

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 cultivars 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 *F. virguliforme* 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 resistance 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 recognition proteins.

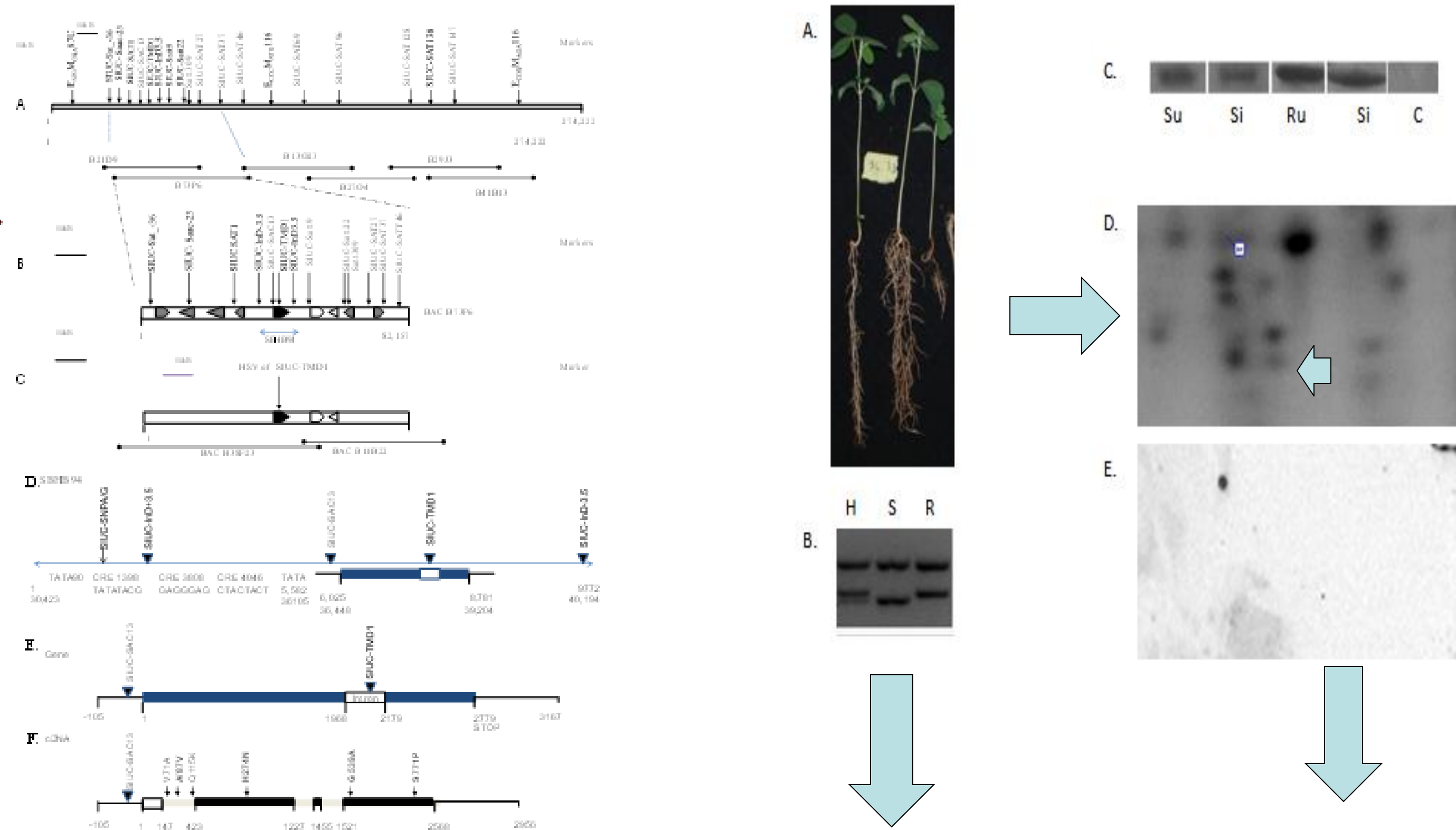
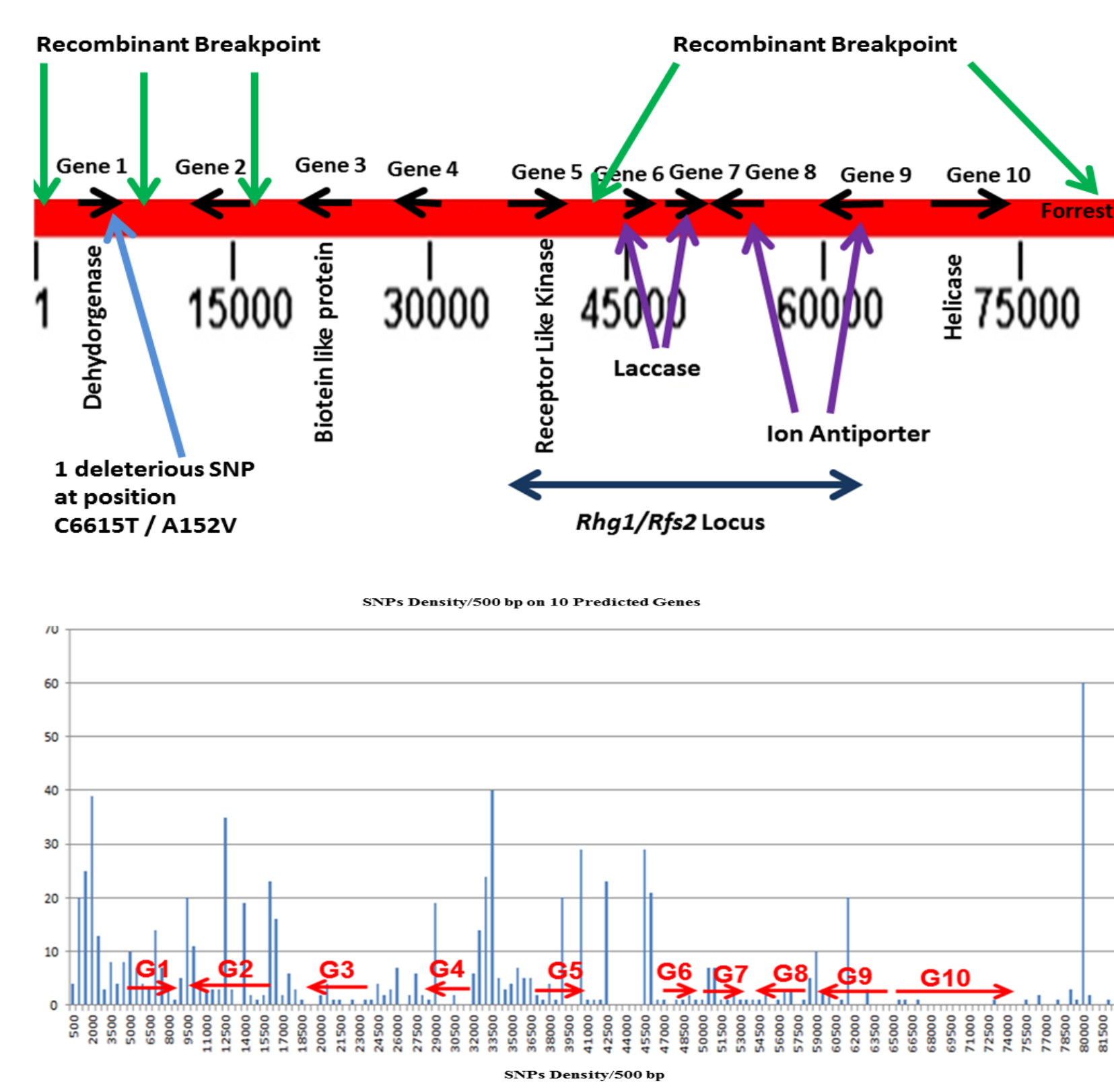


Figure 8: THE RLK at *Rfs2/Rhg1* integrates signals to regulate appropriate root development and growth or giant cell death and reduced root growth.

