New Approaches to Selecting Resistance or Tolerance to SDS and Fusarium root rot

David Lightfoot, Samreen Kazi, John Yuan, Ali Srour, Hemlata Sharma
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(2) USDA-ARS, Stoneville, MS, USA Web Site: http://bioinformatics.siu.edu
(3) Agriculture Canada

WSRC, Saturday 3.50pm (O446).

Soybean Biotechnology and SIUC: The case of SDS and Fusarium root rot

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Transgenics, QTL stacks and NIL verification for SDS and Fusarium root rot

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(3) Agriculture Canada

SBW, Tuesday 4.35pm.

SGC Annotation

cqSDS001 → (D2, 97)

cqSDS002 → (G, 3)

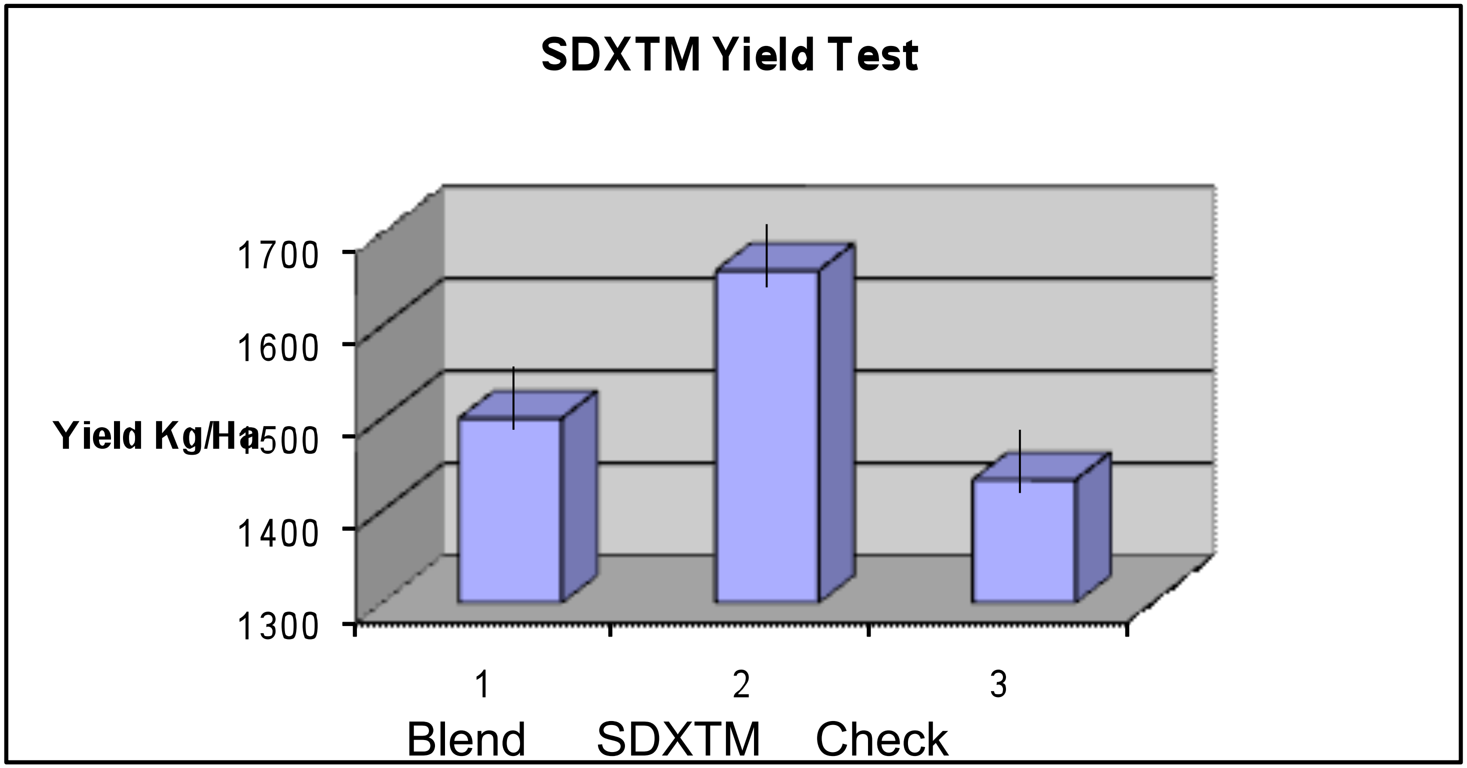
Linkage group	Map Position cM	Essex x Forrest in USA	Pyramid x Dougals	Flyer x Hartwig	Ripley x Spencer	Asgrow x Cordell	GC87018-12-2B-1xGC89045-13-1	Minsoy x Noir 1	93B41 x P9362	Misuzudaizu x Moshidou Gong 503	Essex x Forrest in Argentina	Loci (QTL = q)	Marker
A1	31								■	■		*cqRfs12*	Satt300
	71									■		*cqRfs14*	Satt506
C1	91				■			■				*Dt3/ qRfs9*	Sat_311
C2	146	■	■	■							■	*Rfs4*	Satt371
D1b	76								■			*qRfs13*	Satt282
D2	97		■	■	■	■						*qRfs7*	Sat_001
F	42	■						■				*qRfs12*	Satt160
G	0	■										*qRfs3*	Satt163
	3	■	■	■								*Rfs2*	Satt309
	9	■										*Rfs1*	Satt570
	38	■		■								*Rfs*	Sat_403
	140		■									*Dt2/ qRfs11*	Satt138
H	8										■	*Rfs12*	Satt353
I	46	■		■		■				■		*qRfs5*	Satt354
J	42	■					■ (dark grey)					*qRfs10*	Satt183
K	67									■		*qRfs13*	Satt381
L	78				■			■				*Dt1/ (qRfs8)*	Sat_99
N	45		■	■							■	*qRfs6*	Satt80
Primary Reference		Iqbal et al 2001	Njiti et al., 2001	Prabhu et al., 1999	Farias et al., 2007	Farias et al 2007	Sanichon et al., 2004	Njiti and Lightfoot 2005	Huang et al patent application		Salas, Bashir & DAL unpublished	*Now called cqSDS001-012*	

THE EFFECT OF STACKED QTL FOR RESISTANCE TO SDS SHOWING THE EXCLUSION OF DISEASE

Ordinary
Cultivars

Resistant SDS
QTL Stack

THE YIELD EFFECT OF SDX^{TM} SHOWING THE EFFECT OF MIXES



Resistant **SDS QTL Stack**

Ordinary Cultivar

QTL on D in PxD (from 1996) was D2 found in FxH and RxS

TABLE 12

Intervals most likely to contain the SDS QTL from Pyramid that show significant associations with mean SDS disease index across five locations.

DNA Marker	Linkage group †	R^2	P > F	QTL LOD‡	QTL var.§	MEAN DI for RILs with alleles from Douglas	MEAN DI for RILs with alleles from Pyramid
B1t65	A2	0.16	0.009	2.0	16.0	53.4 ± 15	37.6 ± 14
A85H	A2	0.11	0.0099	2.0	9.1	52.3 ± 12	41.7 ± 10
$OG01_{390}$	D	0.14	0.0051	2.9	14.3	51.6 ± 13	31.1 ± 10
SZ19	D	0.01	0.006	2.0	11.1	51.7 ± 12	33.5 ± 10
SATT38	G	0.17	0.0005	3.8	17.0	51.7 ± 12	31.3 ± 10
SATT309	G	0.24	0.0001	4.4	25.0	55.5 ± 12	29.5 ± 10
$OD04_{950}$	G	0.09	0.0097	1.0	10.0	52.0 ± 12	39 ± 13

U.S. Patent Oct. 9, 2001 Sheet 5 of 23 US 6,300,541 B1

US006300541B1

(12) **United States Patent** (10) Patent No.: US 6,300,541 B1
Lightfoot et al. (45) Date of Patent: Oct. 9, 2001

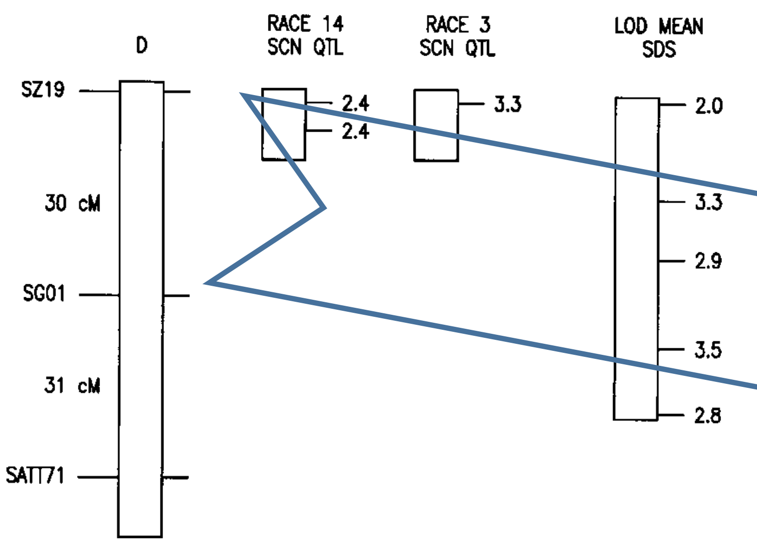
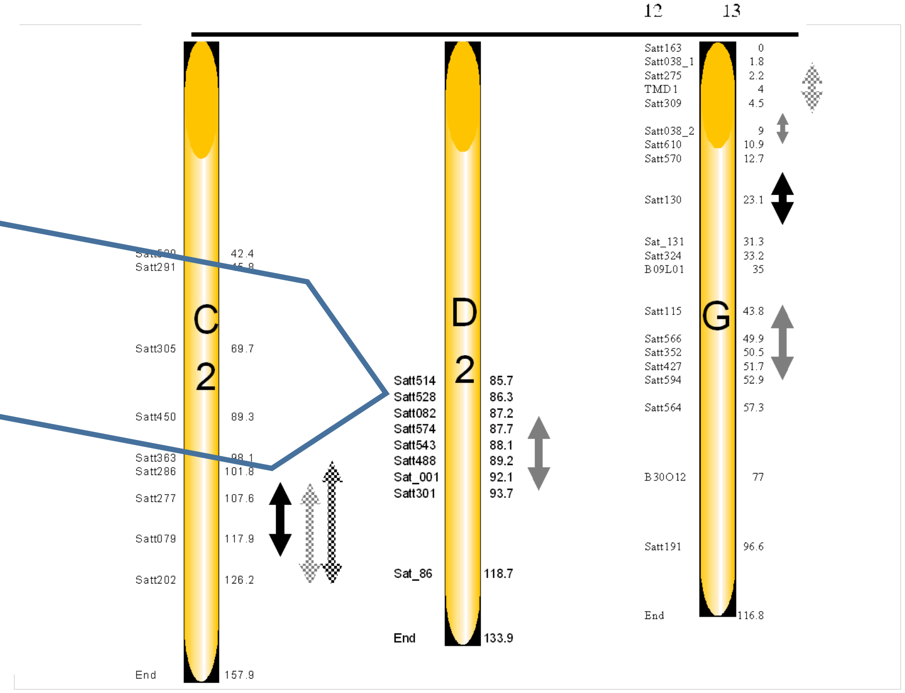


