ABSTRACT: 295

SOYBEAN RESISTANCE TO *PHAKOPSORA PACHYRHIZI* AND *PHYTOPHTHORA SOJAE* THROUGH SILICON FEEDING

GUERIN V[1], DESHMUKH R[1], VIVANCOS J[1], COGLIATI E[1], GENDRON M[1], MENZIES J G[2], BELZILE[1] F & BELANGER R R[1]

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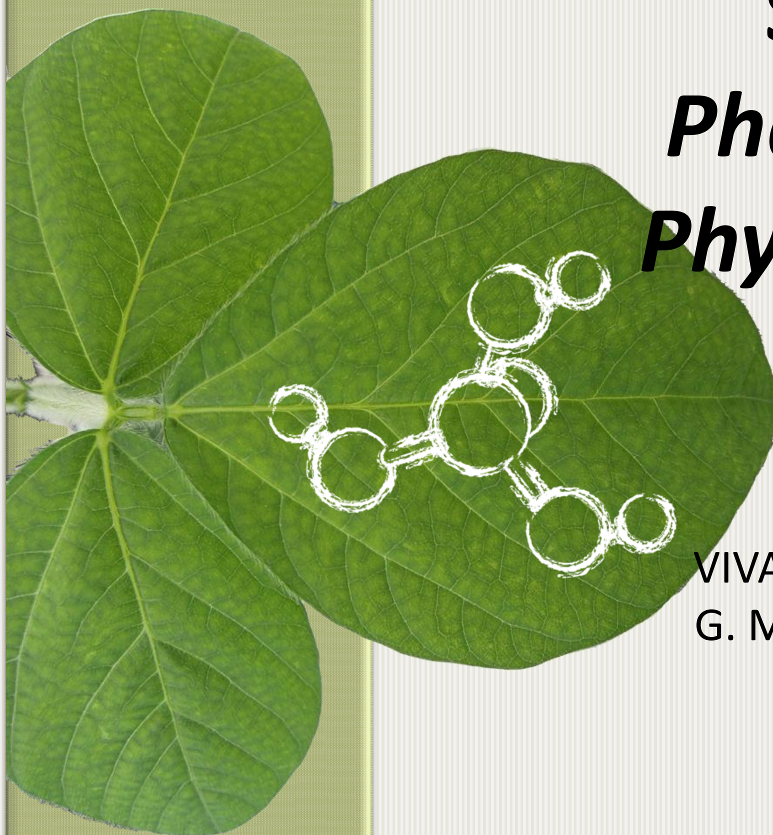
Soybean crops are constantly threatened by plant pathogens such as *Phakopsora pachyrhizi* and *Phytophthora sojae* causing soybean rust and stem and root rot, respectively. Chemical methods of control are expensive and of limited efficacy, and genetic control has only yielded partial or temporary resistance so far although many efforts are underway to characterize resistance traits. In recent months, a few reports have highlighted the potential of silicon (Si) feeding for reducing soybean rust infection, an approach easy to implement under different conditions. Si has long been known for its prophylactic properties against plant pathogens especially in the case of Si-competent plants such as rice, wheat and cucumber. Previously, soybean has rarely been tested for its response to Si feeding mainly because it was mostly considered a low Si absorber. New evidence indicates that soybean plants do possess Si transporters but that there exists a great variability among soybean cultivars in terms of their ability to absorb Si. To determine if this ability could be exploited, we tested different soybean cultivars fed, or not fed, with Si against *P. pachyrhizi* and *P. sojae*. Firstly, the results indicated that some cultivars absorbed Si in quantities as high as 1.8%, a value within the range of some Gramineae plants known as high Si absorbers. Secondly, plants supplemented with Si displayed a much higher level of resistance against both *P. pachyrhizi* and *P. sojae*, with a strong correlation between the quantity of Si absorbed and the resistance to the pathogens. This was particularly evident in the case of one cultivar that was systematically the most sensitive when tested in the absence of Si and the most resistant if fed with Si. This cultivar absorbed more Si than any other cultivar, a trait that could seemingly be exploited to increase soybean resistance to plant pathogens.



Soybean resistance to *Phakopsora pachyrhizi* and *Phytophthora sojae* through silicon feeding

Valérie GUERIN, Rupesh DESHMUKH, Julien VIVANCOS, Erik COGLIATI, Maryse GENDRON, James G. MENZIES, François BELZILE, Richard R. BELANGER

UNIVERSITÉ LAVAL

World Soybean Research Conference IX
Durban, South Africa, 2013


Carnaval de Québec

Soybean diseases

Phytophthora Root and Stem Rot of soybean (PRSR)

Phytophthora sojae

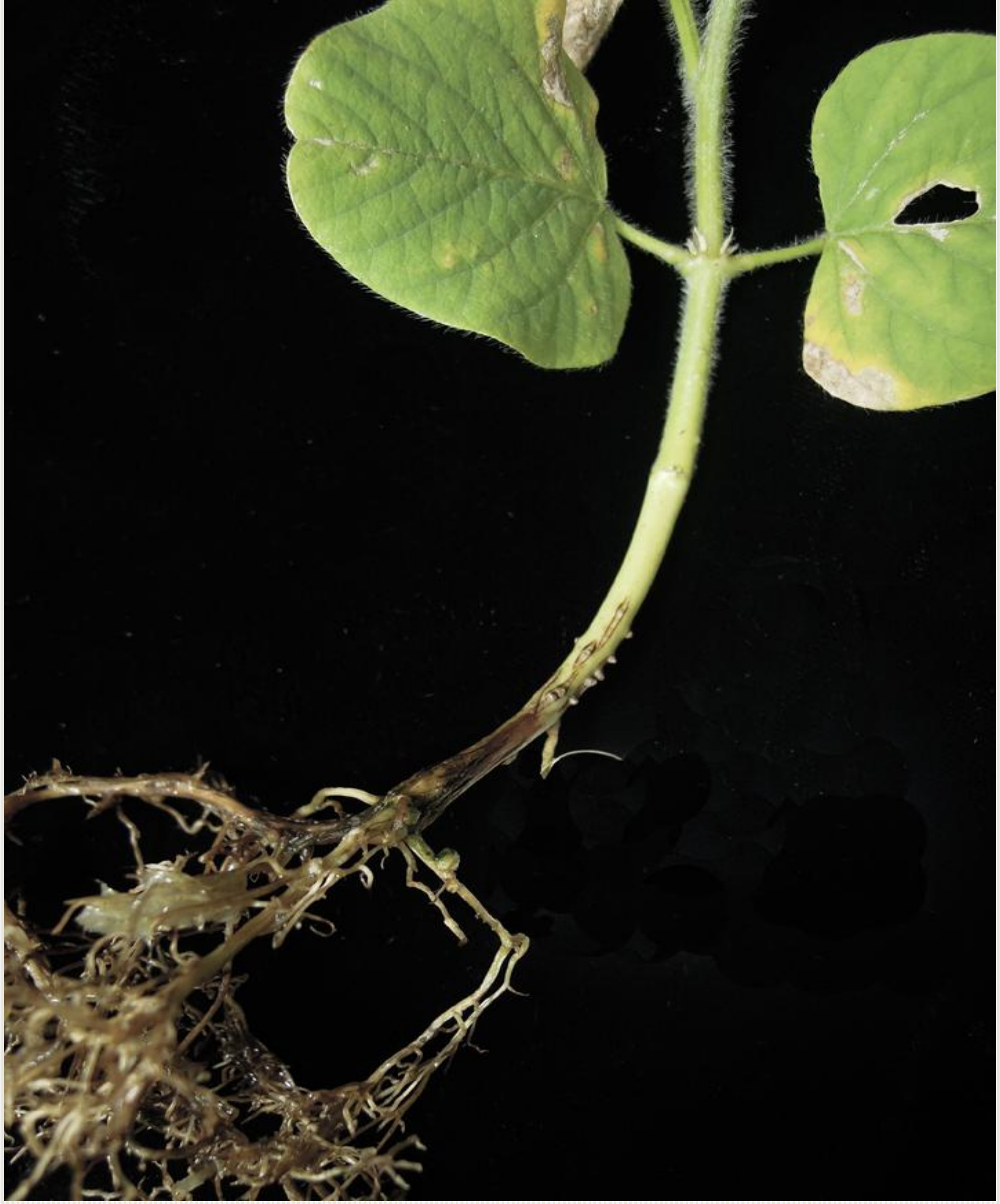
Asiatic Soybean Rust (ASR)