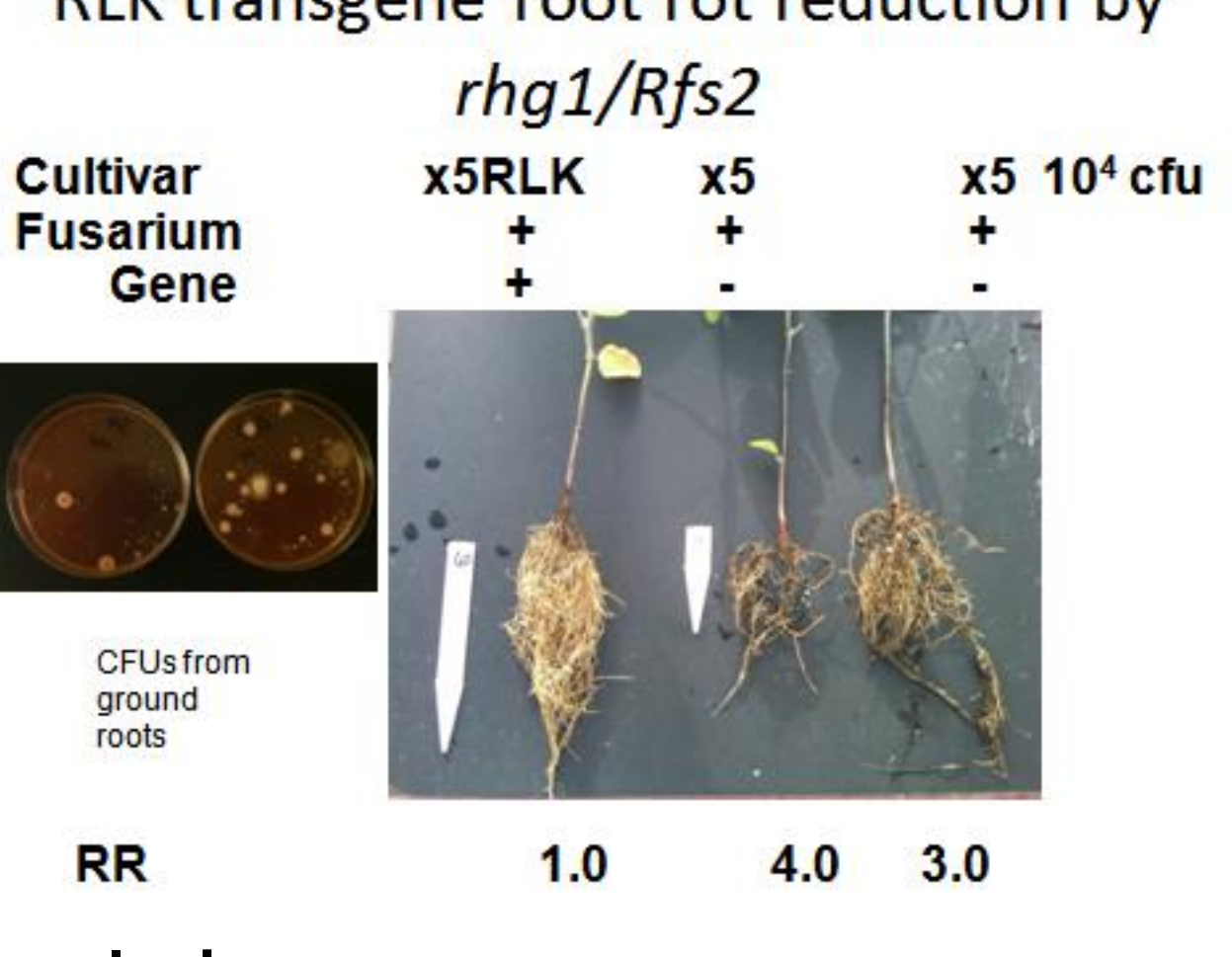
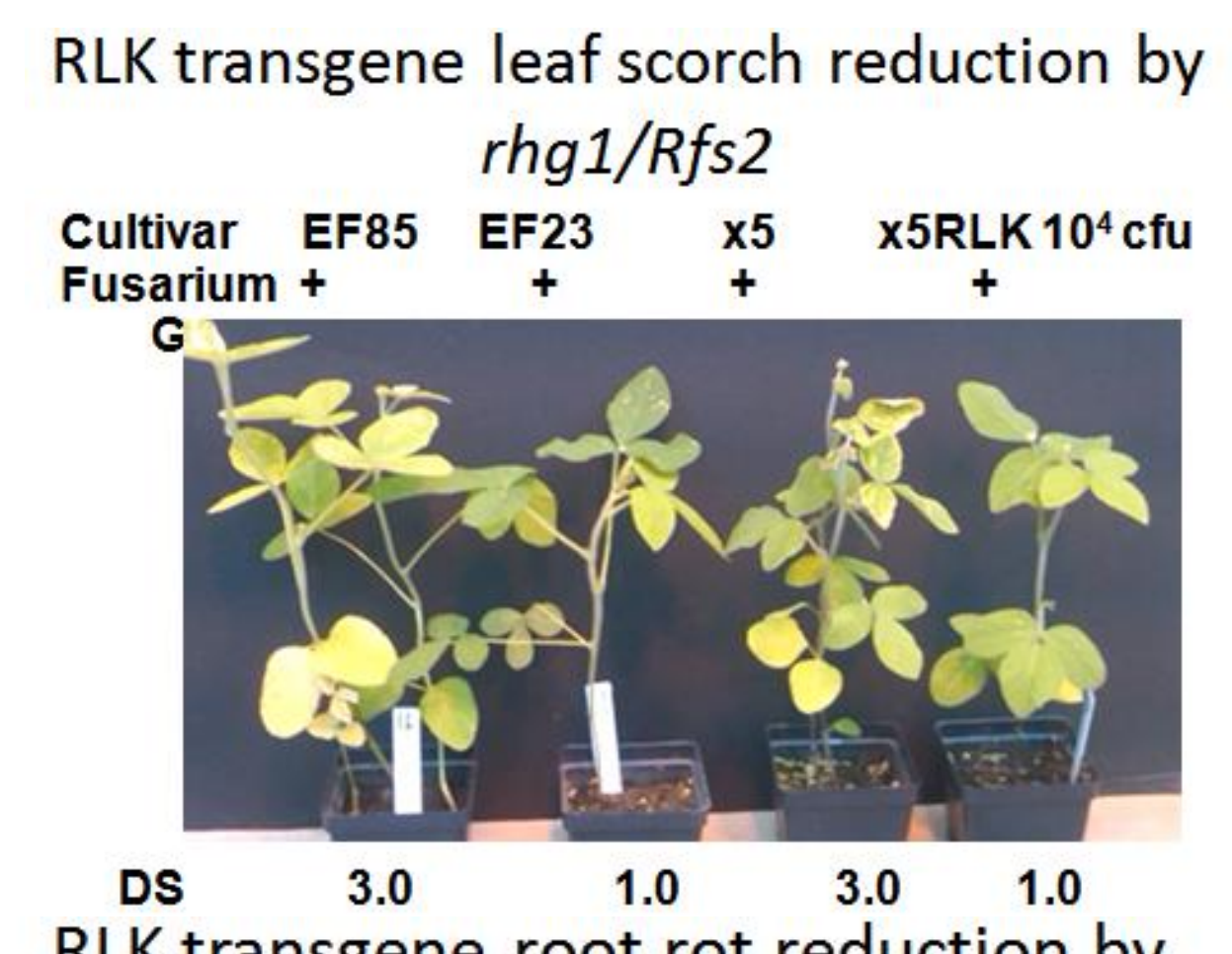
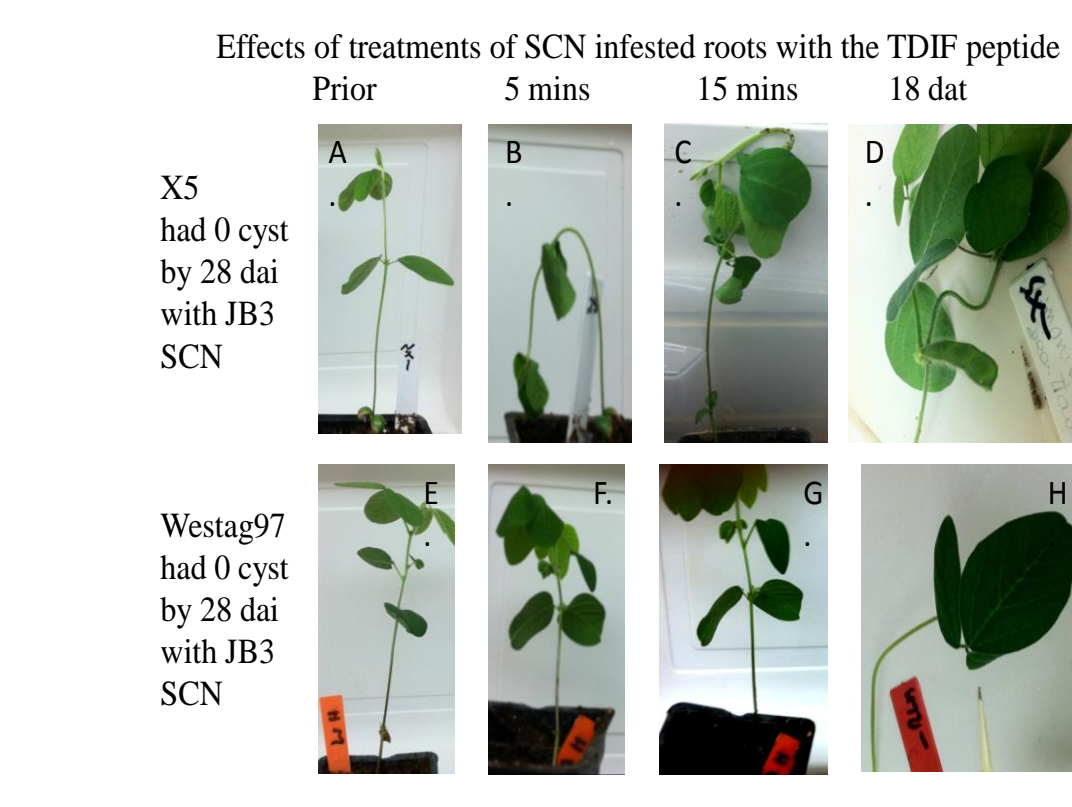
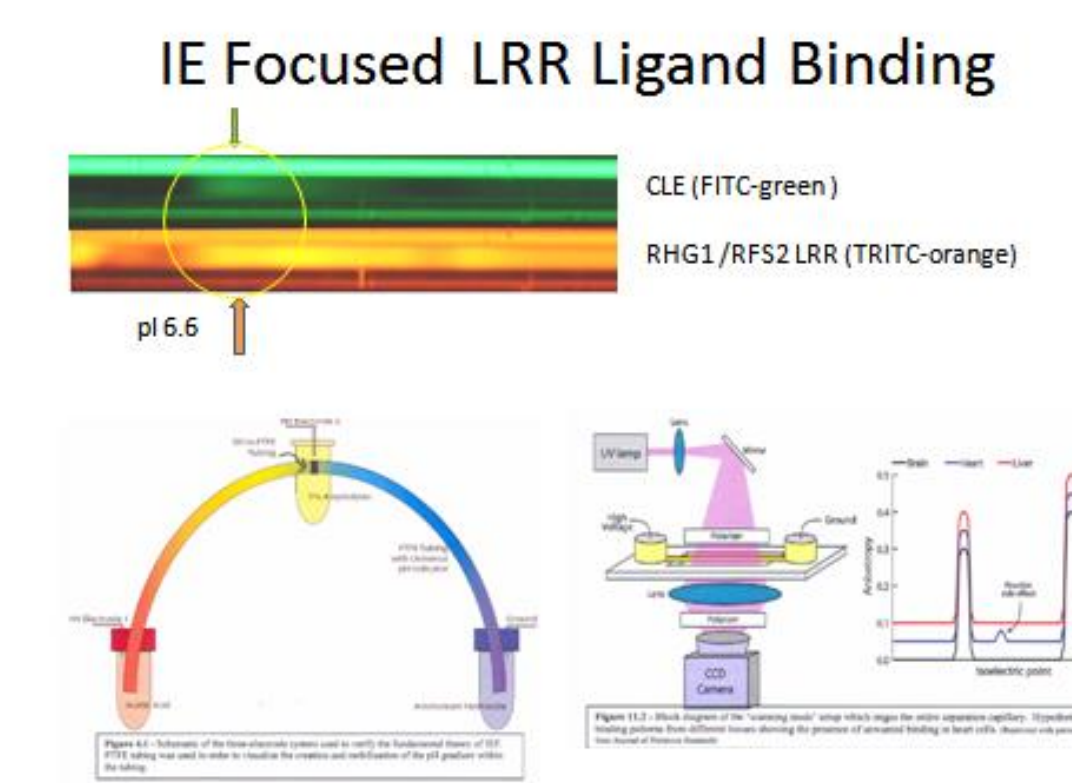
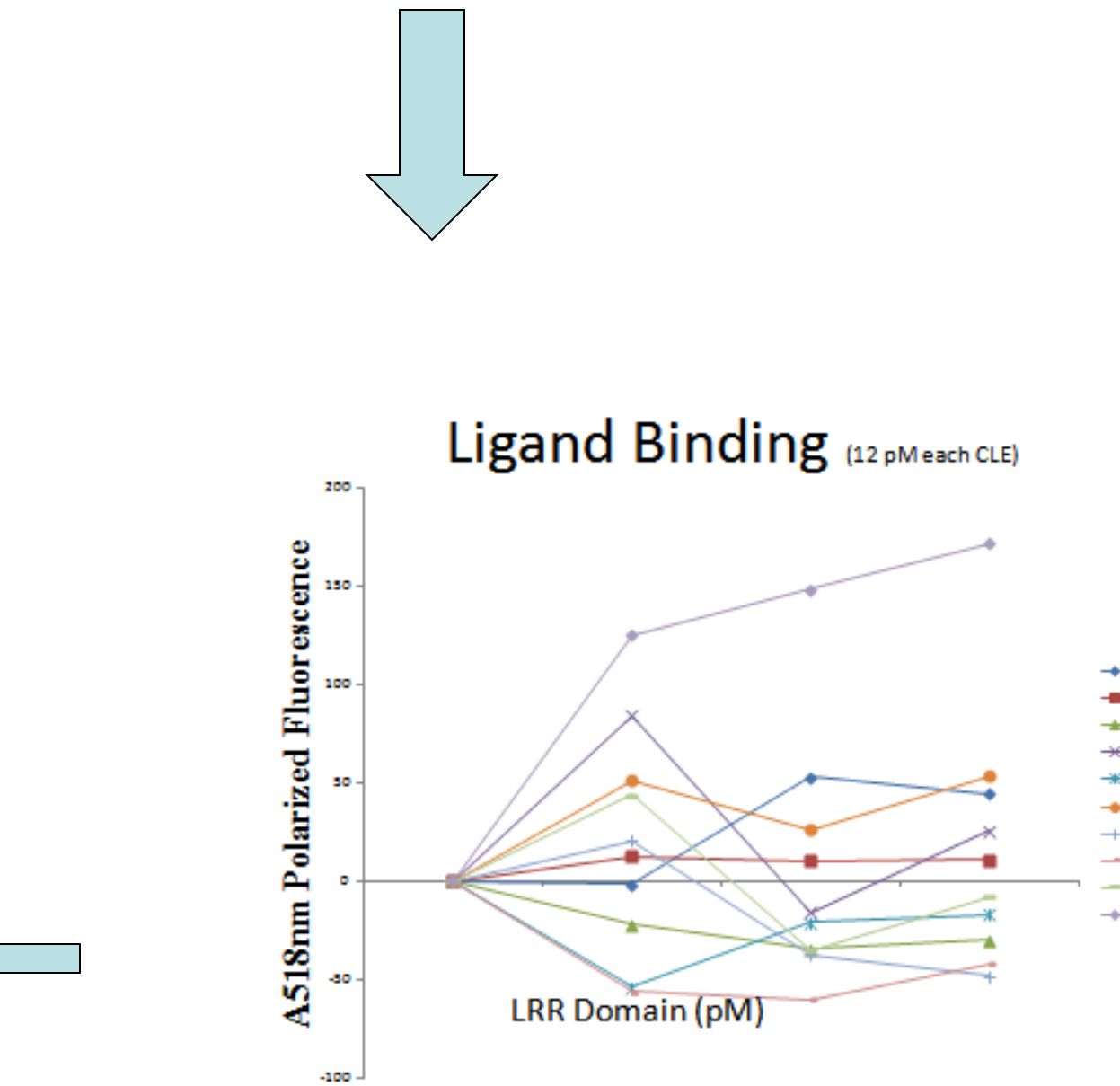
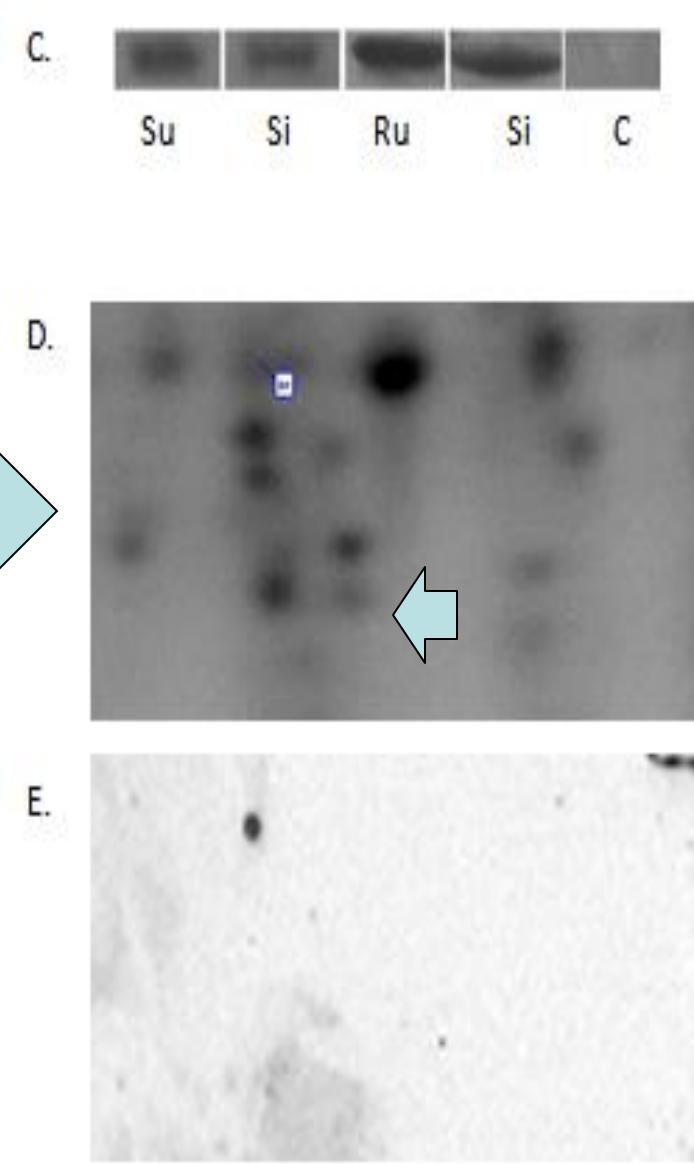
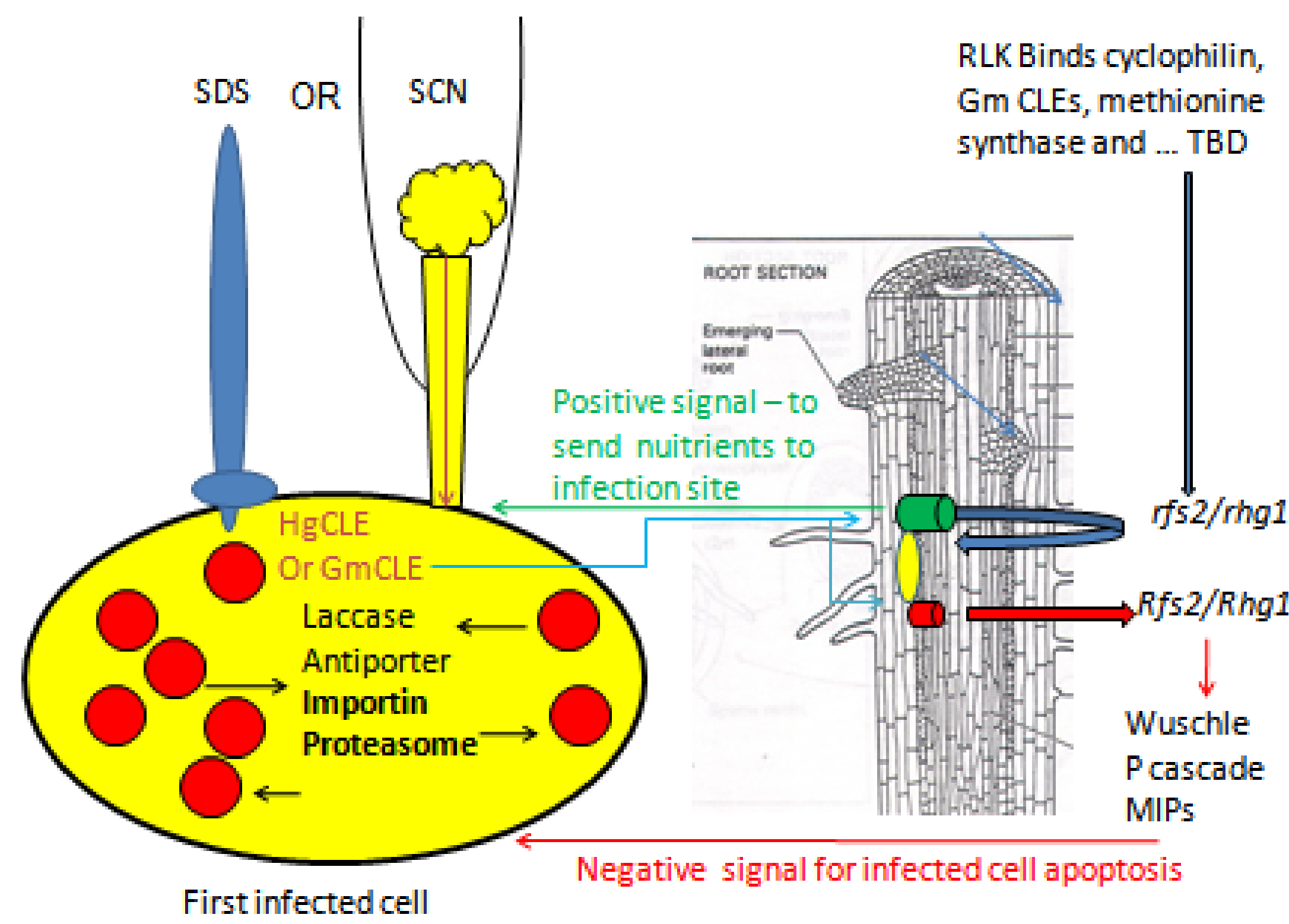