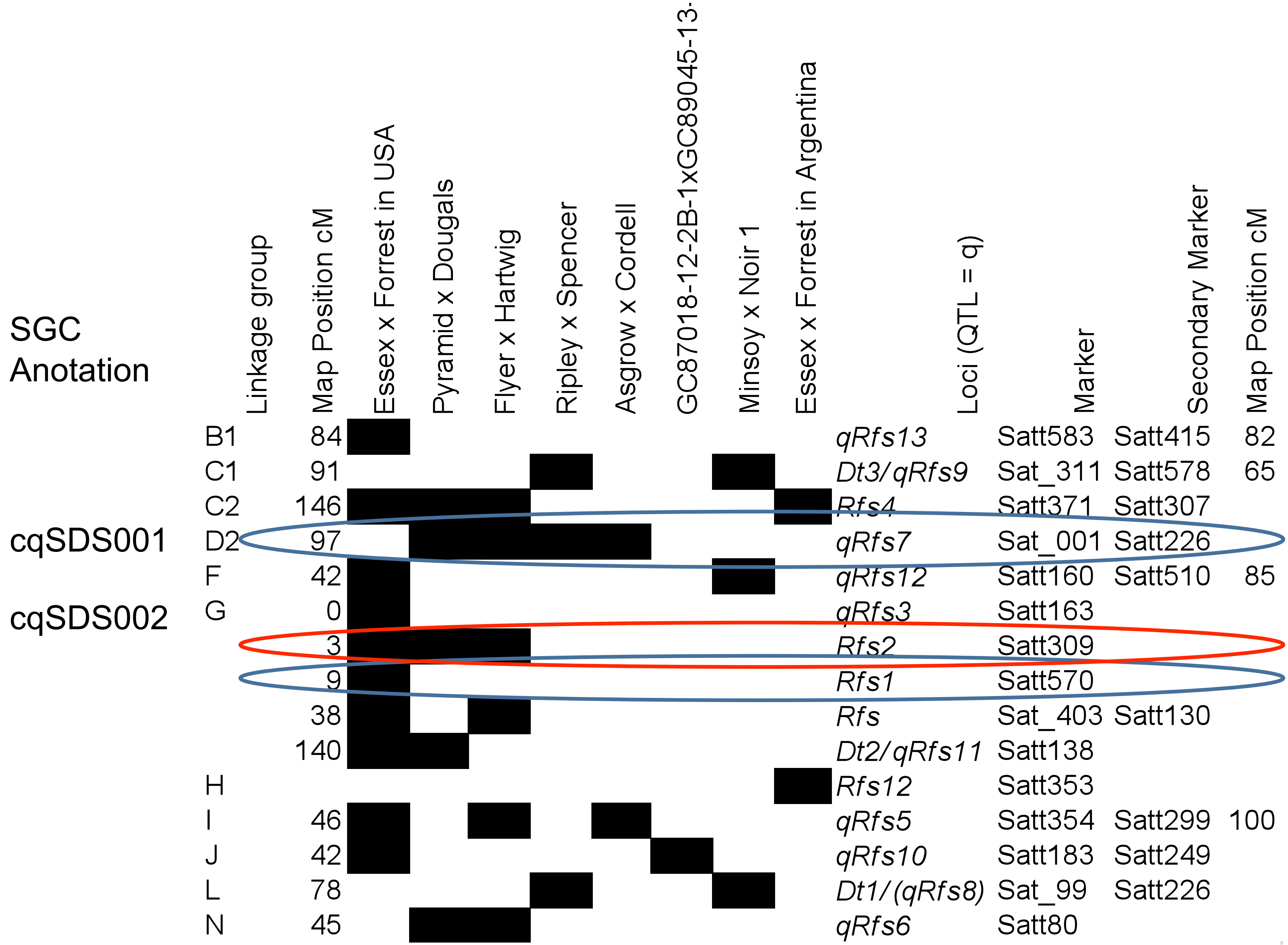
FIG. 2



Due Diligence And the Patent Landscape

The Original SDS Patent
Lightfoot D.A., Meksem K., P.T. Gibson. 2001. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants: DNA markers. US Patent # 6,300,541. Filed Jan19 1996
*Claims loci on B1, C2, **D, G,** I, J, **N.***

The Submarine Patent
Lightfoot D.A., Meksem K., **P.T. Gibson**. 2007. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants: Greenhouse Assays. US Patent #7,288,386. Filed Jan19 1996
Claims all greenhouse assays predicting field SDS are pale imitations


SGC
Anotation
cqSDS001
cqSDS002
Linkage group
Map Position cM
Essex x Forrest in USA
Pyramid x Dougals
Flyer x Hartwig
Ripley x Spencer
Asgrow x Cordell
GC87018-12-2B-1xGC89045-13-
Minsoy x Noir 1
Essex x Forrest in Argentina
Loci (QTL = q)
Marker
Secondary Marker
Map Position cM
B1 84 qRfs13 Satt583 Satt415 82
C1 91 Dt3/qRfs9 Sat_311 Satt578 65
C2 146 Rfs4 Satt371 Satt307
D2 97 qRfs7 Sat_001 Satt226
F 42 qRfs12 Satt160 Satt510 85
G 0 qRfs3 Satt163
3 Rfs2 Satt309
9 Rfs1 Satt570
38 Rfs Sat_403 Satt130
140 Dt2/qRfs11 Satt138
H Rfs12 Satt353
I 46 qRfs5 Satt354 Satt299 100
J 42 qRfs10 Satt183 Satt249
L 78 Dt1/(qRfs8) Sat_99 Satt226
N 45 qRfs6 Satt80