Phakopsora pachyrhizi

PRSR

Agent : *Phytophthora sojae*

Symptoms:



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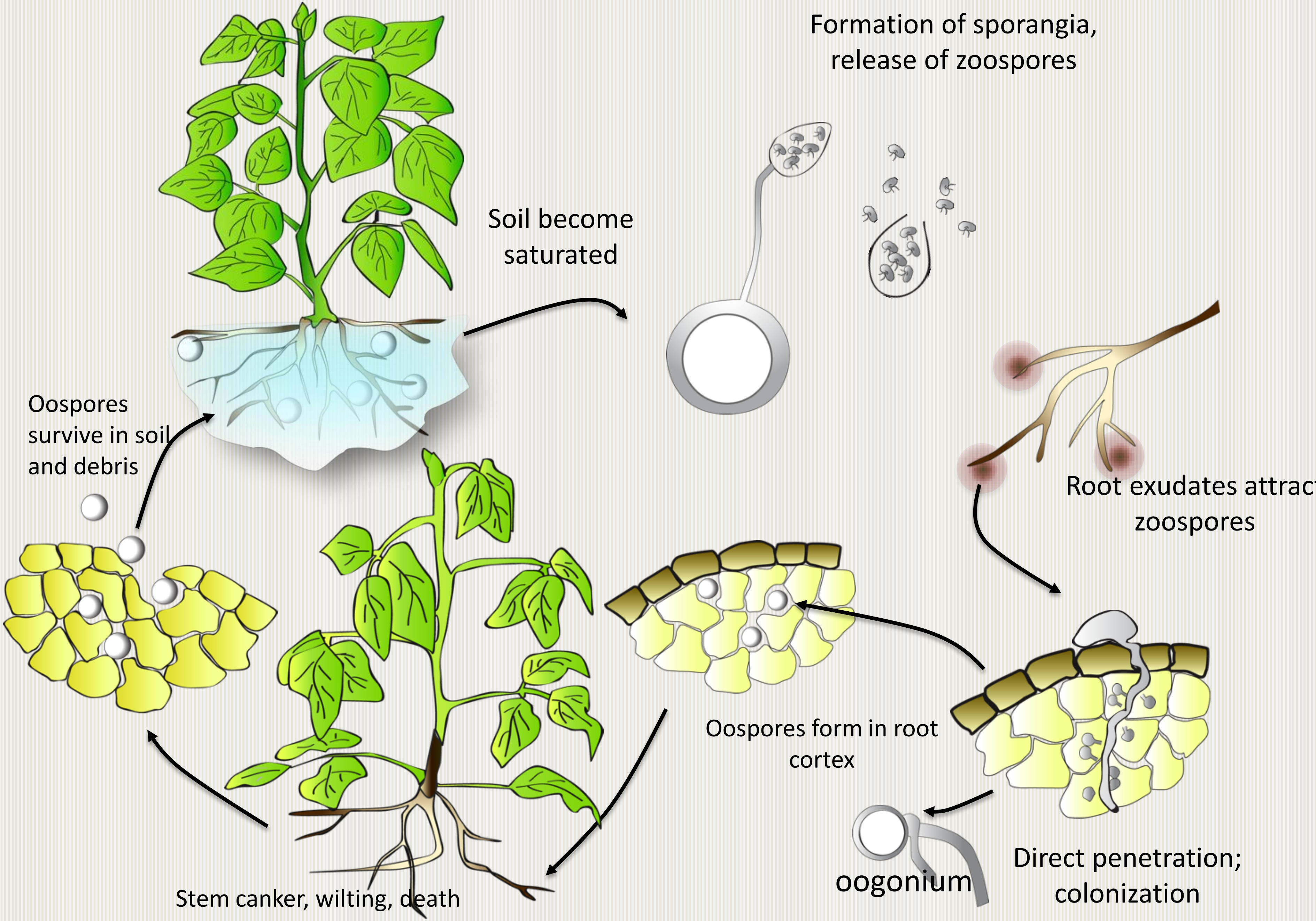


Adapted from Dorrance et al. 2007

Soybean diseases

Phytophtora Root and Stem Rot of soybean (PRSR)

Phytophthora sojae

Asiatic Soybean Rust (ASR)

