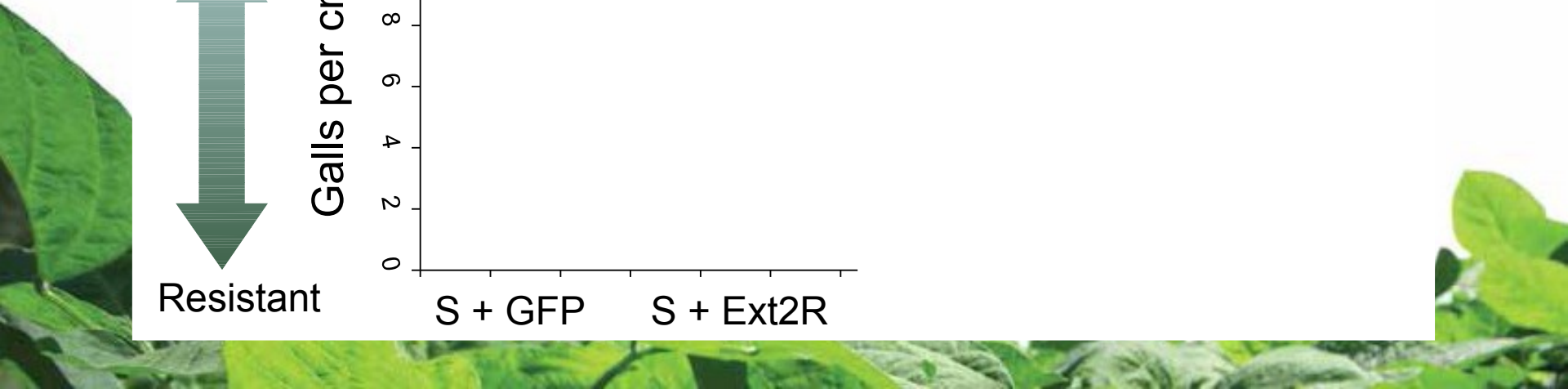
Galls per cm

12
10
8
6
4
2
0

Average Number of galls per cm root length

S + GFP

S + Ext2R



OE of Pectin Esterases from R

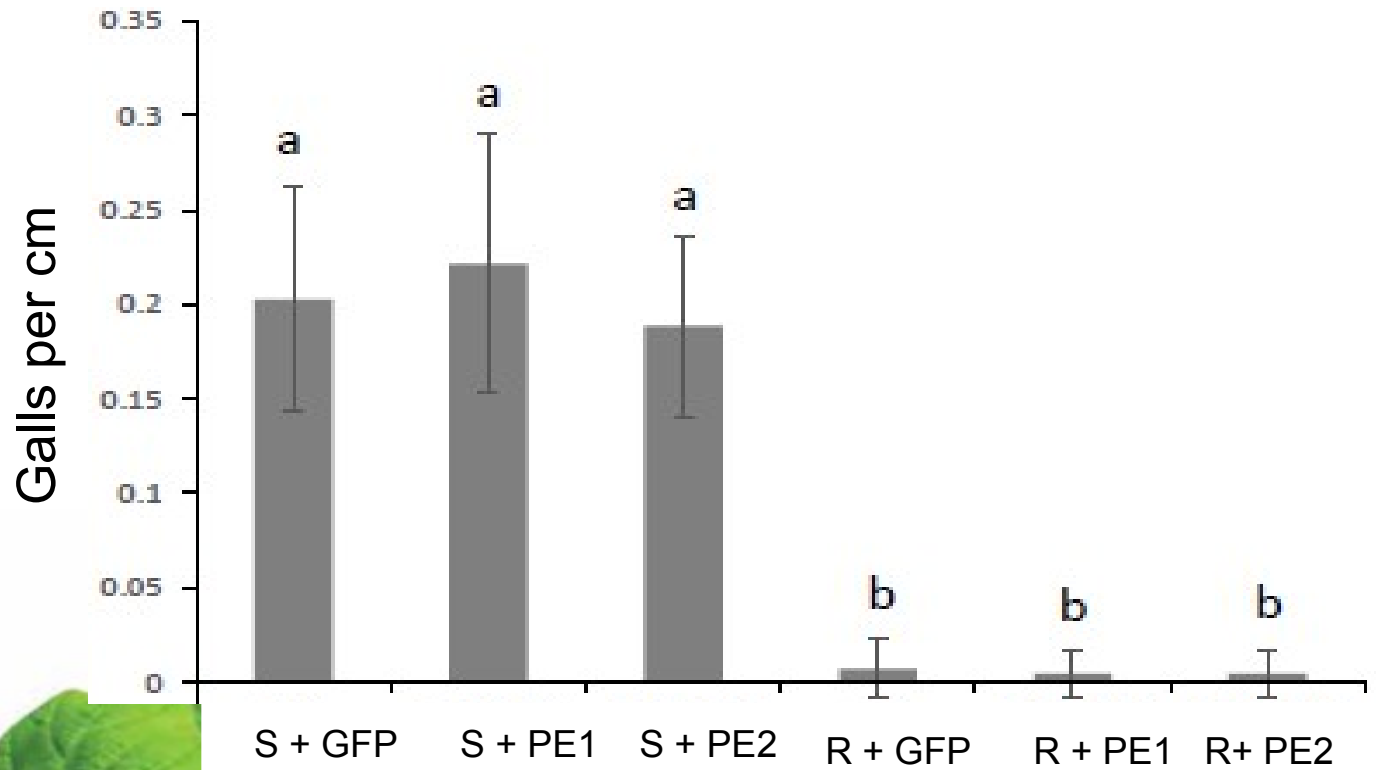