Root and Leaf Diseases Differ

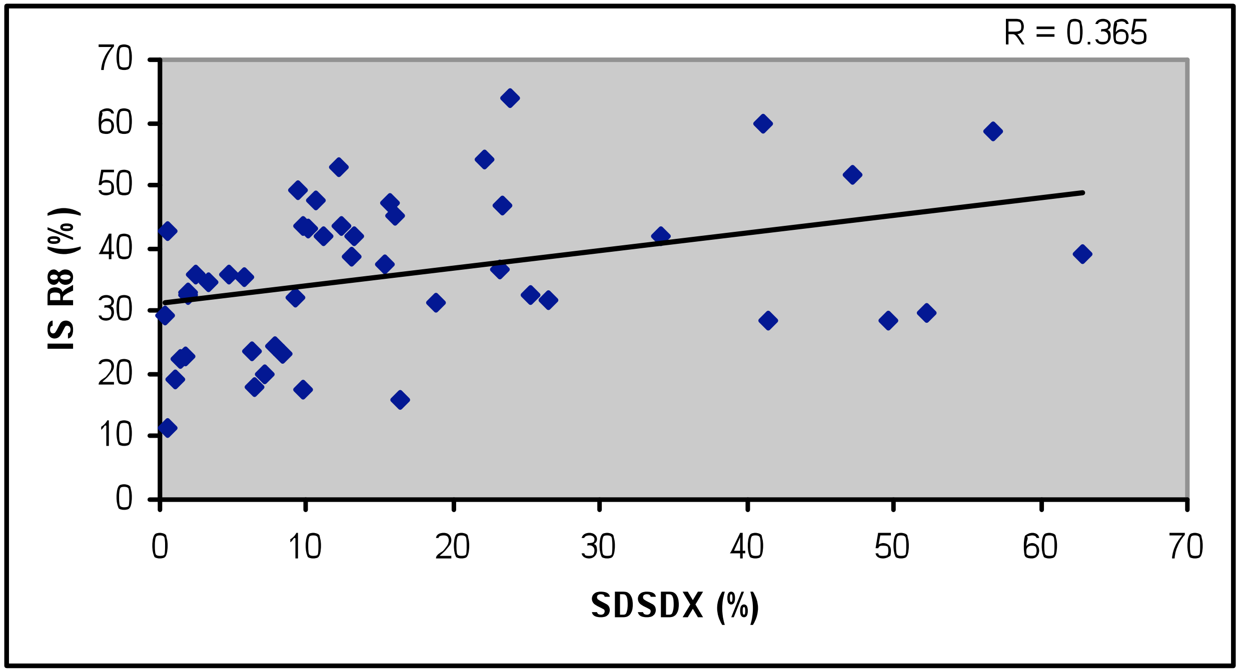
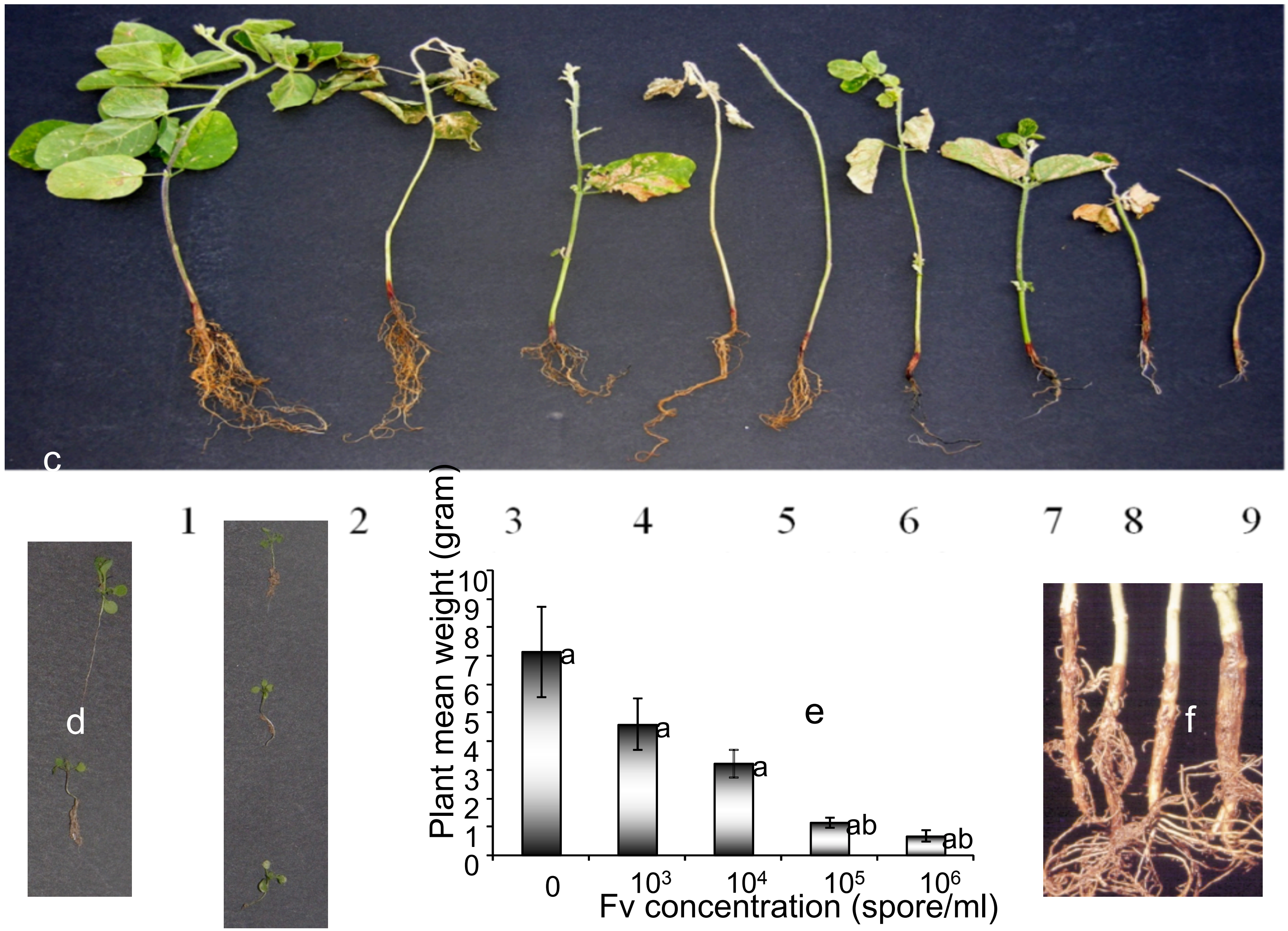


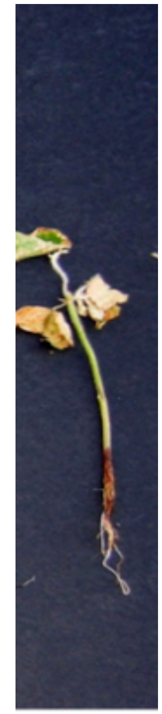
Figure 1: Correlation between leaf scorch measured as mean disease index at the R6 and root infection measured as infection severity at the R8. Among the metrics used to measure leaf and root SDS these two showed the closest correlation. The data was from different years. Only lines with IS scores are shown. The correlation was significant $P<0.05$ with 49 df.

SDS PATHOGEN CAUSES ROOT DISEASE IN ALL PLANTS TESTED



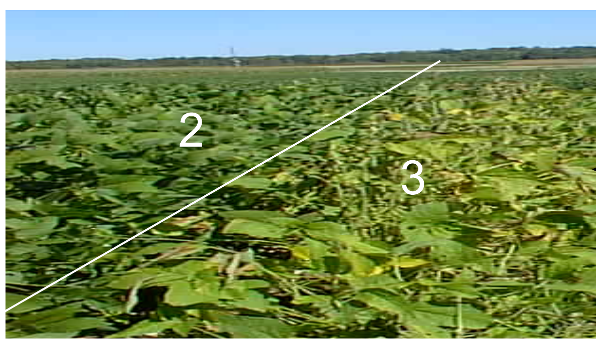
THE INTERMEDIATE EFFECT OF MIXES SHOWS THER IS NO “ROOT ZONE PROTECTANT FACTOR”