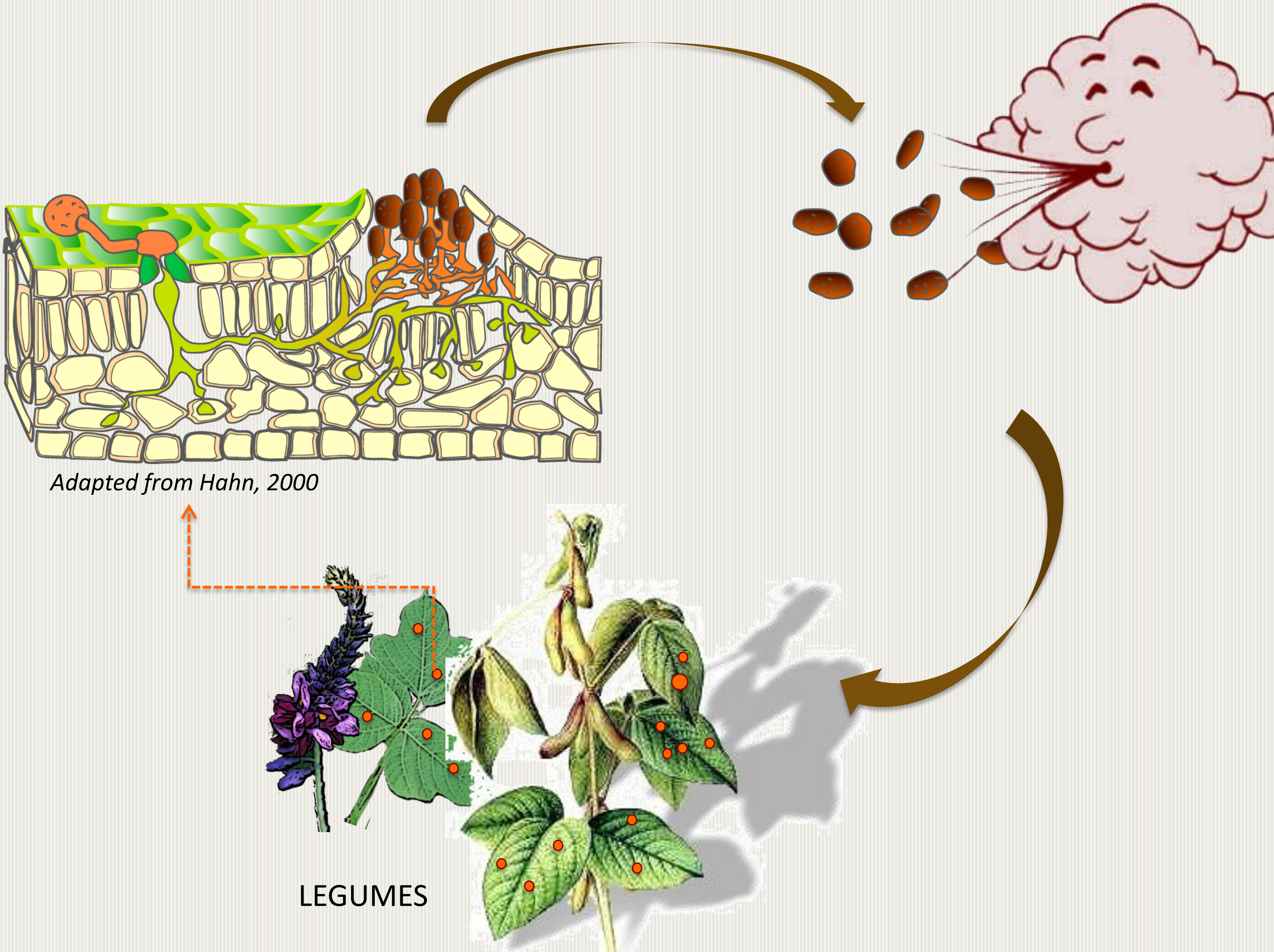
Phakopsora pachyrhizi

Asian Soybean Rust

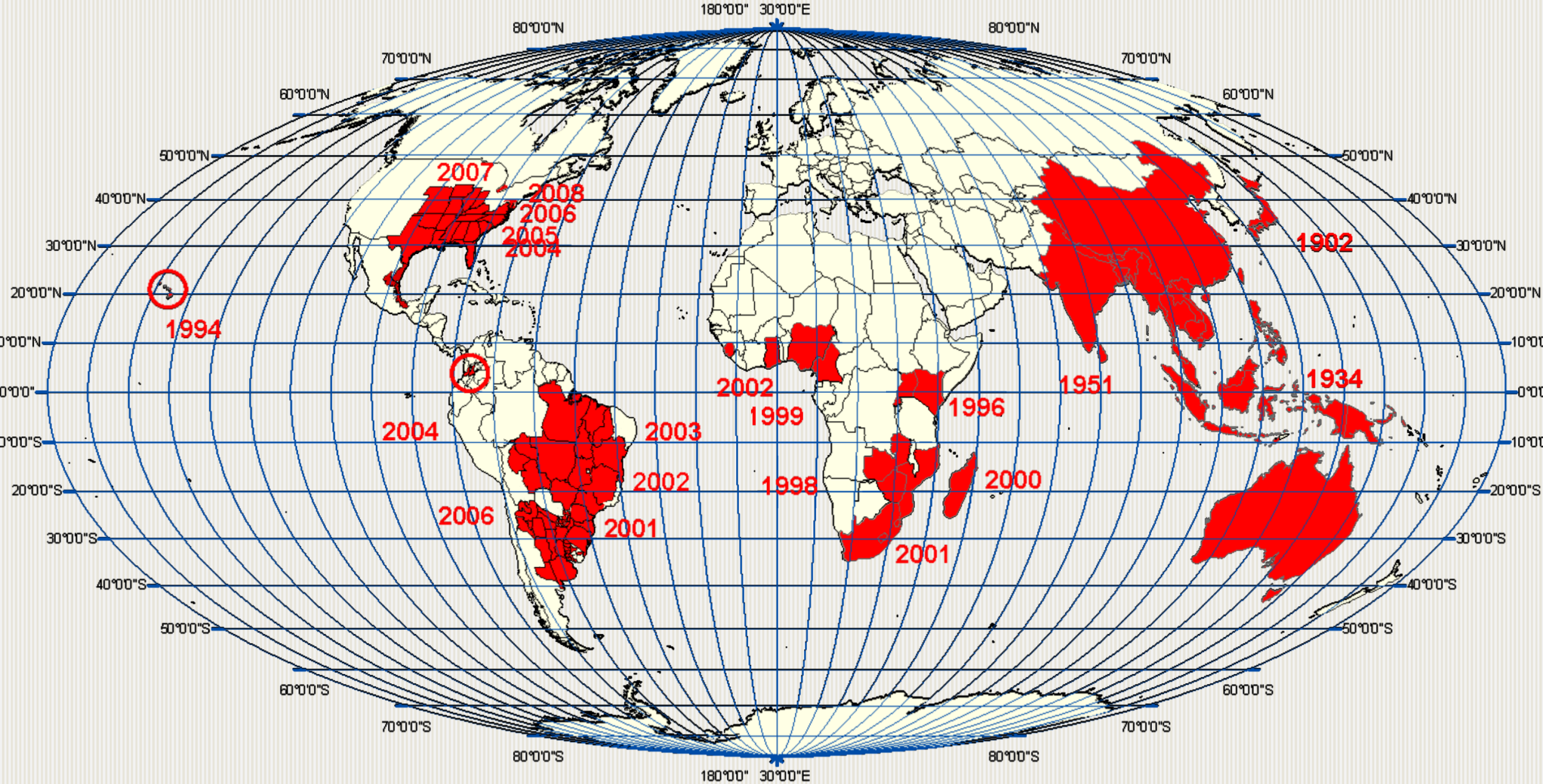
Agent : *Phakopsora pachyrhizi*

Symptoms:

http://www.scientificamerican.com/


Adapted from Hahn, 2000
LEGUMES

GLOBAL EXPANSION OF SOYBEAN RUST



Projection: Mollweide
Central Meridian: 0.00

N

0 2,500 5,000 7,500 10,000 Kilometers

Source: Bromfield K.R., Frederick R., Godoy C.V., Hartman G.L., Levy C., Miles M.

Author: Annalisa Ariatti, Penn State University

Disease management

PRSR: Gene for gene resistance used with success

BUT new races of the pathogen agent appear quickly

ASR: No known resistance

Fungicides (?)

Hypothesis

Utilization of silicon on soybean crops may help to control diseases such as PRSR and ARS

Silicon in agriculture

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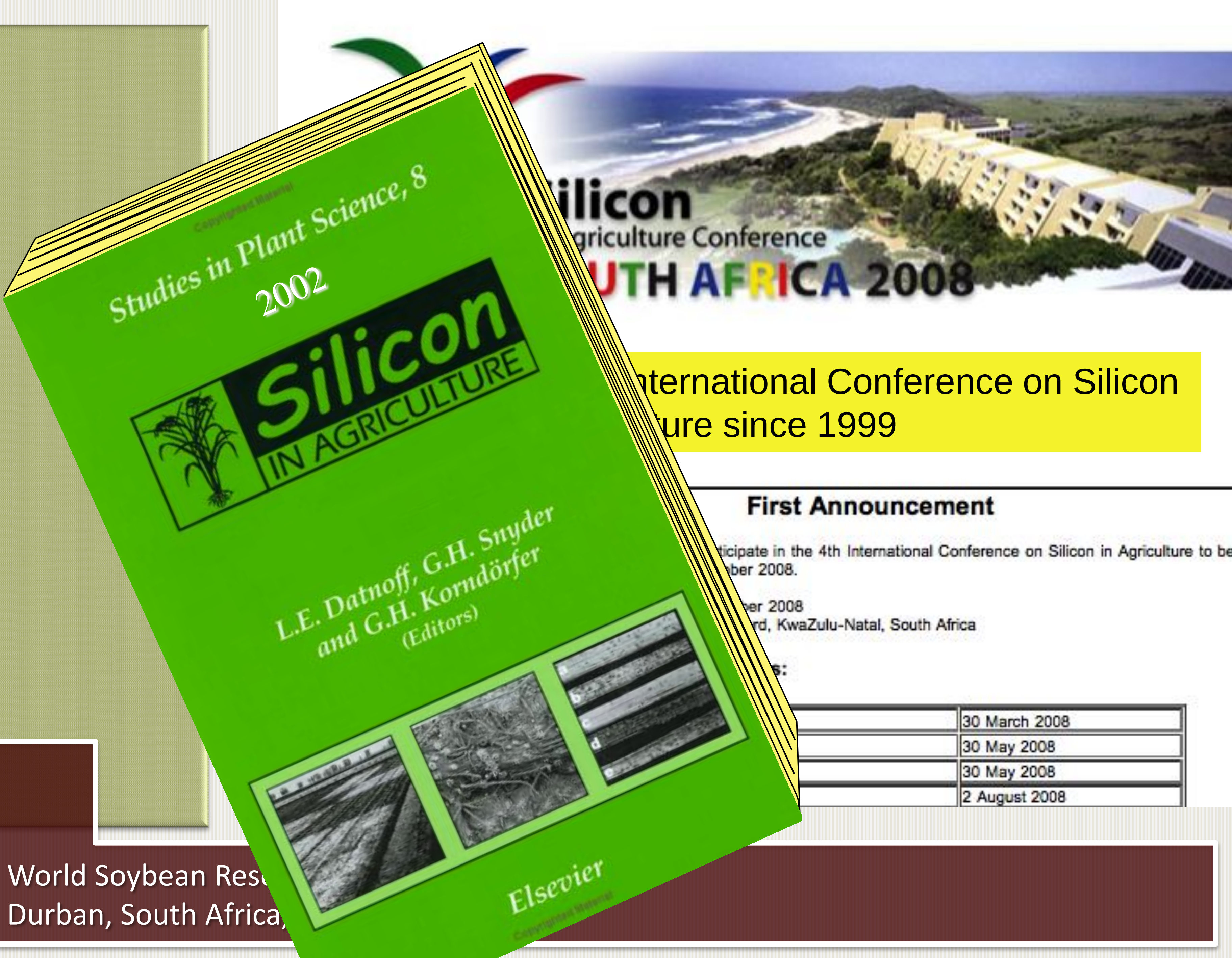

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Durban, South Africa,

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11–17, January 1994

Review

The anomaly of silicon in plant biology

Emanuel Epstein

Department of Land, Air and Water Resources, Soils and Biogeochemistry, University of California, Davis, CA 95616-8627

Contributed by Emanuel Epstein, September 15, 1993

ABSTRACT **Silicon is the second most abundant element in soils, the mineral substrate for most of the world's plant life. The soil water, or the "soil solution," contains silicon, mainly as silicic acid, H_4SiO_4, at 0.1–0.6 mM—concentrations on the order of those of potassium, calcium, and other major plant nutrients, and well in excess of those of phosphate. Silicon is readily absorbed so that terres-**

plants and, hence, of their consumers. It is the solid phase of the soil that is the reservoir of the mineral elements, both nutrient and non-nutrient, that the roots of plants ultimately draw upon. The immediate source of most of these elements, however, is the soil water, or more accurately, the soil solution, supplied by the solid phase through the processes of weathering: solution, ion ex-

element in plant matter varies greatly. The table is based on analyses of crop species, and leaf material for the most part, because that is the provenance of most published values. For any one species, the range of concentrations would be much narrower. (Values below and above those listed may be encountered, but most would fall within the ranges indicated.)