No effect in either genotype



Susceptible



Resistant



Knock out 4 genes individually



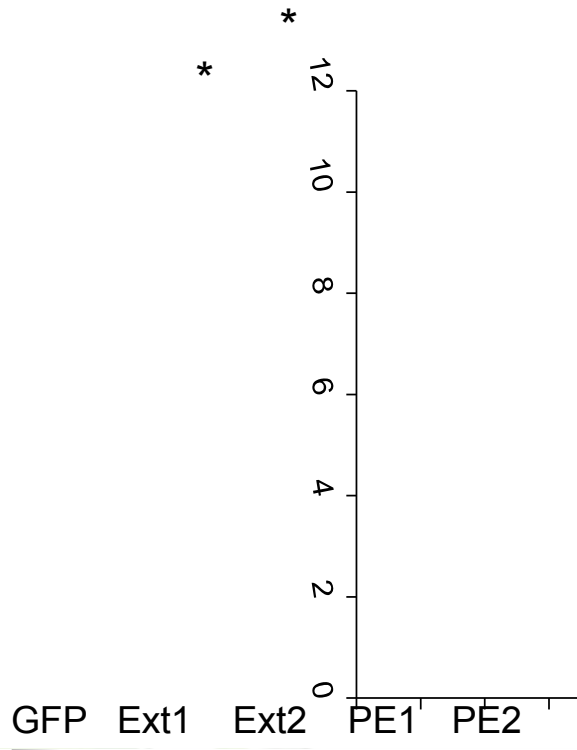
Small effects of Extensin/ no effects of pectin esterase