1

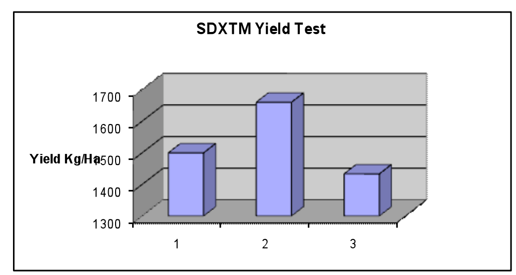


8

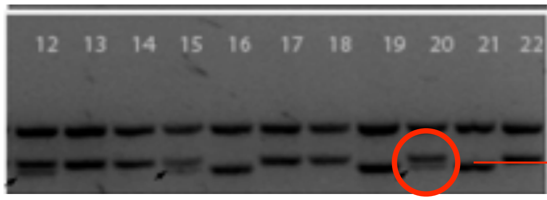
Resistant **SDX™**



Ordinary Cultivar

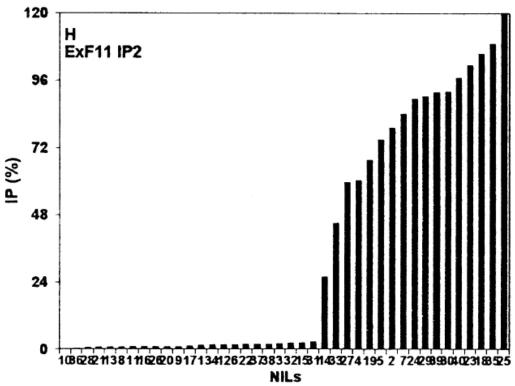


NILs of LG G (Chr 18) for QTL Confirmation, Fine Mapping and Proteomics



2,000 Single Seed Selections

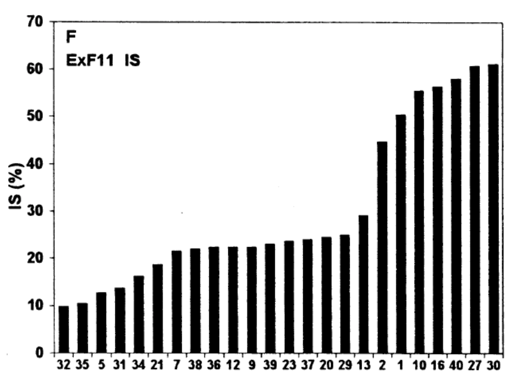
Heterozygous Plant

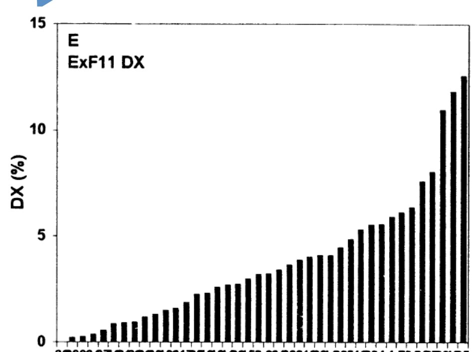


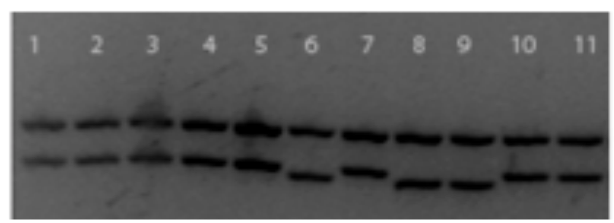
40-200 Single Plant Selections

RILs to NILs

Segregation in NILs







Homeoelog

Rhg1/Rfs2

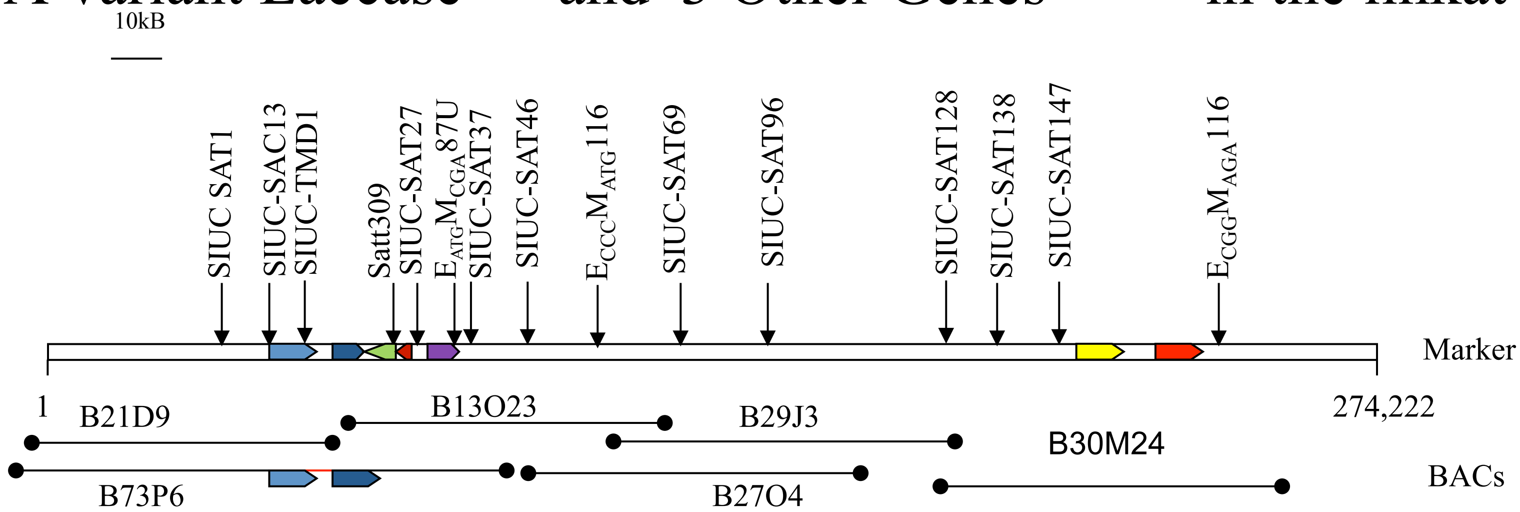
SDS Proteomics
RvS NILs Uninfested

Protein	Acc no	Mowse score/ P<0.05	No of peptides matched	Exp pI, mol wt/ theor pI, mol wt	Fold difference
Glucose 6-phosphate isomerase	21256302	142/51	6	5.3, 60 kDa/ 5.5, 67	3.22±0.25*
Isoflavone reductase	6573171	88/52	2	6.1,30 kDa/ 5.6,34$	3.04±0.56#

SDS Metabolomics
RvS NILs Uninfested

Genotypes Compared	R/S		R/S
Glucose	1.00	Glucaric acid	0.67
Sorbose	2.32	Fumaric acid-like	2.71
Mannose major	1.36	Malonic acid	1.24
Galactose	1.08	Glyceric acid (ox. glycerol)	1.44
Fructose	1.80	Ribonic acid (deC. ribose)	0.59
Maltose	**0.06**	Citric acid	0.70
Trehalose	0.65	Fumaric Acid	1.37
Phenylalanine	0.84	Agmatine (polyamine precursor)	1.01
Aspartic acid	1.25	Inositol	1.31
Alanine	0.96	Myo-inositol	0.53
Valine	1.04	Aminovaleric acid (deC valine)	0.57
Leucine	1.00	3-Hydroxypyruvic acid (de-NH ser)	1.26
Isoleucine	0.95	yCHO_854.975	**0.29**
Glycine	1.22	yCHO_556.425	2.40
Glutamate	0.85	Gamma-amino-N-butyric acid (deCglu)	0.73
Lysine	1.02	Oxoproline	1.35

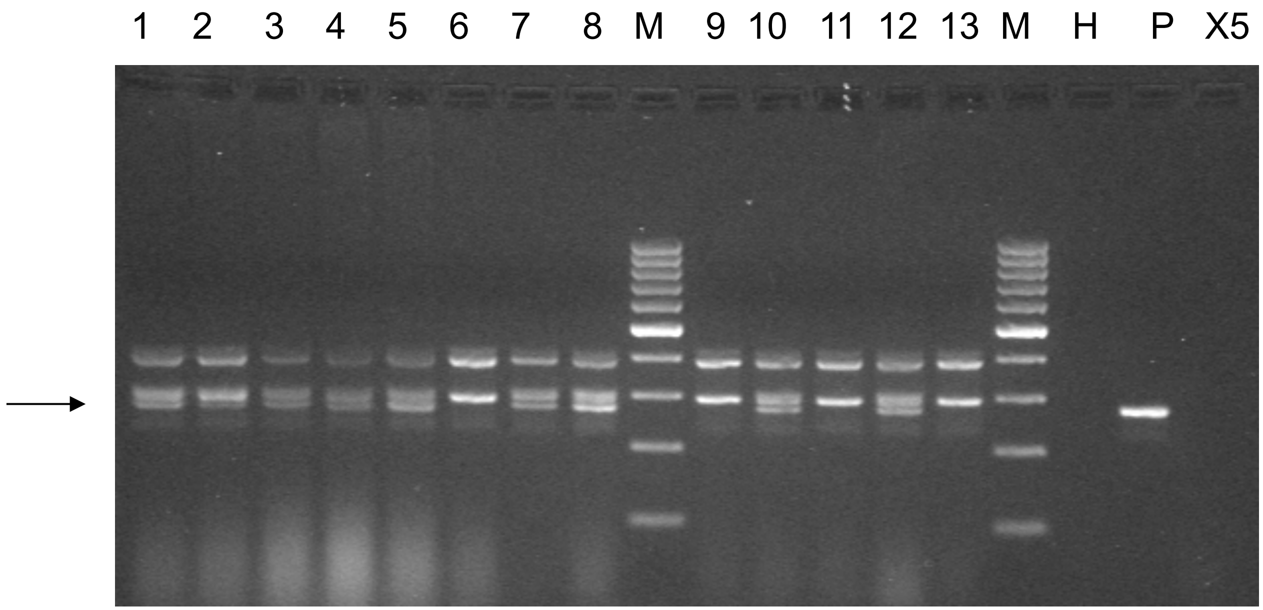
The *rhg1/Rfs2* Locus Encompassed a Receptor Like Kinase A Variant Laccase and 3 Other Genes in the linkat



The *Rfs1* Locus Encompassed a Stress Response Protein and A Serine Lyase Between Flanking Markers

Soybean
Transgenics with the RLK at *Rfs2/rhg1*

PCR from leaf samples
6B3-7D2(1) with TMD1 primers



Arrow shows region of double band for positive samples ~ 300 bp.
M =marker, H = water control, P = Rhg1 plasmid, X5 =control plant

Transgene root Stunting by *rhg1/Rfs2*

Fusarium	**-**	**-**	**+**	**+**	**10^3cfu**
Gene	**-**	**+**	**+**	**-**	

6.4 3.2 4.6 6.3 (g)

-

RLK transgene leaf scorch reduction by *rhg1/Rfs2 14 dai*

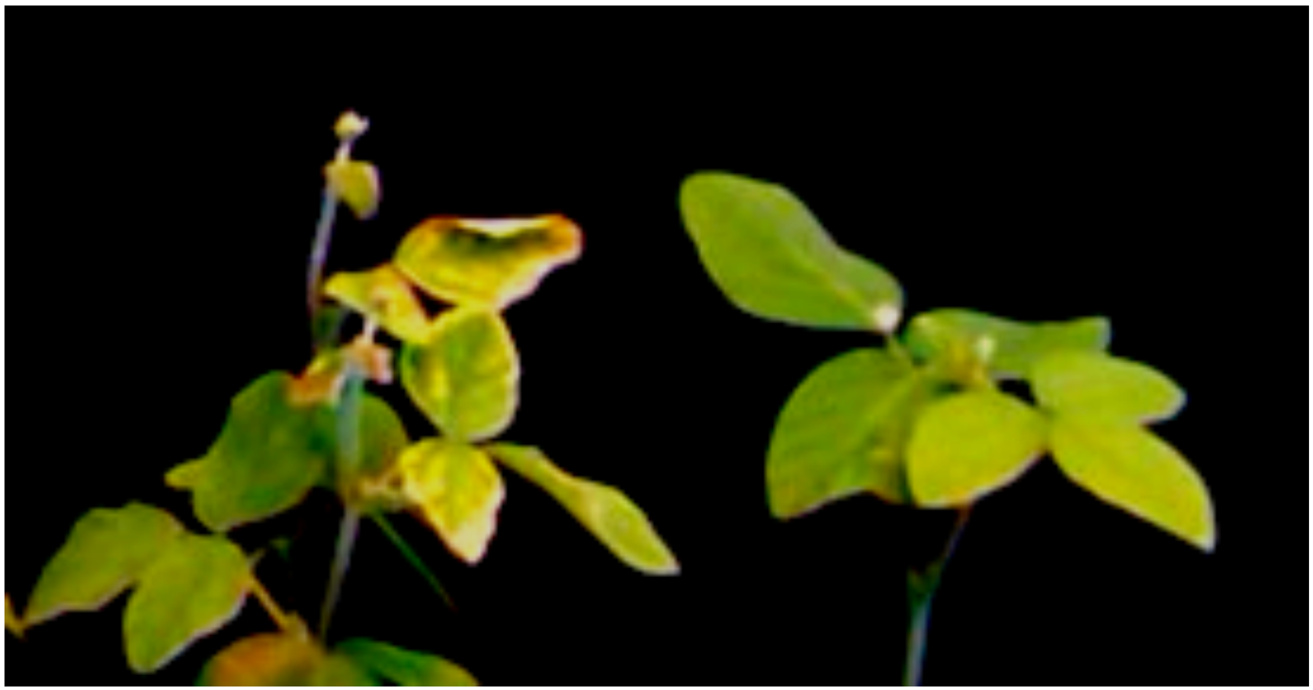
Cultivar	**x5**	**x5RLK**	**EF23**	**EF85**	
Fusarium	**+**	**+**	**+**	**+**	**10^4 cfu**
Gene	**-**	**+**	**+**	**-**	