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Indirect effects of silicon on plants

- Support for cell walls
- Reduction of water loss by evapotranspiration
- Reduction in certain heavy metal toxicities
- Resistance to pest and pathogens

Consensus: prophylactic role

Beneficial in many plant/pathogen interactions

- cucumber - powdery mildew
- cucumber - *Pythium*
- rice - blast
- wheat, barley, oat - powdery mildew
- sugarcane - leaf diseases

Long English cucumber/Powdery mildew

Control

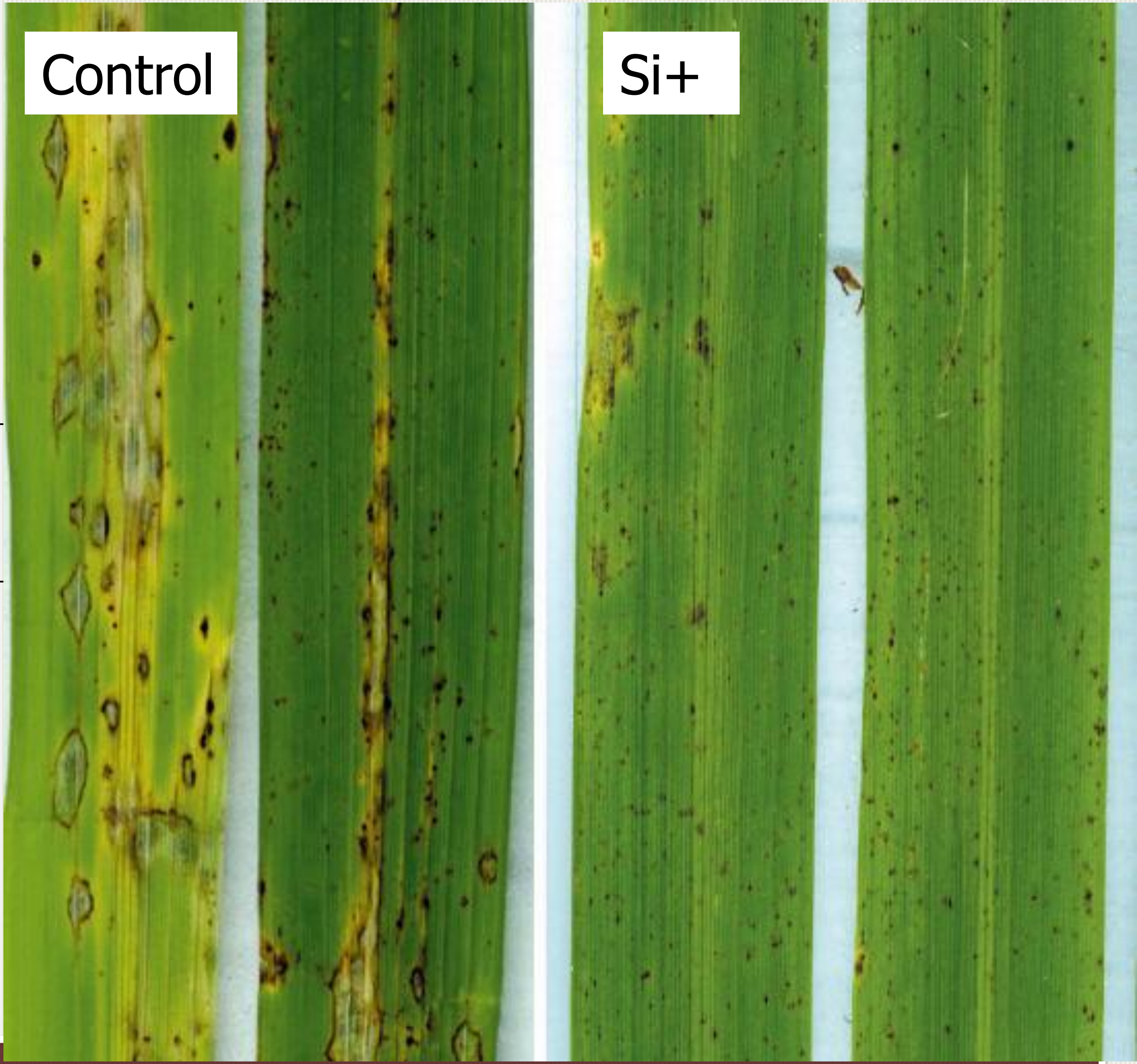
Si+

Long English cucumber -*Pythium*

Wheat-Powdery mildew

Rice-blast

(*Magnaporthe grisea*)



The enhancement of plant resistance by Si appears to be a universal response …

…as long as Si is absorbed by the plant.

How do plants absorb silicon ??

nature Vol 440|30 March 2006|doi:10.1038/nature04590

LETTERS

A silicon transporter in rice

Jian Feng Ma[1], Kazunori Tamai[1*], Naoki Yamaji[1*], Namiki Mitani[1*], Saeko Konishi[2], Maki Katsuhara[1], Masaji Ishiguro[3], Yoshiko Murata[3] & Masahiro Yano[4]

Silicon is beneficial to plant growth and helps plants to overcome abiotic and biotic stresses by preventing lodging (falling over) and increasing resistance to pests and diseases, as well as other stresses[1–3]. Silicon is essential for high and sustainable production of rice[4], but the molecular mechanism responsible for the uptake of silicon is unknown. Here we describe the Low silicon rice 1 (*Lsi1*) gene, which controls silicon accumulation in rice, a typical

13.9 kb between the markers lsi1-a and lsi1-6 (Fig. 2a, b). Using gene prediction software we predicted a gene in the candidate region (Fig. 2b), sequenced it and made comparisons between the wild type and *lsi1* mutant. We found a mutation in the DNA sequence of the candidate gene (G in the wild type; A in the mutant) that results in an amino acid change from alanine in the wild type to threonine in the mutant at position 132 (Fig. 2d). Thus, we considered this candidate