Susceptible



Galls per cm

Resistant

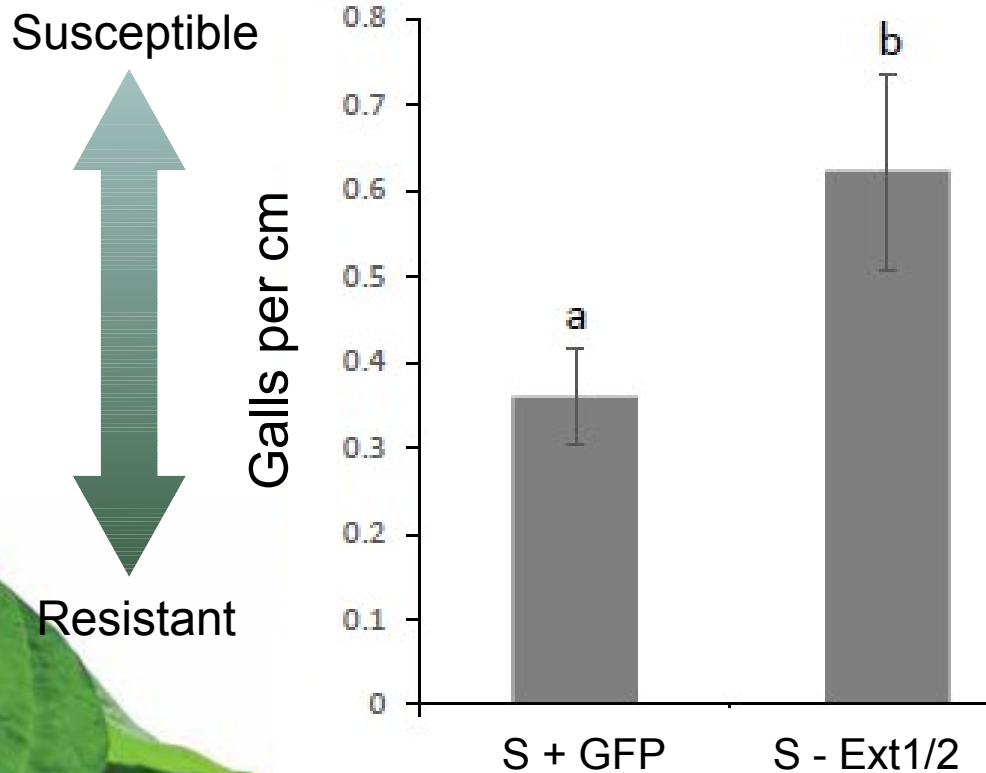


Average number of galls per cm root length

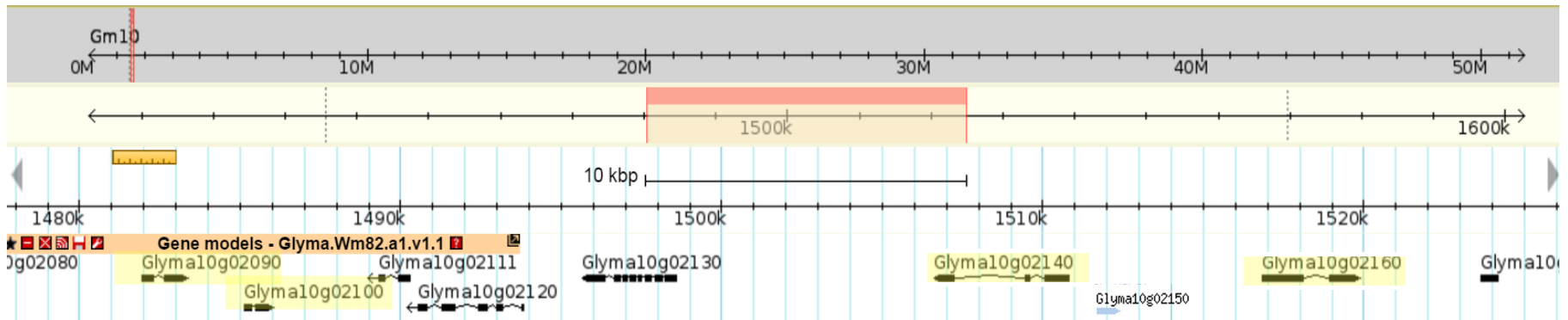
Susceptible genotype

Double extensin knockouts

Susceptibility increases in both genotypes



Four candidate genes



Extensin 2
Glyma.10G016700



Extensin 1
Glyma.10G016600

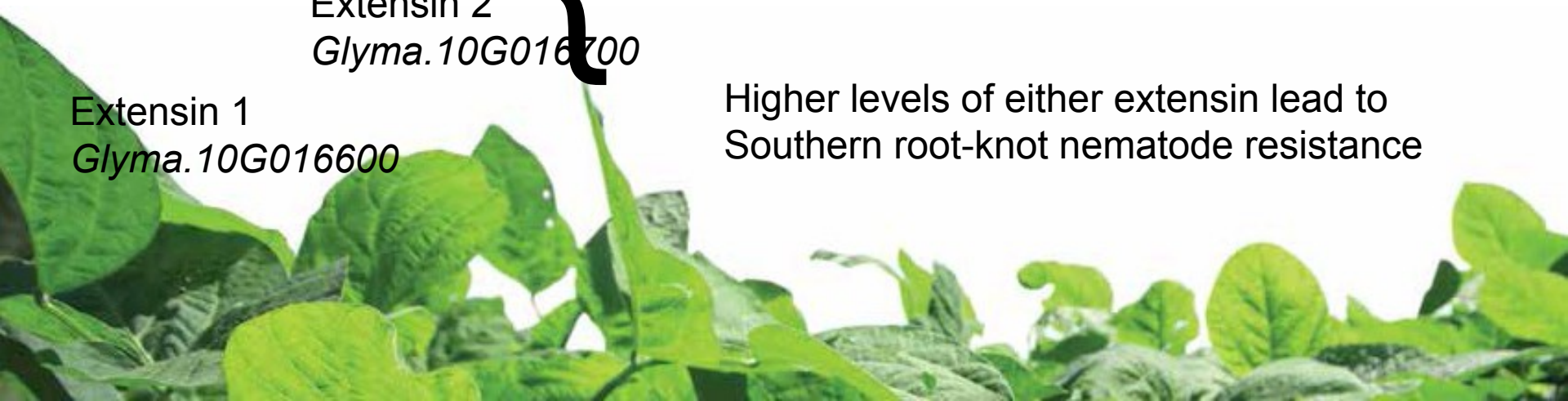


Pectin Esterase 1



Pectin Esterase 2

Higher levels of either extensin lead to Southern root-knot nematode resistance



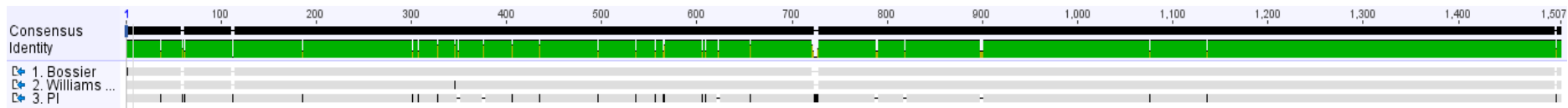
Extensin 2 promoter

Relative to Williams 82



⚡ Resistance associated with expression level

⚡ Related to promoter sequence



	SNPs	Insertions	Deletions
Bossier	2	0	3
PI96354	19	7	10



So what is it?



~~Extensin~~

Pollen Ole e I allergen

Gene family, expressed in various tissues

Is it really an allergen?

80mer Sliding Window Search Results

Database	AllergenOnline Database v16 (January 27, 2016)
