Field Analysis of SDS Resistance in Soybean Transgenic With the RLK From Rhg1 and Rfs2.

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Soybean (Glycine max (L. Merr.)) resistance to any population of Heterodera glycines (I.), or Fusarium virguliforme (Akoi, O'Donnell, Homma & Lattanzi) required a functional allele at Rhg1/Rfs2. H. glycines, the soybean cyst nematode (SCN) was an ancient, endemic, pest of soybean whereas F. virguliforme causal agent of sudden death syndrome (SDS), was a recent, regional, pest. This study examined the role of a receptor like kinase (RLK) GmRLK18-1 (gene model Glyma_18_02680 at 1,071 kbp on chromosome 18 of the genome sequence) within the Rhg1/Rfs2 locus in causing resistance to SCN and SDS. A BAC (B73p06) encompassing all the DNA from Peking introgressed into Forrest and so the Rhg1/Rfs2 locus was sequenced from a resistant cultivar and compared to the sequences of two susceptible cultvars from which 800 SNPs were found but only 18 SNPs between susceptibles. Sequence alignments inferred that the resistance allele was an introgressed region of about 59 kbp at the center of which the GmRLK18-1 was the most polymorphic gene and encoded protein. Analyses of plants that were either heterozygous at or transgenic and so hemizygous with the resistance allele of *GmRLK18-1* at a new location were made. Those plants infested with either *H. glycines* or *F. virguliforme* showed that the allele for resistance was dominant. In the absence of Rhg4 the RLK was sufficient to confer nearly complete resistance to both root and leaf symptoms of SDS caused by *Evirguliforme* and provided partial resistance to three different populations of nematodes (mature female cysts were reduced by 30-50%). In the presence of *Rhg4* the plants with the transgene were nearly classed as fully resistant to SCN as well as SDS (females reduced to 11% of the susceptible control). A reduction in the rate of early seedling root development was also shown to be caused by the resistance allele of the GmRLK18-1. Field trials of transgenic plants in 2010, 2011, 2012 and 2013 showed an increase in resistance to SDS, an increase in rsistance to SCN and in 2010 an increase in foliar susceptibility to insect herbivory. The inference that soybean has adapted part of an existing pathogen recognition and defense cascade (H.glycines; SCN and insect herbivory) to a new pathogen (F. virguliforme; SDS) has broad implications for crop improvement. Stable resistance to many pathogens might be achieved by

manipulation the genes encoding a small number of pathogen r a a litia a la kata ina









Figure 8: THE RLK at Rfs2/Rhg1 integrates signals to regulate appropriate root development and growth or giant cell death and reduced root growth.



Figure: The RHG1 protein alters root development by interaction with a cyclophilin. Panel A; Soybean NILS at 2 weeks pre SCN inoculation show different root morphologies, post inoculation root masses are not different (by 6 weeks). Therefore. *rhg1* inhibits germination and early root growth. Panel B; the root morphologies cosegregated perfectly with the allele in the RLK at the rhg1 locus as shown by the intragenic marker TMD1 (satellite in the intron). Panel C; The RLK protein detected by anti-RHG1 antibody is more abundant in resistant NILs (R) than susceptible (S) but is not altered by inoculation (i) or non-inoculation. Panel D; shows a sector of a 2 D gel stained with Coomassie. Panel E; a duplicate of this gel used for Western transfer and probed with the RHG1 LRR domain protein to identify interacting partners. Arrowed is the cyclophilin.







Table 1: Current state of soybean germplasm field testing by 2013. Yields for transgenics and OTL stack crosses were from a drought affected field in 2012 and so were lower than usual.

	Maturity Group	Growth Habit	-SDS Yield	+SDS Yield	DX	SDS R QTL	SDS Root R	SCN R Race
Convention	al Germpl	asm						
FxH 13	4.5	Det	3.15	3.09	0.1	6	Yes	3
FxH 34	4.5	Det	3.09	2.95	18.9	5	Yes	2, 3, 14
FxH 45	4.5	Det	3.33	2.65	62.6	0	No	None
		D .	0.00	12.40		1	1	1
ExF 23	D.1	Det	5.05	5.48	1.1	0	Yes	5
ExF 85	5.1	Det	3.62	3.05	24.5	0	No	None
EF23xFH13	4.7	Det	TBD	1.97	0.7	8	Yes	3
EF85xFH45	4.7	Det	TBD	1.06	23.2	0	No	None