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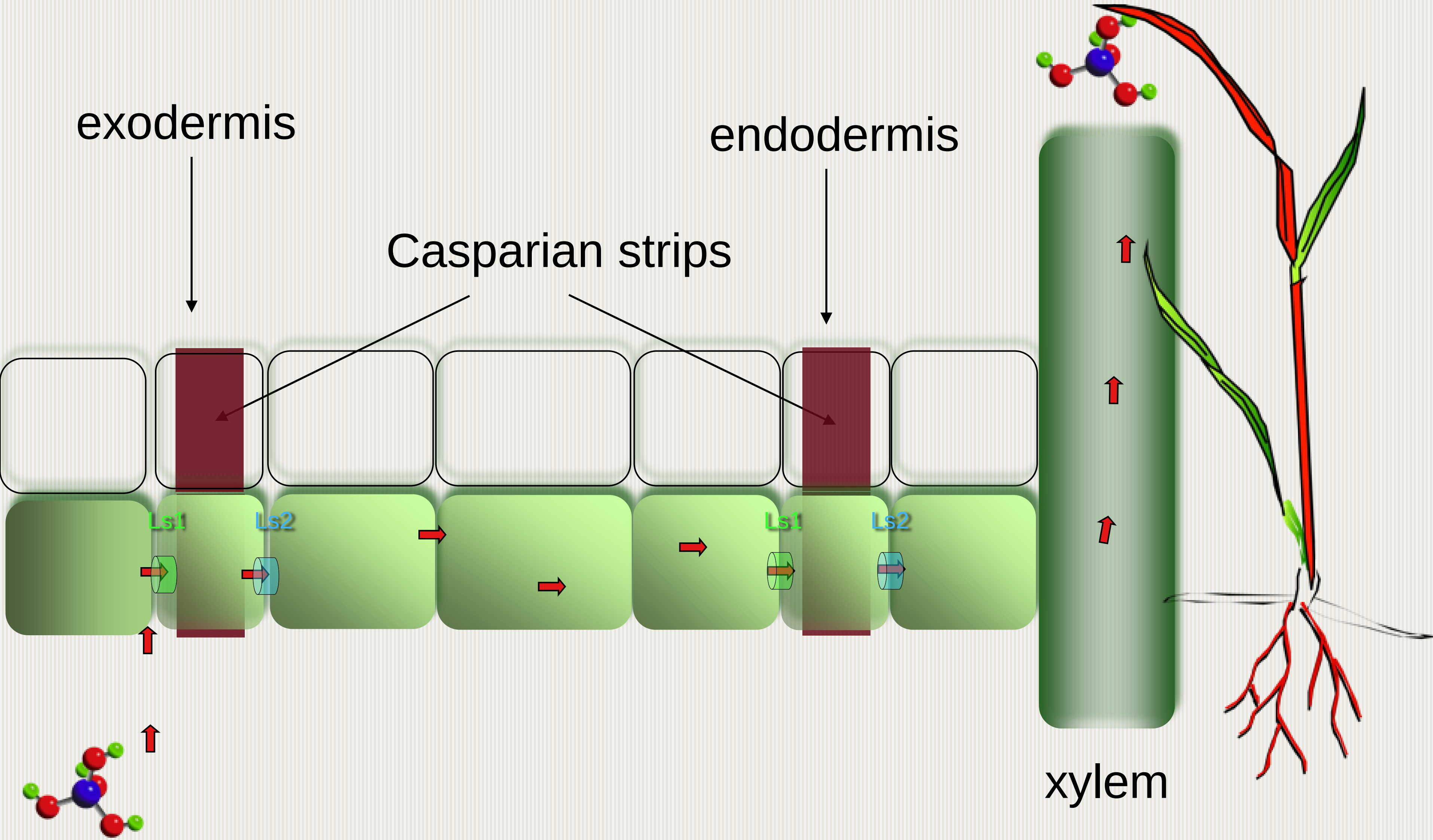


exodermis
endodermis
Casparian strips
Ls1
Ls2
Ls1
Ls2
xylem

…so what plants in agriculture can benefit from silicon?

- Plants known to absorb silicon

  Sugarcane
  Cucumber
  Rice
  Wheat
  Banana

What about soybean?

nature International weekly journal of science

Search

Article

Nature **463**, 178-183 (14 January 2010) | doi:10.1038/nature08670; Received 19 August 2009; Accepted 12 November 2009

There is a Corrigendum (6 May 2010) associated with this document.

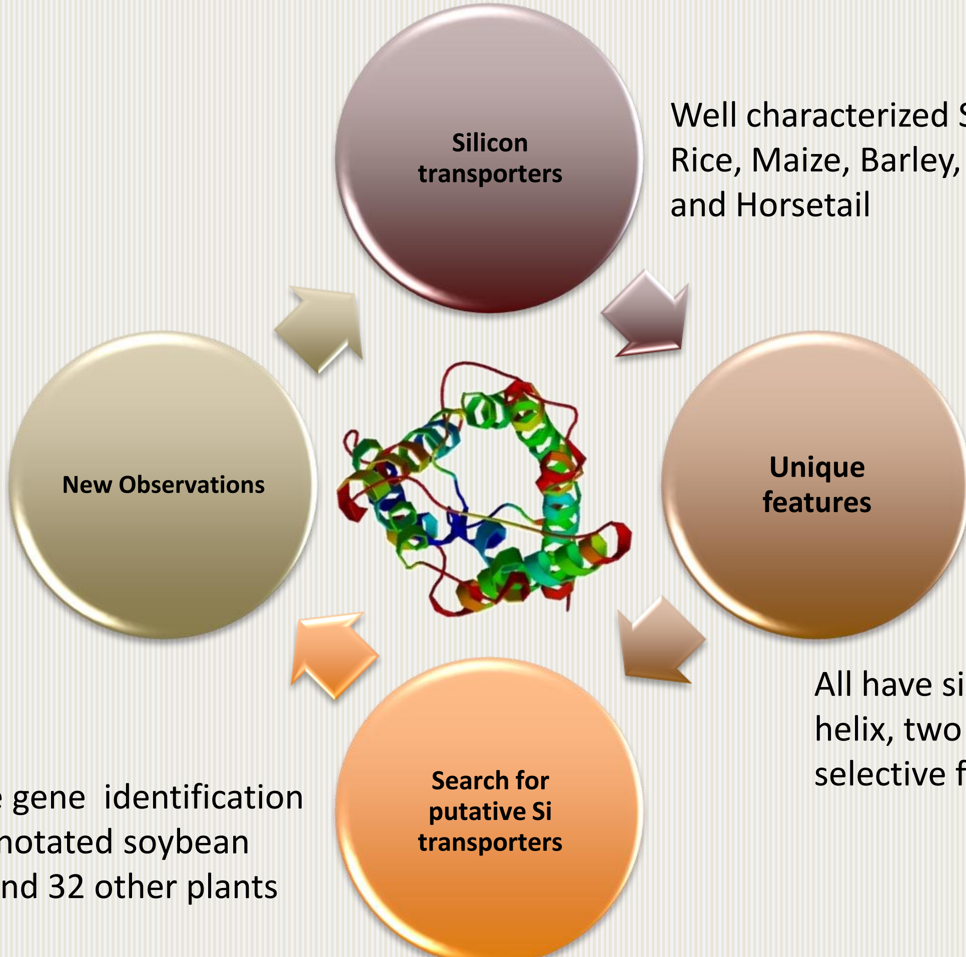
Genome sequence of the palaeopolyploid soybean

Jeremy Schmutz[1,2], Steven B. Cannon[3], Jessica Schlueter[4,5], Jianxin Ma[5], Therese Mitros[6], William Nelson[7], David L. Hyten[8], Qijian Song[8,9], Jay J. Thelen[10], Jianlin Cheng[11], Dong Xu[11], Uffe Hellsten[2], Gregory D. May[12], Yeisoo Yu[13], Tetsuya Sakurai[14], Taishi Umezawa[14], Madan K. Bhattacharyya[15], Devinder Sandhu[16], Babu Valliyodan[17], Erika Lindquist[2], Myron Peto[3], David Grant[3], Shengqiang Shu[2], David Goodstein[2], Kerrie Barry[2], Montona Futrell-Griggs[5], Brian Abernathy[5], Jianchang Du[5], Zhixi Tian[5], Liucun Zhu[5], Navdeep Gill[5], Trupti Joshi[11], Marc Libault[17], Anand Sethuraman[1], Xue-Cheng Zhang[17], Kazuo Shinozaki[14], Henry T. Nguyen[17], Rod A. Wing[13], Perry Cregan[8], James Specht[18], Jane Grimwood[1,2], Dan Rokhsar[2], Gary Stacey[10,17], Randy C. Shoemaker[3] & Scott A. Jackson[5]

1. HudsonAlpha Genome Sequencing Center, 601 Genome Way, Huntsville, Alabama 35806, USA
2. Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California 94598, USA
3. USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, Iowa 50011, USA

Does soybean possess Si transporters?