DS	**2.0**	**0**	**1**	**3**

-

Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 21 dai

Cultivar	x5	x5RLK	10^4 cfu
Fusarium	+	+	
Gene	+	-	



DS	3.0	1.0

RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 21 dai*

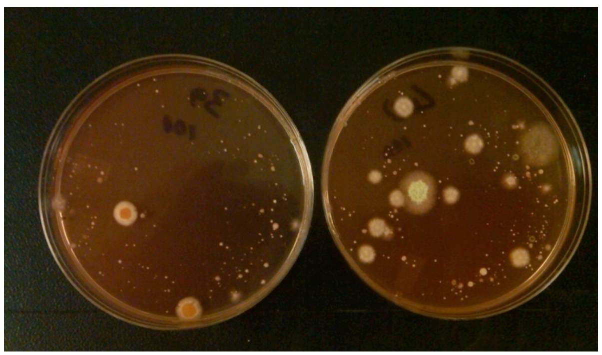
Cultivar	EF85	EF23	x5	x5RLK 10^4 cfu
Fusarium	+	+	+	+
Gene	-	+	-	+

DS	3.0	1.5	3.0	1.0

-

RLK transgene root rot reduction by *rhg1/Rfs2 at 28 dai*

Cultivar	**x5RLK**	**x5**	**x5 10^4 cfu**
Fusarium	**+**	**+**	**+**
Gene	**+**	**-**	**-**



CFUs from ground roots

Root Rot score	**1.0**	**4.0**	**3.0**

Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 28 dai

Cultivar	x5	x5RLK
Fusarium 10^4 cfu	+	+
RLK transgene	-	+

DS	4.0	1.0

Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 56 dai

Cultivar	**x5**	**x5RLK**
Fusarium 10^4 cfu	**+**	**+**
RLK transgene	**-**	**+**

DS

Run 2 of Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 28 dai

Fusarium 10^4 cfu

DS	**4.0**	**2.5**	**1.5**
Cultivar	**x5**	**F2-3**	**F2-2RLK**
RLK transgene	**-**	**-**	**+**

Cultivar	**x5RLK**	**F2-1**	**x5**
RLK transgene	**+**	**+**	**-**
DS	**3.0**	**2.5**	**5.5**

Run 2 RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 56 dai*

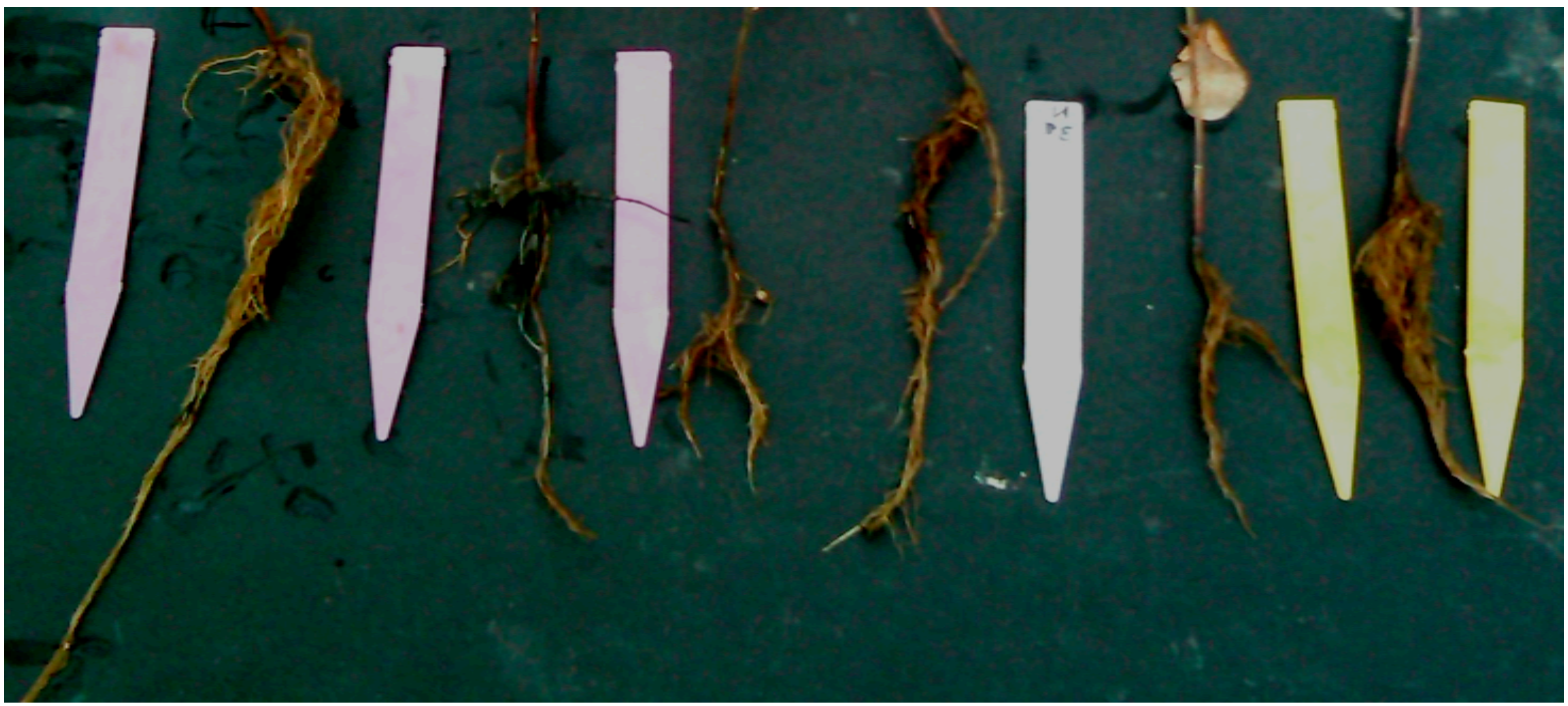
Fusarium	**+**	**+**	**+**	**+**	**+**	**+**
Cultivar	**F2-1**	**F2-2**	**F2-3**	**x5RLK**	**x5**	**x5**
Gene	**+**	**+**	**-**	**+**	**-**	**-**

DS	**2.0**	**2.5**	**7.0**	**3.5**	**7.0**	**9.0**
		-				

Run 2 RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 56 dai*

Fusarium

Cultivar	**F2-2**	**F2-3**	**F2-1**	**x5RLK**	**x5**	**x5**
Gene	**+**	**-**	**+**	**+**	**-**	**-**



RR	**1.0**	**4.0**	**2.5**	**2.0**	**4.5**	**3.0**
DS	**2.5**	**7.0**	**2.0**	**3.5**	**7.0**	**9.0**

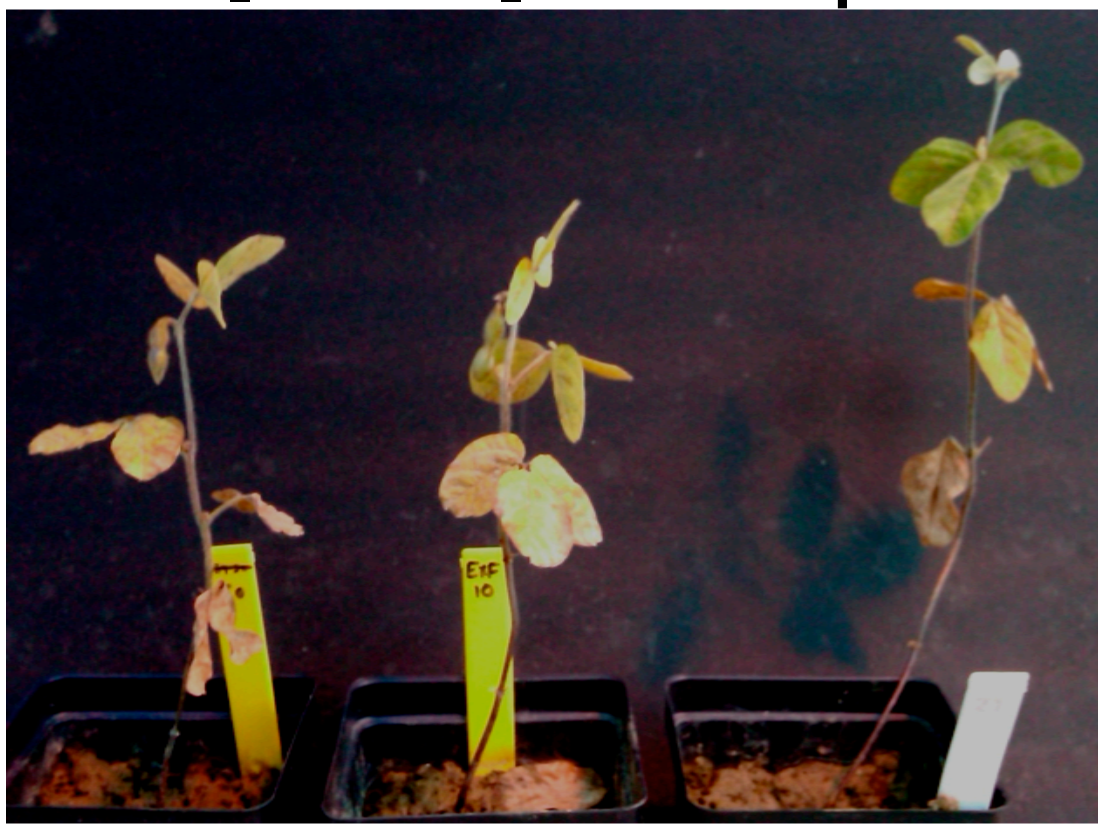
Run 2 RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 56 dai*

Fusarium	**+**	**+**		**+**	**+**	**+**
Cultivar	**F2-2**	**F2-3**	**F2-1**	**x5RLK**	**x5**	**x5**
Gene	**+**	**-**	**-**	**+**	**-**	**-**