Table 2. Association of CLE treatments with resistance to SCN JB3 and mean root growth in non-transgenic lines. SCN female index in greenhouse grown seedlings at 28 days after SCN infestations. Pots were watered daily with 100 ml. Female index (FI) was the mean percentage of cysts of Hg Type 0 found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

Line: gene	SCN infested	Root mass (g)	Significant differences	Range (g)	n	SCN FI (%)
X5	No	1.05	a	0.81-1.44	15	0±0.0
X5	Yes	0.98	a	0.73-1.31	15	100±13
X5 + HgCLE	Yes	1.42	a	0.95-1.81	5	15±6
X5 + TDIF	Yes	1.40	a	0.92-1.78	5	8±3
Westag97	Yes	4.20		3.50-4.82	5	120±13
Wes97+ HgCLE	Yes	3.14		2.66-3.53	4	10±3
Wes97+ TDIF	Yes	3.10		2.66-3.53	4	5±3

- ### Conclusions
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
 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 can 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

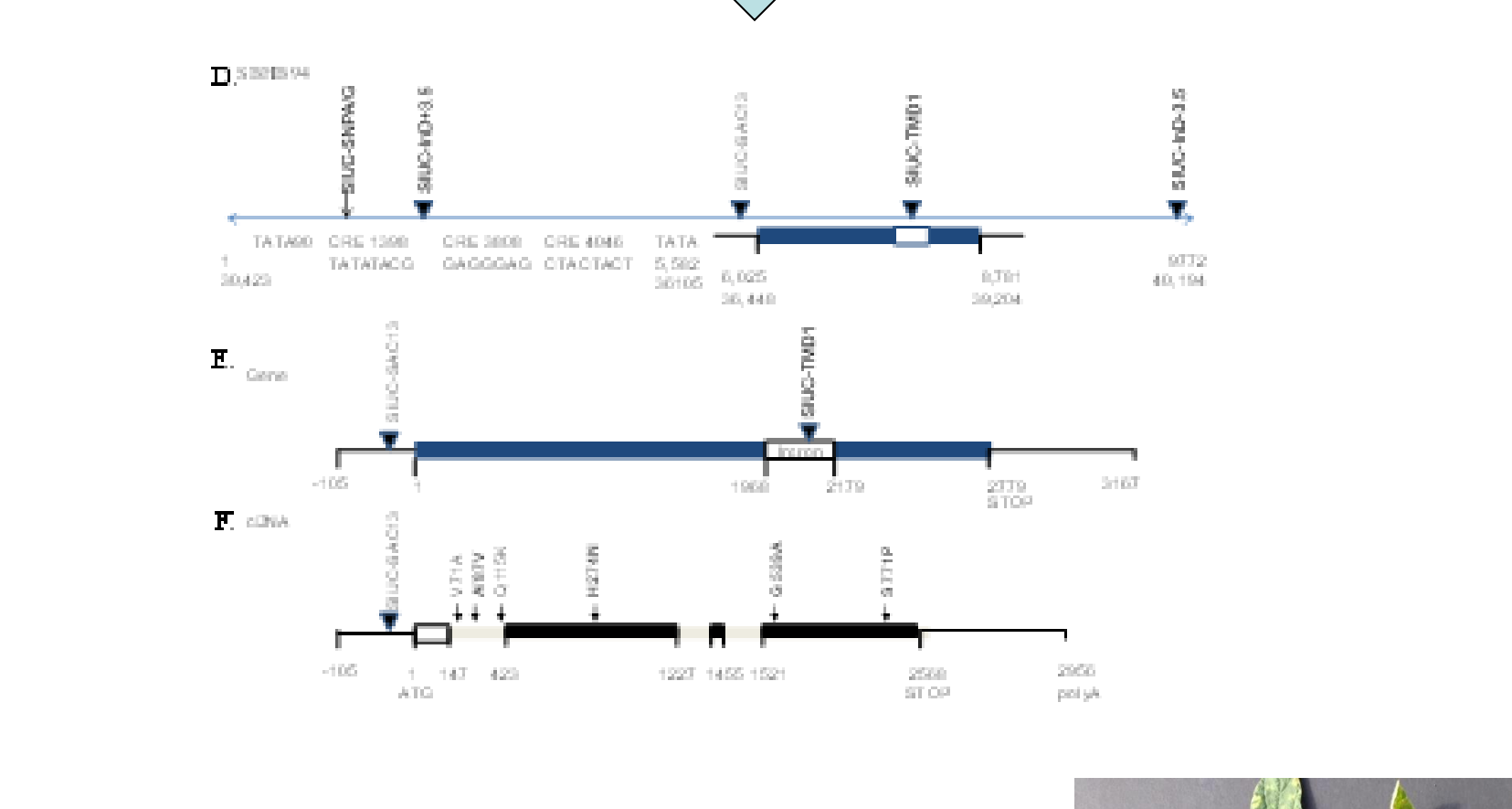
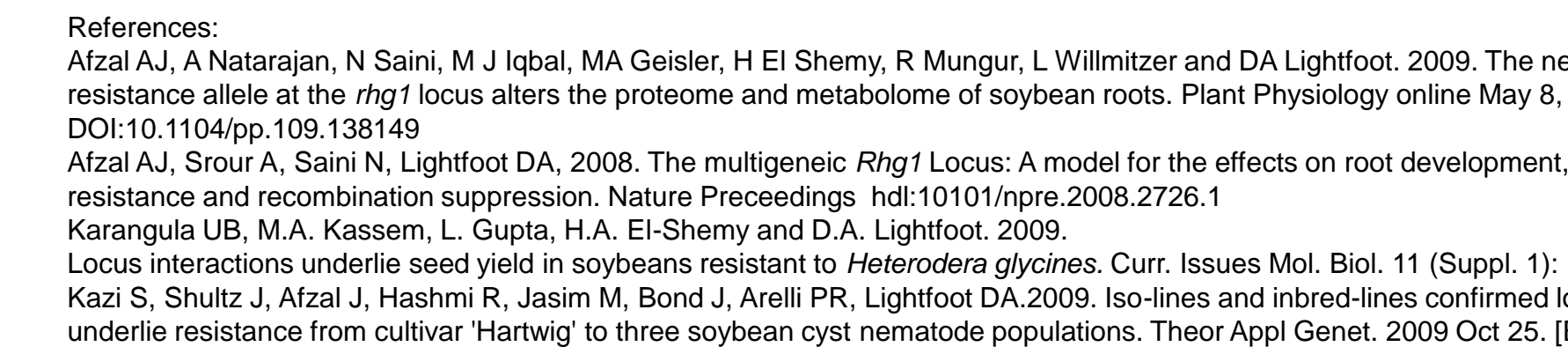
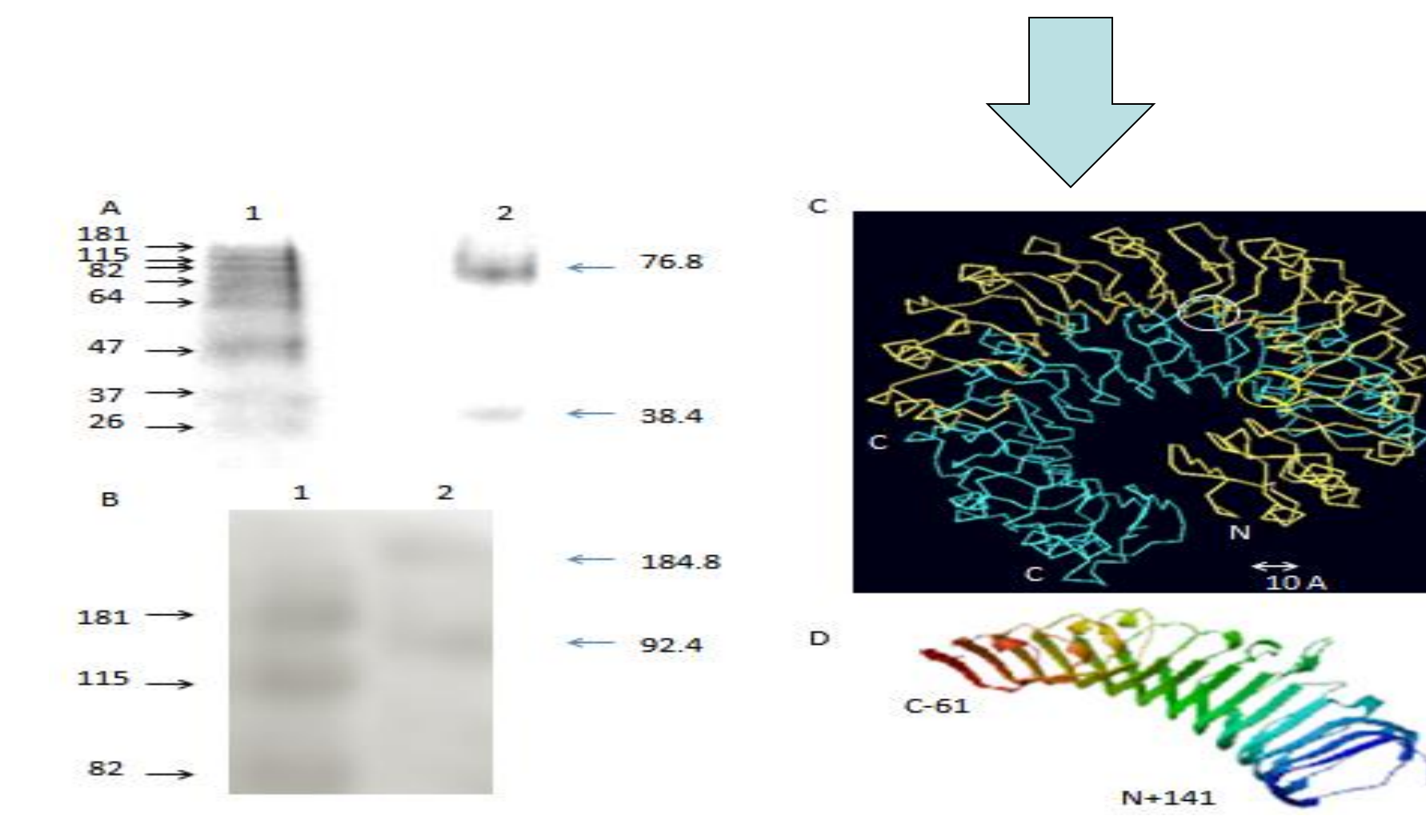
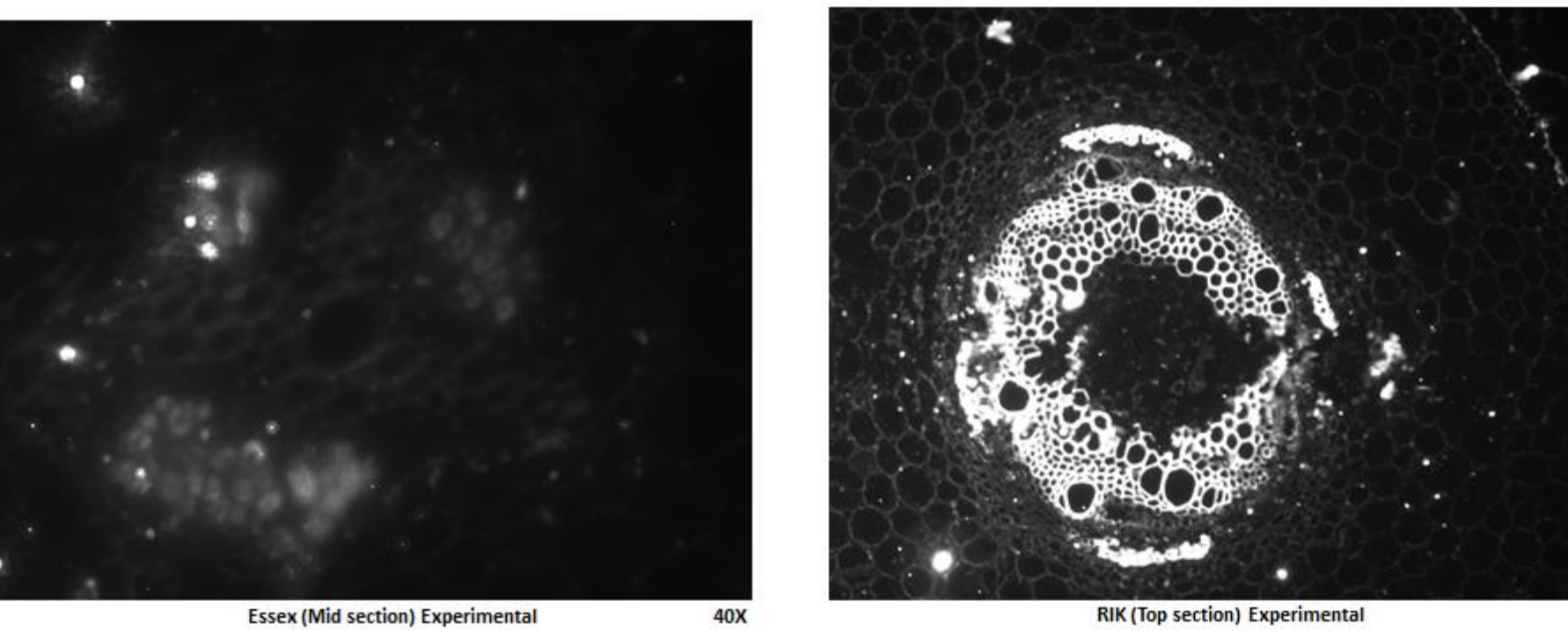
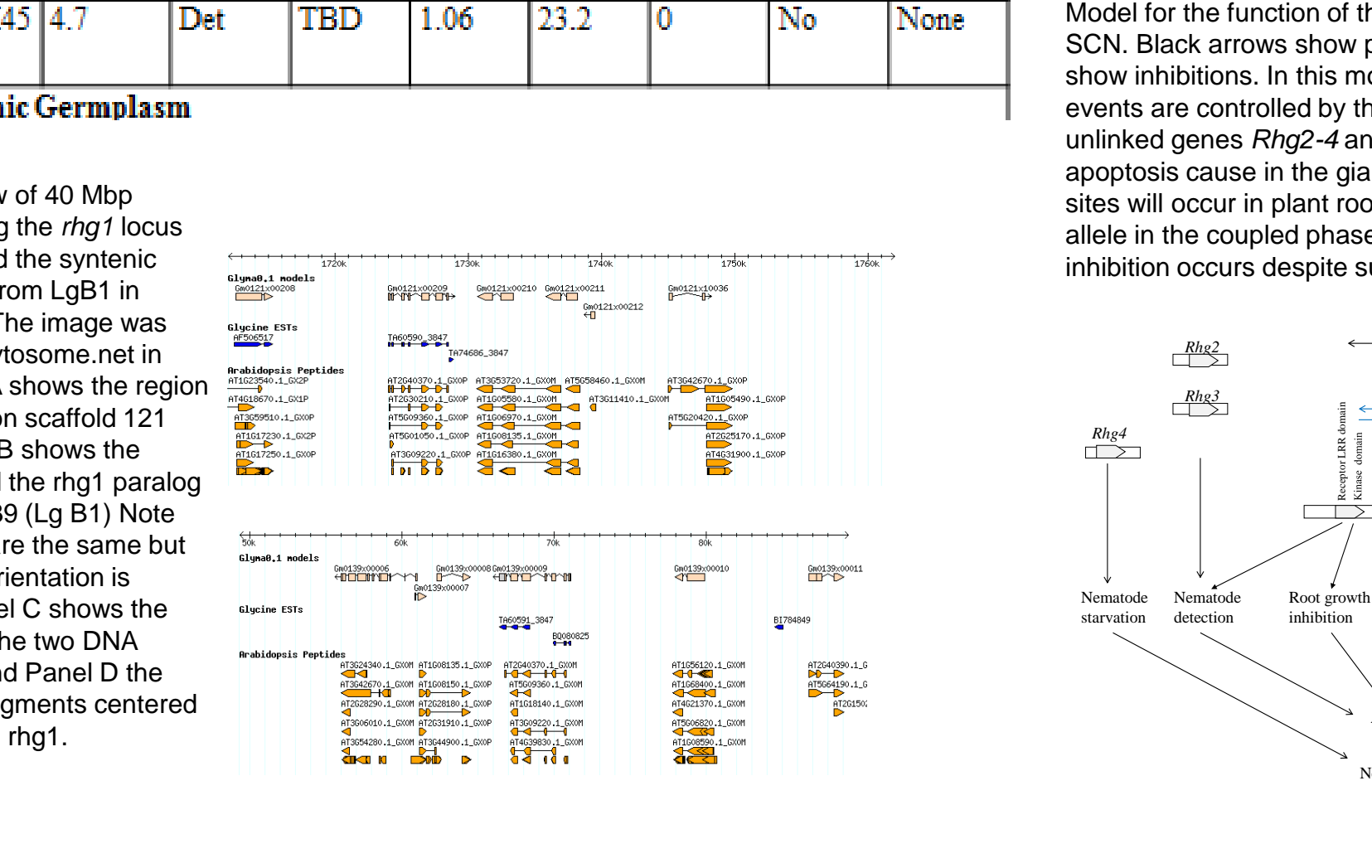


Table 1: Current state of soybean germplasm field testing by 2013. Yields for transgenics and QTL stack crosses were from a drought affected field in 2012 and so were lower than usual.

Group	Maturity Group	Growth Habit	-SDS Yield	+SDS Yield	DX	SDS R QTL	SDS Root R	SCN R Race
Conventional Germplasm								
FxH 13	4.5	Det	3.13	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
Transgenic Germplasm								
ExF 23	5.1	Det	3.63	3.48	1.1	6	Yes	3
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



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 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 of print]