Attributes to be an active silicon transporter protein



Well characterized Si transporters in Rice, Maize, Barley, Wheat, Cucumber, and Horsetail

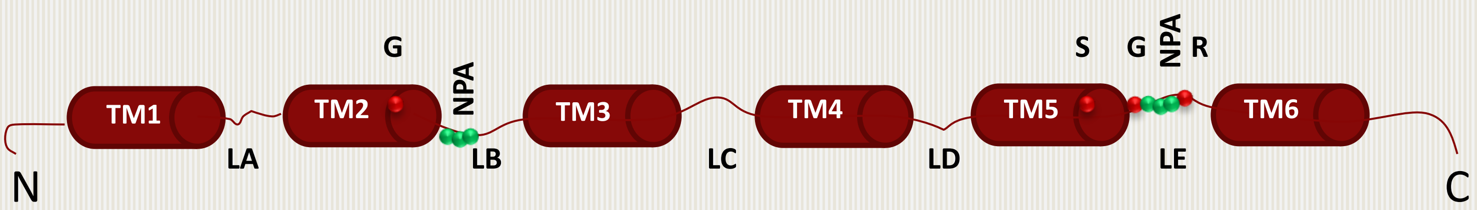
All have six trans-membrane helix, two NPA domain, GSGR selective filter,

Candidate gene identification in well annotated soybean genome and 32 other plants

Study of several features in known and putative genes

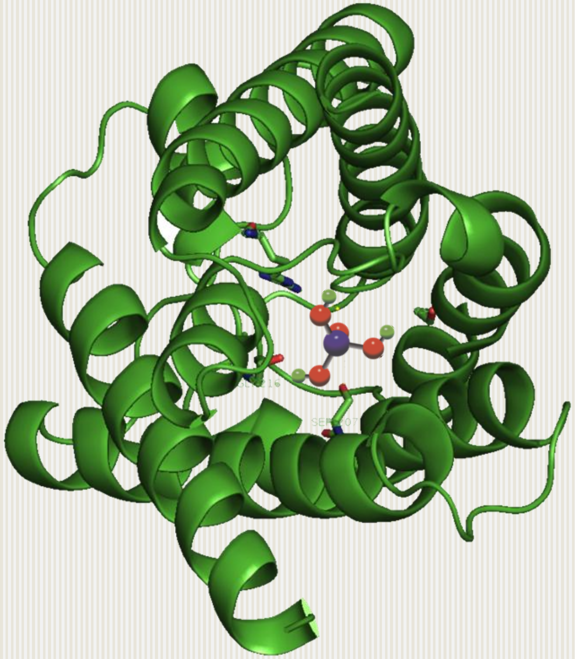
Characterized silicon transporter genes in different plant species

Plant species	Si transporter	Features	Reference
Monocots			
Rice	OsLsi1	NPA –NPA, GSGR, 6TM	Ma et al., 2006
Wheat	TaLsi1	NPA –NPA, GSGR, 6TM	Montpetit et al., 2012
Barley	HvLsi1	NPA –NPA, GSGR, 6TM	Chiba et al., 2008
Maize	ZmLsi1	NPA –NPA, GSGR, 6TM	Mitani et al., 2009
Dicots			
Pumpkin	CmLsi1	NPA –NPV, GSGR, 6TM	Mitani et al.,2011
Primitive plants			
Horsetail	EaNIP3,2; EaNIP3,4a; …….	NPA –NPA, STAR, 6TM	Grégoire et al., 2012



Prot	Gene	Loops		Pore			
GmNIP1-2	Glyma14g35030	NPA	NPA	S	V	A	R
GmNIP1-3	Glyma07g34150	NPA	NPA	W	V	A	R
GmNIP1-5	Glyma02g41400	NPA	NPA	W	V	A	R
GmNIP1-6	Glyma14g07560	NPA	NPA	W	V	A	R
GmNIP1-7	Glyma05g29500	NPA	NPV	W	V	A	R
GmNIP1-8	Glyma08g12650	NPA	NPA	W	V	A	R
GmNIP1-9	Glyma05g29510	NPA	NPA	W	V	A	R
GmNIP1-10	Glyma08g12660	NPA	NPA	W	V	A	R
GmNIP1-11	Glyma13g29690	NPA	NPA	W	V	A	R
GmNIP1-12	Glyma15g09370	NPA	NPA	W	V	A	R
GmNIP2-1	**Glyma09g37280**	**NPA**	**NPA**	**G**	**S**	**G**	**R**
GmNIP2-2	**Glyma18g49410**	**NPA**	**NPA**	**G**	**S**	**G**	**R**
GmNIP3-1	Glyma15g00620	NPA	NPV	T	I	G	R
GmNIP3-2	Glyma08g23230	NPA	NPV	N	I	S	R
GmNIP3-5	Glyma10g36560	NPS	NPV	A	I	G	R
GmNIP4-1	Glyma02g15870	NPA	NPA	A	V	G	R
GmNIP4-2	Glyma10g03870	NPA	NPA	A	V	G	R

Is it functional?



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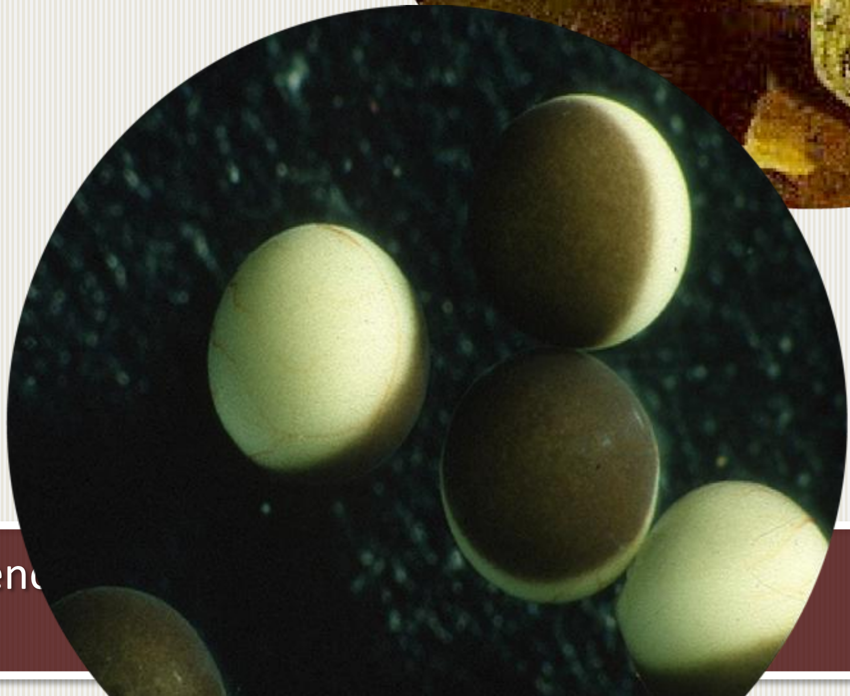
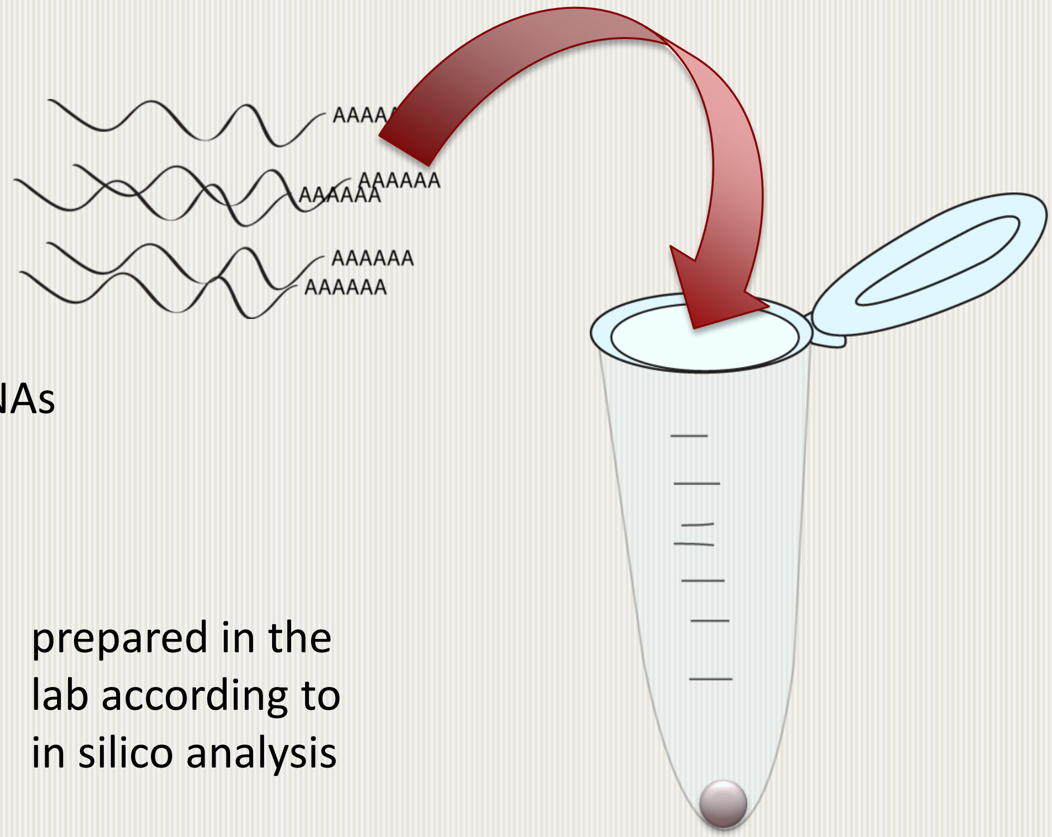
Protein functionality