Input Query	>query atgaaaatggcctatattccatagtagtacctgctttctggtttttatgcttatcgatcactg ttggtccccggcagaggcggtgactacggtccccgaagaagaaaaacttctatcaaaaact ataggcattcaaggcattgtctattgcaaatctgcctctaaactcaccctgaaggg gccttgacaaggatatcatgcgaggctggtgatgaatatgggtttgaaacgacaccggtt tctttctta
Length	249
Number of 80 mers	170
Number of Sequences with hits	0

No Matches of Greater than 35% Identity Found

So what is it?



Previously cloned in soybean

DOI: 10.1007/s10535-012-0258-0

BIOLOGIA PLANTARUM 57 (1): 85-90, 2013

***GmPOI* gene encoding a Pollen_Ole_e_I conserved domain is involved in response of soybean to various stresses**

W.W. SONG¹, F.M. DUAN¹, W.B. LI², Q. LIN¹, H.X. ZHOU¹, X. HAN³, and J.A. WANG^{2*}

Associated with drought tolerance

- Upregulated in roots during stress

- Transgenics have higher drought tolerance

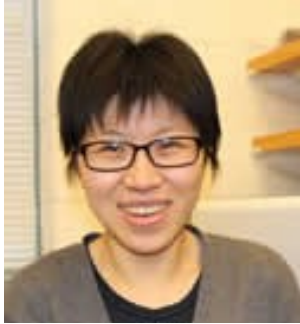
 - Higher SOD levels

 - Higher proline levels

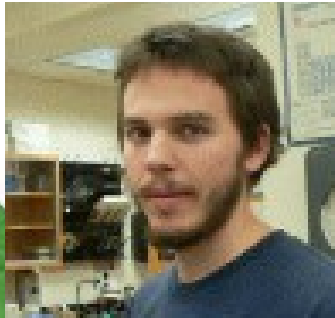
Acknowledgements



Peter LaFayette



Shi Li



Tom Jacobs



Tim Chappell



Karim Abdullah