RIK (Top section) Experimental

H S R



RLK tran	sgene	e leaf s <i>rha1/</i>	corch <i>Rfs2</i>	redu	ction by	/
Cultivar	EF85	EF23	x5	x5R	LK 10 ⁴ cfu	
G						
DS PLK trai	3.0	1 o root	.0	3.0	1.0	
NLK LI di	isgen	e 1001	101 H	euuci	ION Dy	
Cultivar	v	rng1/i	x5	,	v5 10 ⁴ cft	
Fusarium	^	+	+	Ĩ	+	
Gene		+				

LRR Domain (pM)

Ligand Binding (12 pM each CLE)

Table 2: Association of CLE treatments with resistance to SCN JB3 and mean root growth in nontransgenic lines. SCN female index in greenhouse grown seedlings at 28 days after SCN nfestations. Pots were watered daily with 100 ml. Female index (FI) was the mean percentage of cysts of Hg Type 0 found on five plants per repetition compared to a susceptible genotype Essex. Plant treated with CLE peptides received 50 pM dip treatments with HgCLE or GmTDIF

Line::gene X5	SCN infested No	Root mass (g) 1.05	Significant differences a	Range (g) 0.81-1.44	n 15	SCN FI (%) 0 <u>+</u> 0.0
X5	Yes	0.98	a	0.73-1.31	15	100 <u>+</u> 13
X5 + HgCLE	Yes	1.42	a	0.95-1.81	5	15 <u>+</u> 6
X5 + TDIF	Yes	1.40	a	0.92-1.78	5	8 <u>+</u> 3
Westag97	Yes	4.20		3.50-4.82	5	120 <u>+</u> 13
Wes97+ HgCLE	Yes	3.14		2.66-3.53	4	10 <u>+</u> 3

2.66-3.53 4 5+3 **RLK transgene SCN reduction by** rhg1/Rfs2

SCN IP	x5RLK 60 <u>+</u> 11	x5 100 <u>+</u> 13	
RLK Gene	+	1	
No chlorosis	and a second		Nonspecific chlorosis

Conclusions

RR

CFUsfrom ground roots

1. Transgenic plants showed the RLK at rhg1/Rfs2 had a significant effect on resistance to SDS by root resistance to Fusarium virguliforme and partial resistance to SCN this resulted in increased field yield

GBrowse view of 40 Mbp encompassing the rhg1 locus from Lg G and the syntenic gene cluster from LgB1 in Williams 82. The image was viewed at Phytosome.net in 2008. Panel A shows the region around rhg1 on scaffold 121 (LgG). Panel B shows the region around the rhg1 paralog on scaffold 139 (Lg B1) Note gene orders are the same but the scaffold orientation is inverted. Panel C shows the alignment of the two DNA sequences and Panel D the amino acid aligments centered on the RLK at rhg1.





Model for the function of the *rhg1* locus in resistance to SCN. Black arrows show positive interactions, blue arrows

show inhibitions. In this model four primary phenotypic

events are controlled by the 3 genes at *rhg1* and four

unlinked genes *Rhg2-4* and *Rzd1* (*sup-Rhg1*). The



References:

37 ->

181 ->

82 ->

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Locus interactions underlie seed yield in soybeans resistant to Heterodera glycines. Curr. Issues Mol. Biol. 11 (Suppl. 1): i73-84 Kazi S, Shultz J, Afzal J, Hashmi R, Jasim M, Bond J, Arelli PR, Lightfoot DA.2009. Iso-lines and inbred-lines confirmed loci that underlie resistance from cultivar 'Hartwig' to three soybean cyst nematode populations. Theor Appl Genet. 2009 Oct 25. [Epub ahead 2. Cyclophilin at 10 dai, methionine synthase at 42 dai and 8 CLE peptides in vitro were shown to be interacting partners for the RLK that may be involved in elicitor interaction and / or protein folding.

3. 2D PAGE and GCMS showed 2 proteins and eight metabolites differed due to the activity of the RLK R/S allele in the uninfected state but that 30 differentially abundant proteins and 58-112 metabolites differed in response to SCN infection. Those an be used for biomarkers of resistant genotypes and transgene expression.

4. A syntenic cluster of paralogs was found for the RLK and 2 core genes of rhg1 at another locus suggesting functional duplications. That cluster altered plant growth.

5. A systems biology model was developed from the resources for soybean

4.0 3.0

1.0