Heterologous expression of TaLsi1 in *Xenopus* oocytes



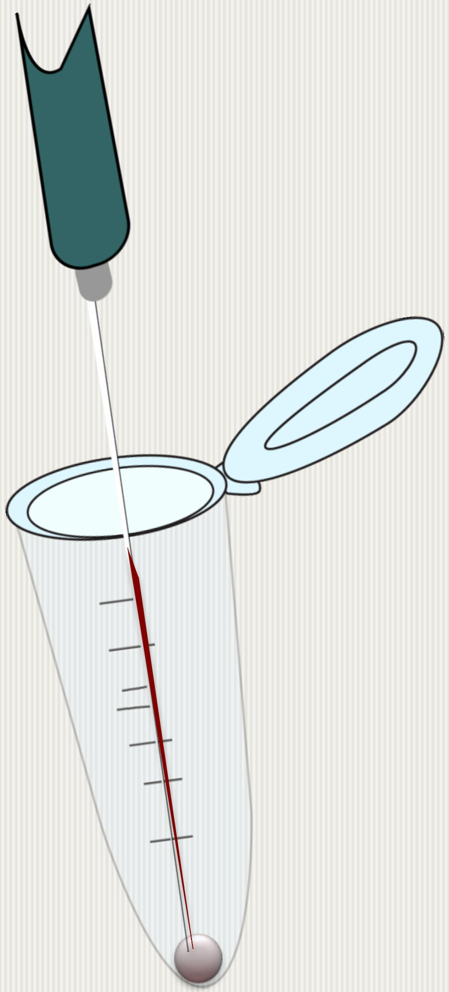
Functional analysis



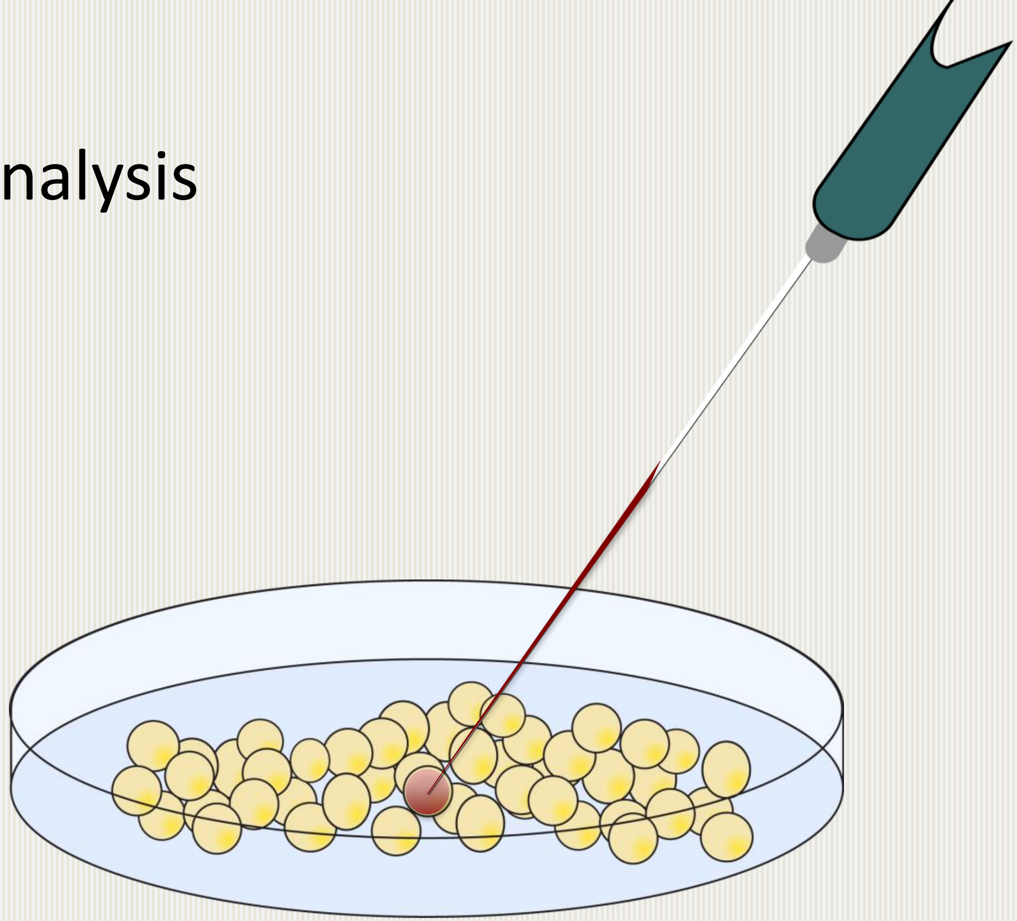
mRNAs

prepared in the lab according to in silico analysis

Functional analysis



Functional analysis



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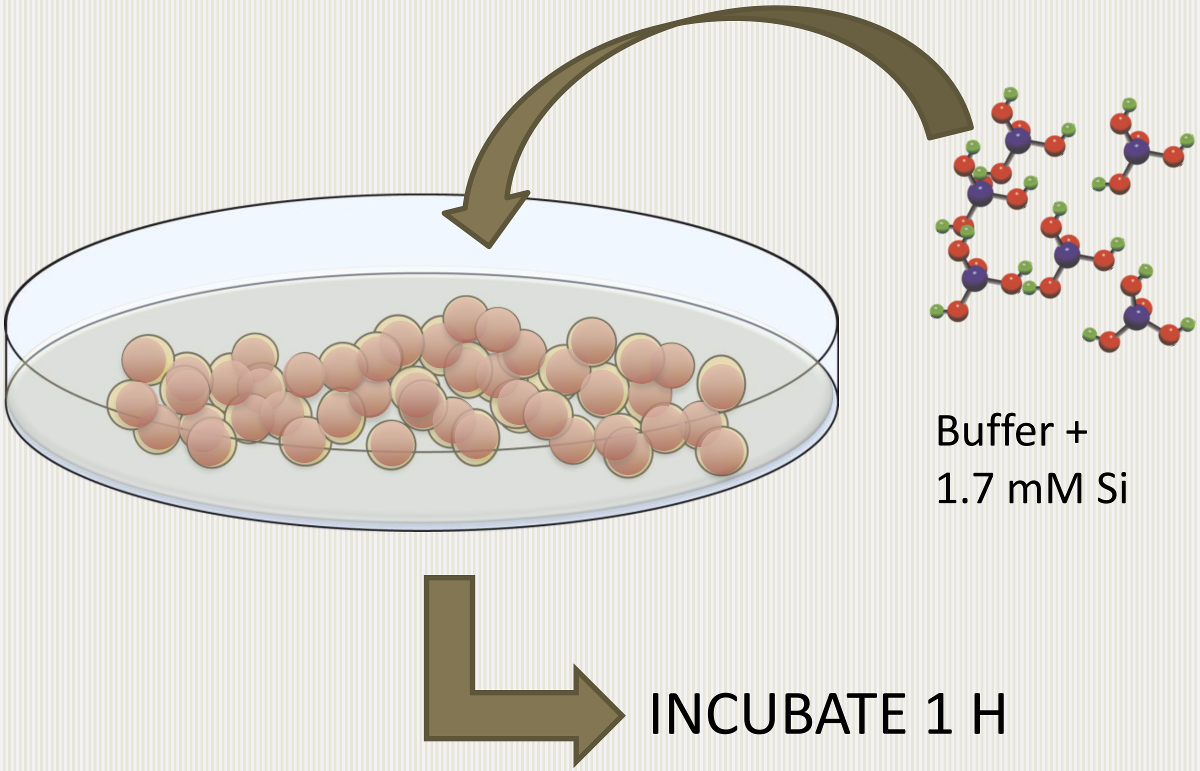
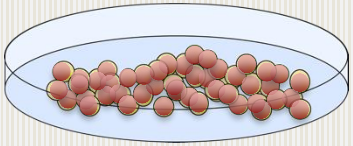
Functional analysis