DS	**2.5**	**7.0**	**3.0**	**3.5**	**7.0**	**9.0**
RR	**1.0**	**4.0**	**2.5**	**2.0**	**4.5**	**3.0**

Run 2 of Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 28 dai

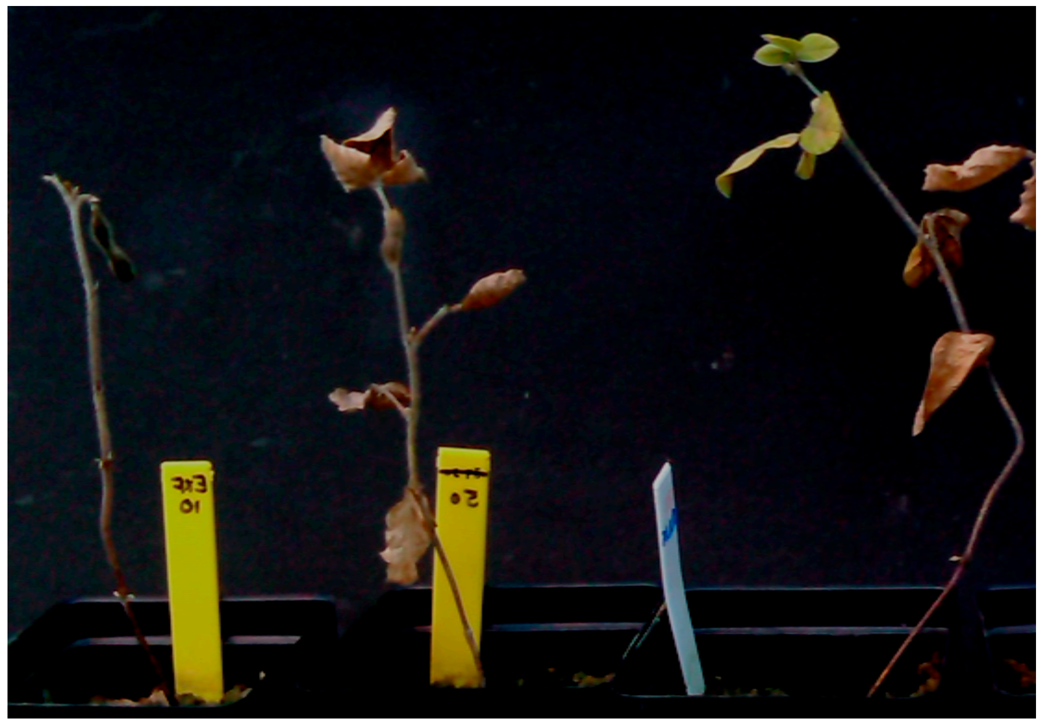
Cultivar	**x5**	**x5**	**x5RLK**
Fusarium 10^4 cfu	**+**	**+**	**+**
RLK transgene	**-**	**-**	**+**



DS **4.0** **1.5**

Run 2 of Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 56 dai

Cultivar	**x5**	**x5RLK**
Fusarium 10^4 cfu	**+**	**+**
RLK transgene	**-**	**+**



DS	**8.0**	**2.5**

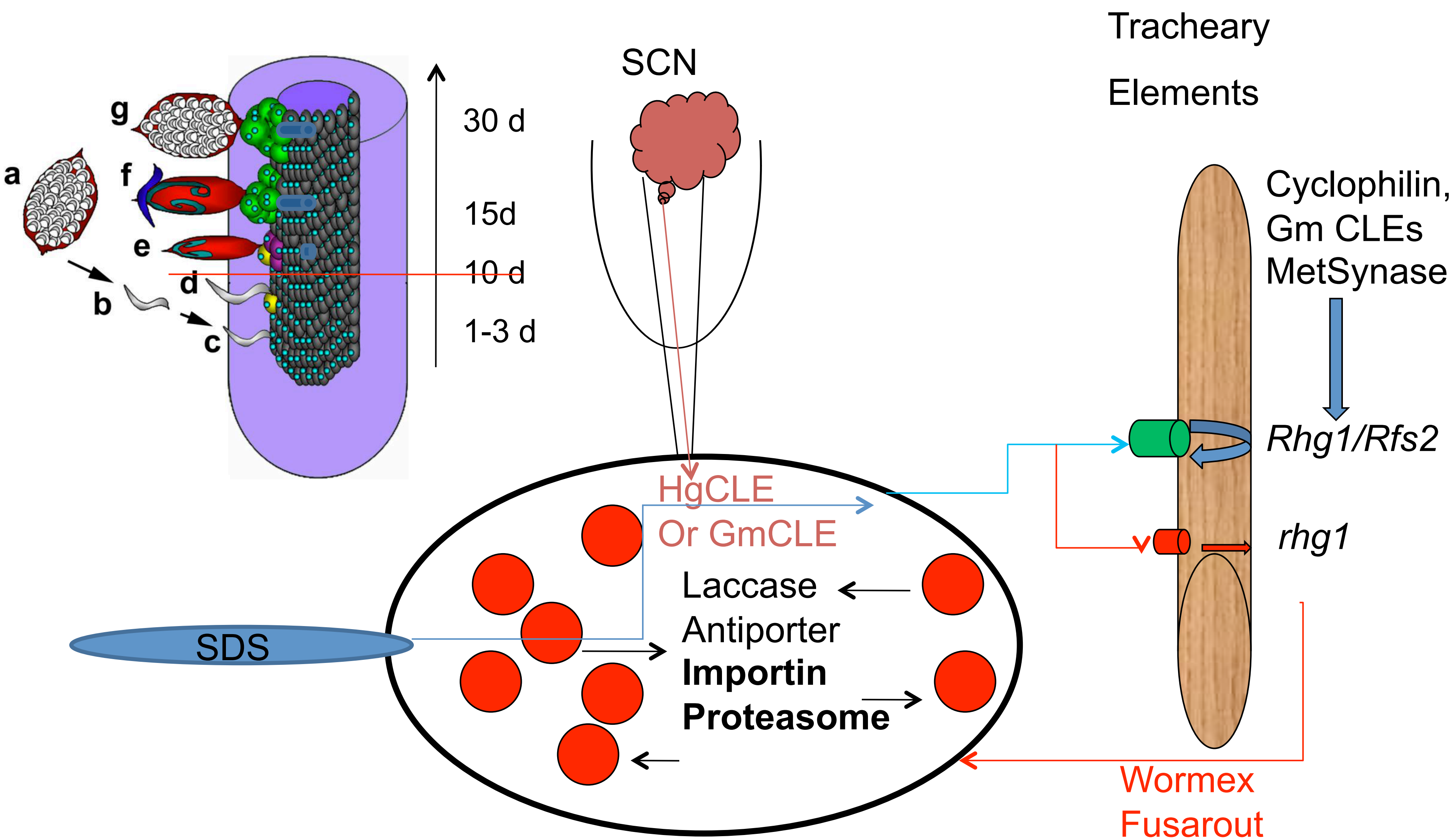
Run 3 of Proof in stable soybean transgenics the 10 kbp rhg1/Rfs2 subclone – 28 dai

Cultivar	**x5**	**x5RLK**
Fusarium 10^4 cfu	**+**	**+**
SCN eggs 10^3	**+**	**+**
RLK transgene	**-**	**+**

DS

5.0 **2.5**

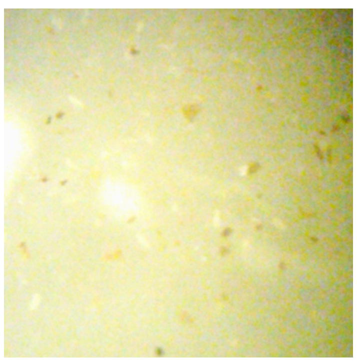
THE RLK at RHG1 integrates signals to regulate appropriate root development and growth or giant cell death and no root growth.



RLK transgene SCN reduction by *rhg1/Rfs2*

Cultivar	x5RLK	x5
SCN IP	60±11	100±13
RLK Gene	+	-

No chlorosis



Nonspecific chlorosis

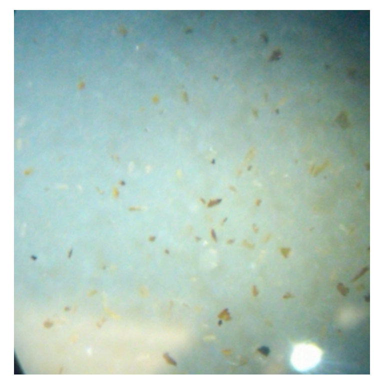
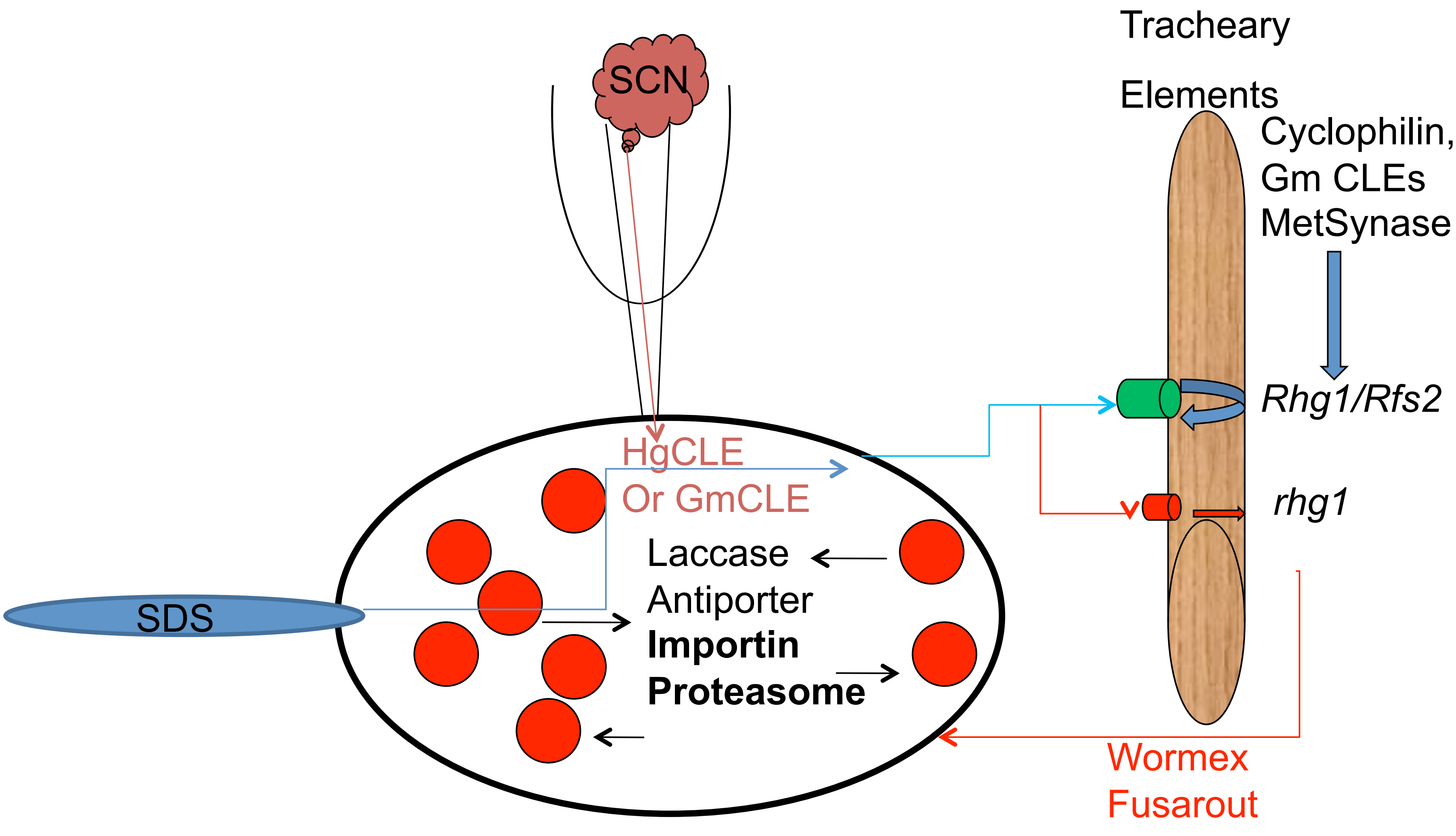


Figure 7: THE RLK at *Rfs2/rhg1* may integrate signals to regulate FRR infection or growth, root development and SCN induced giant cell death.



Conclusions

- SDS001 on D2 was in a region causing SCN resistance, in repulsion with race 14 resistance in PxD in the patent from 2000.
- A patent for the greenhouse assay most commonly used issued on Oct 30, 2007
- Fine Maps of *Rfs1* and *Rfs2* loci have provided gene candidates (*rhg1*/*Rfs2* transgenics disclosed for the first time at SBW).
- Laccase may be part of *rhg1/Rfs2,* but the enzymes substrate remains unknown.
- The RLK has been proven to be part of *Rfs2* reducing leaf scorch by reducing root infection.
- Proteins and Metabolites useful as biomarkers of the LG G effect exist.

Recent Publications

- Ahsan R, **M.J. Iqbal**, A.J. Afzal **A. Jamai**, K.Meksem and D.A. Lightfoot. Analysis of the activity of the soybean laccase encoded within the Rfs2/rhg1 locus. Current Issues in Molecular Biology (2009)
- **Yuan J, Zhu M**, Lightfoot DA, Iqbal MJ, Yang JY, Meksem K. 2008. In silico comparison of transcript abundances during Arabidopsis thaliana and Glycine max resistance to Fusarium virguliforme. BMC Genomics. 2008 2:S6.
- Kazi S., Shultz J, **Bashir, R**., Afzal J, **Njiti V**, Lightfoot D.A. 2008. Identification of loci underlying resistance to soybean sudden death syndrome in 'Hartwig' by 'Flyer'. Theoretical and Applied Genetics 116: 967-977
- Lightfoot D.A., Meksem K., **P.T. Gibson**. 2007. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants: Greenhouse Assays. US Patent #7,288,386
- Kazi S, Njiti VN, Doubler TW, Yuan J, Iqbal MJ, **Cianzio S**, Lightfoot DA. 2007. Registration of the Flyer by Hartwig Recombinant Inbred Line Mapping Population. J Plant Reg. 1: 175-178

Recent and Upcoming Publications

- Iqbal M.J. and D.A. Lightfoot. 2008. Molecular Mapping and Breeding for Biotic Stress Resistance. Chapter 16. In: Kole C, Abbott AG (eds) Principles and Practices of Plant Genomics. Volume 2: Molecular Breeding. Science Publishers, Inc, Enfield, New Hampshire; Edenbridge Ltd, Channel Islands, British Isles: pp475-495

- Lightfoot D.A., Meksem K., P.T. Gibson. 2009. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants: Polynucleotide or polypeptide isolation. US Patent pending. Filing **date 1/19/1996**

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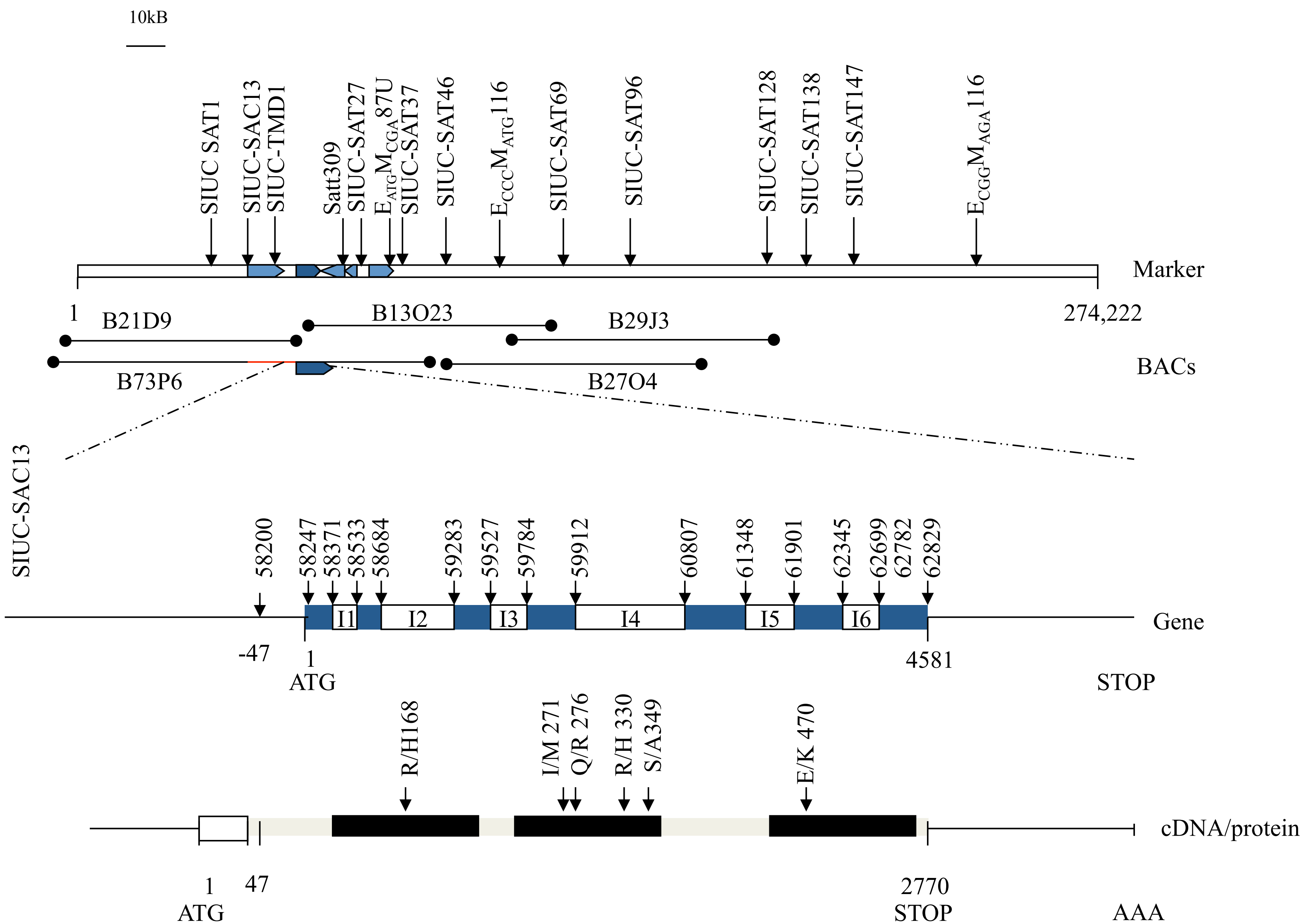
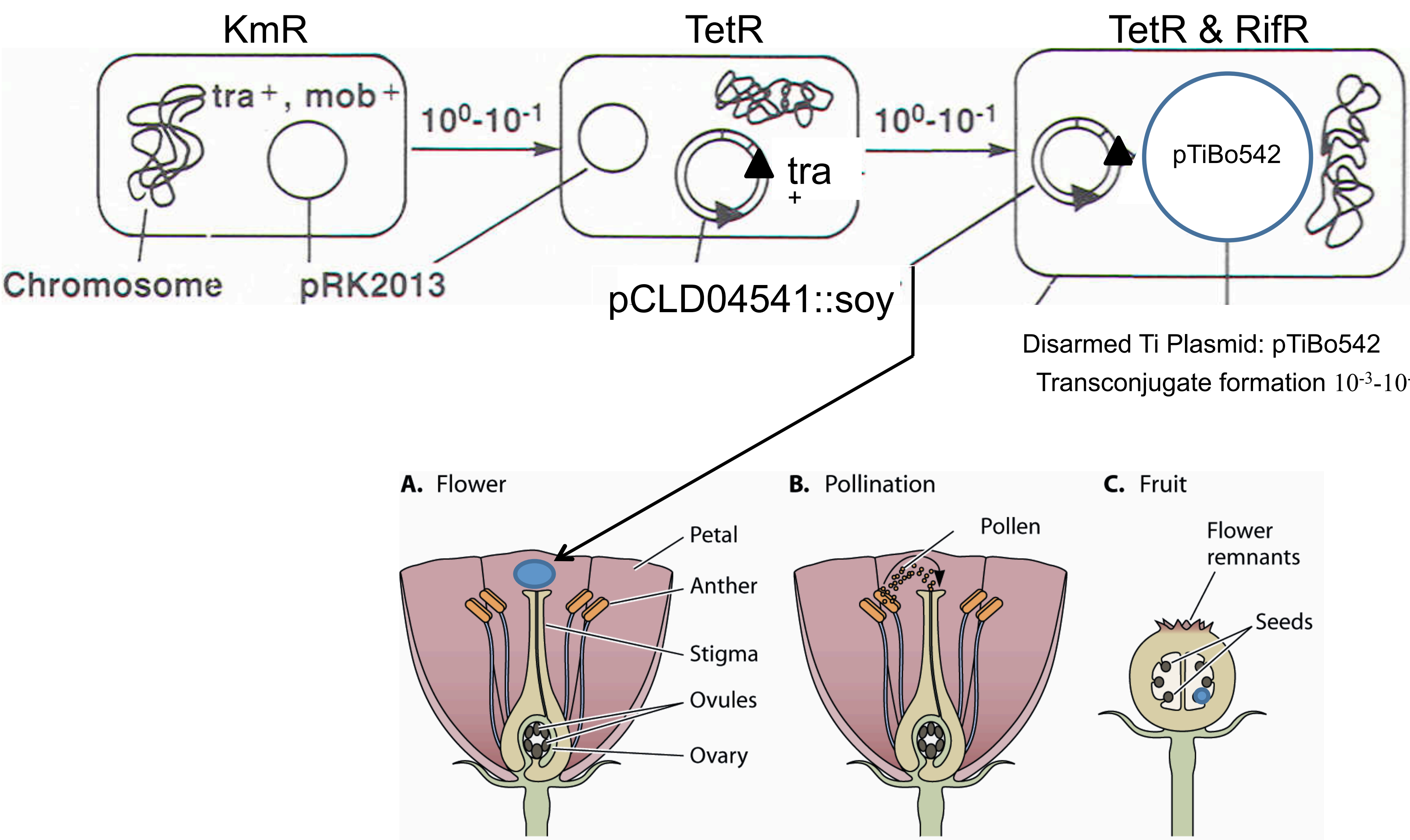
The *rhg1/Rfs2* Locus Encompassed Laccase and 4 Other Genes



Candidate Genes for *Rfs1* on B30M24

Gene Name	Coordinates (bp)		Patent/GenBank#
end rhg1 sequence Overlap	300001	8161	US 7154021 EA048112
EST Rust induced	329510	30205	EH260569
OI03	337163	37695	US 6300541 SIU patent
Phospholipase C	341927	45507	AM269882
EST SALICYLIC ACID	356505	57569	CD416083
O-acetylserine (thiol)lyase	358232	58389	EF535995
BASF stress R protein	384118	85905	WO2006032707 CS486130

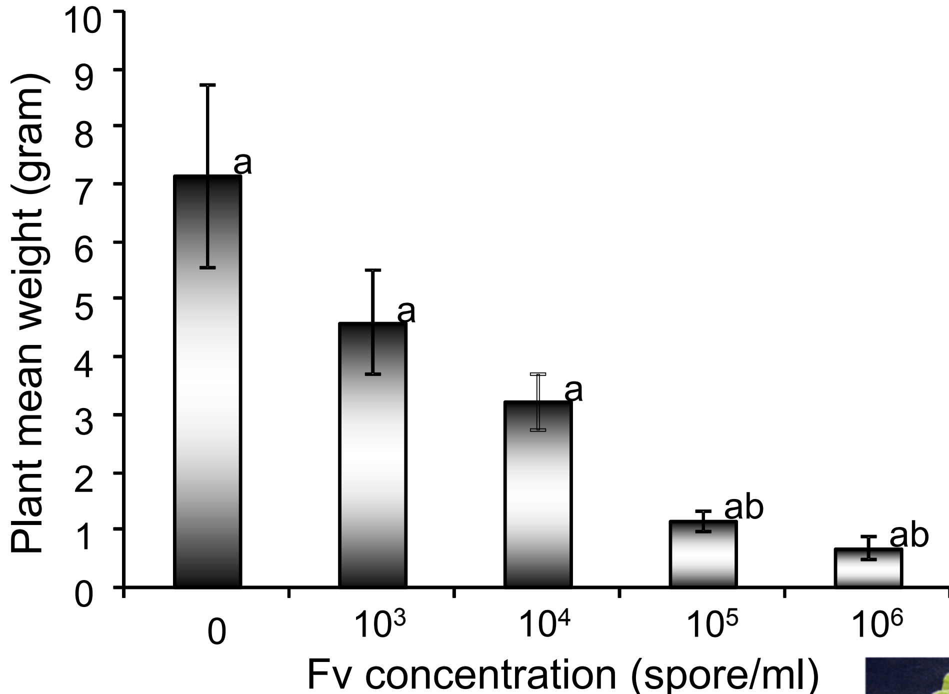
Triparental Mating Schema



Disarmed Ti Plasmid: pTiBo542

Transconjugate formation 10^{-3}-10^{-4}

Fusarium Effects on Arabidopsis and Soybean



Dose applied to transformants

Rfs1 on B30M24

minus plus

Inoculum was virulent on soybean

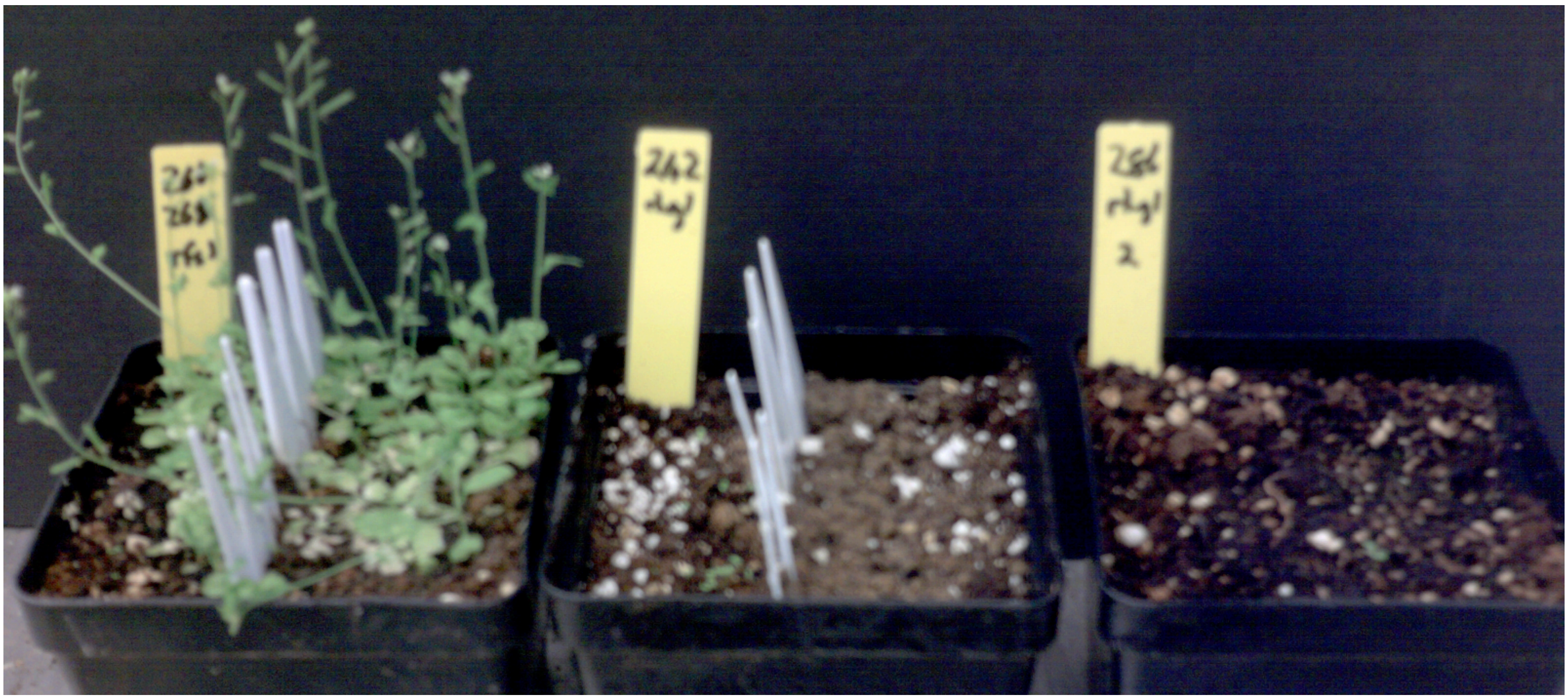
4 5 8 9 2 3 7 9

1 2 3 4 5 6 7 8 9

Root rot scoring from 1-9 does not correlate with leaf symptoms severity

Root resistance to *Fusarium*

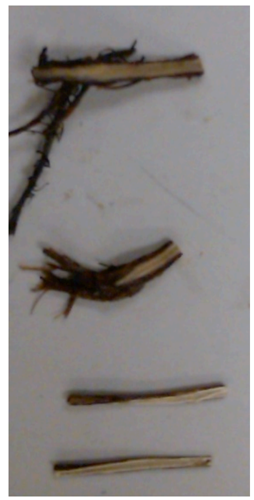
Rfs1 on B30M24 | Rhg1/Rfs2 on 73P0 | Rhg1/Rfs2 on 73P06



minus | plus | minus | plus | minus | *Fusarium*

Run 2 RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 56 dai*

Fusarium	**+**	**+**		**+**	**+**	**+**
Cultivar	**F2-3**	**F2-2**	**F2-1**	**x5RLK**	**x5**	**x5**
Gene	**+**	**-**	**+**	**+**	**-**	**-**



RR	**1.0**	**4.0**	**4.5**	**2.5**	**4.5**	**3.0**
DS	**7.0**	**2.5**	**2.0**	**3.5**	**7.0**	**9.0**