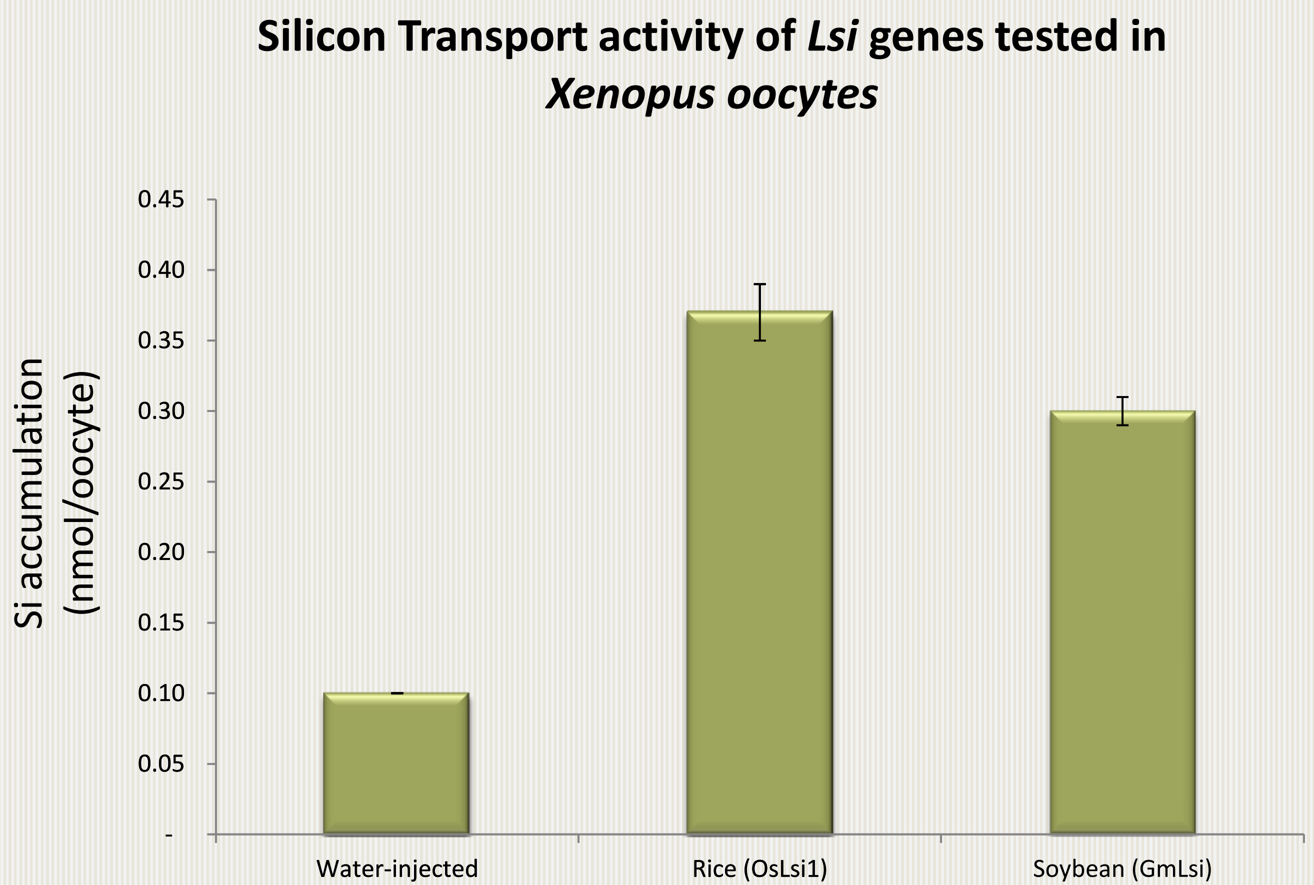
Functional analysis

Zeeman atomic spectrometer



MEASURE OF THE SILICON CONTENT IN OOCYTES

RESULTS



Silicon Transport activity of *Lsi* genes tested in *Xenopus oocytes*
Si accumulation (nmol/oocyte)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
-
Water-injected
Rice (OsLsi1)
Soybean (GmLsi)

Evaluation of Si uptake in different soybean cvs and its effect on disease resistance

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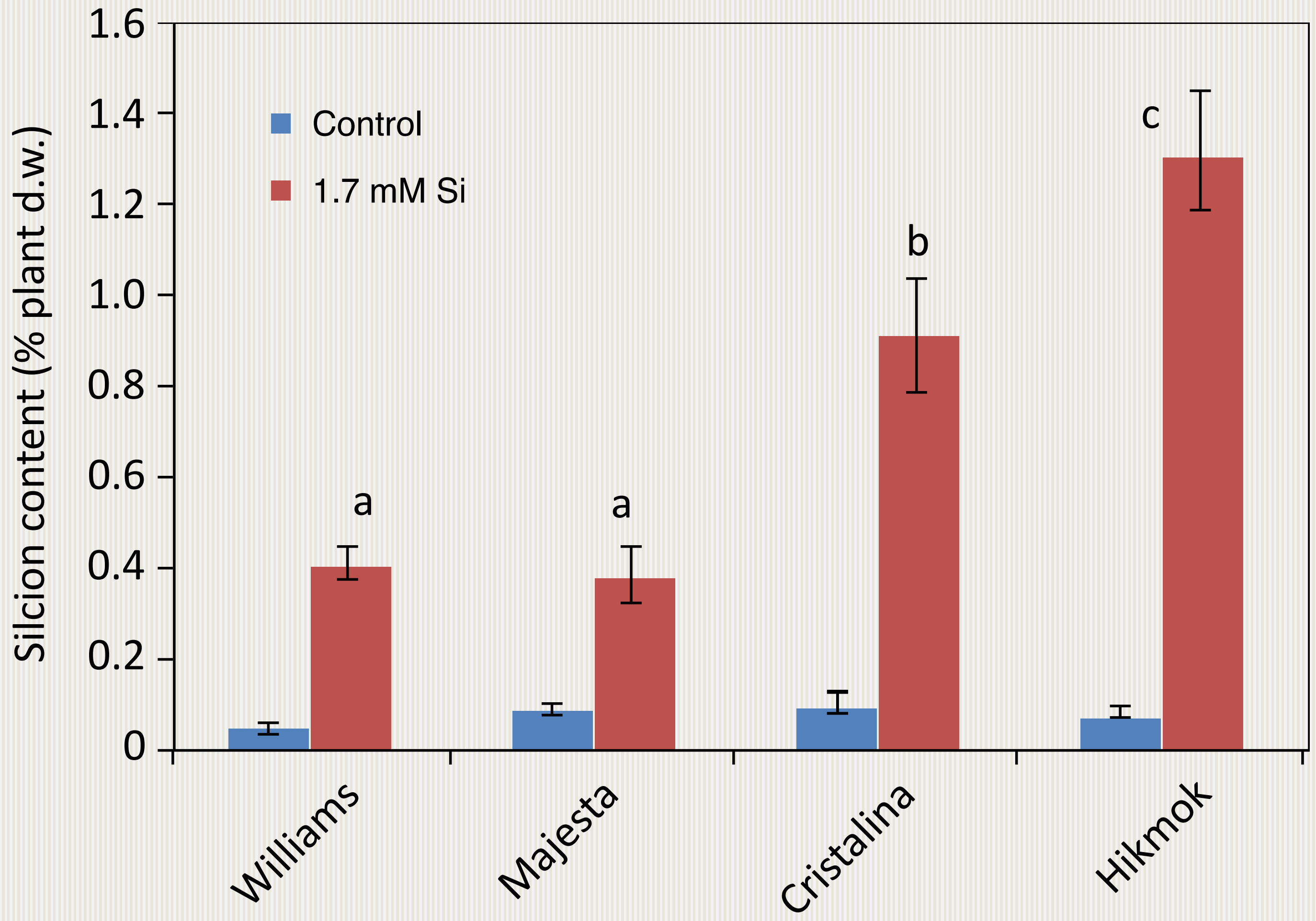
Silicon concentration in shoots of soybean plants treated with potassium-silicate amended solutions (1.7 mM Si).



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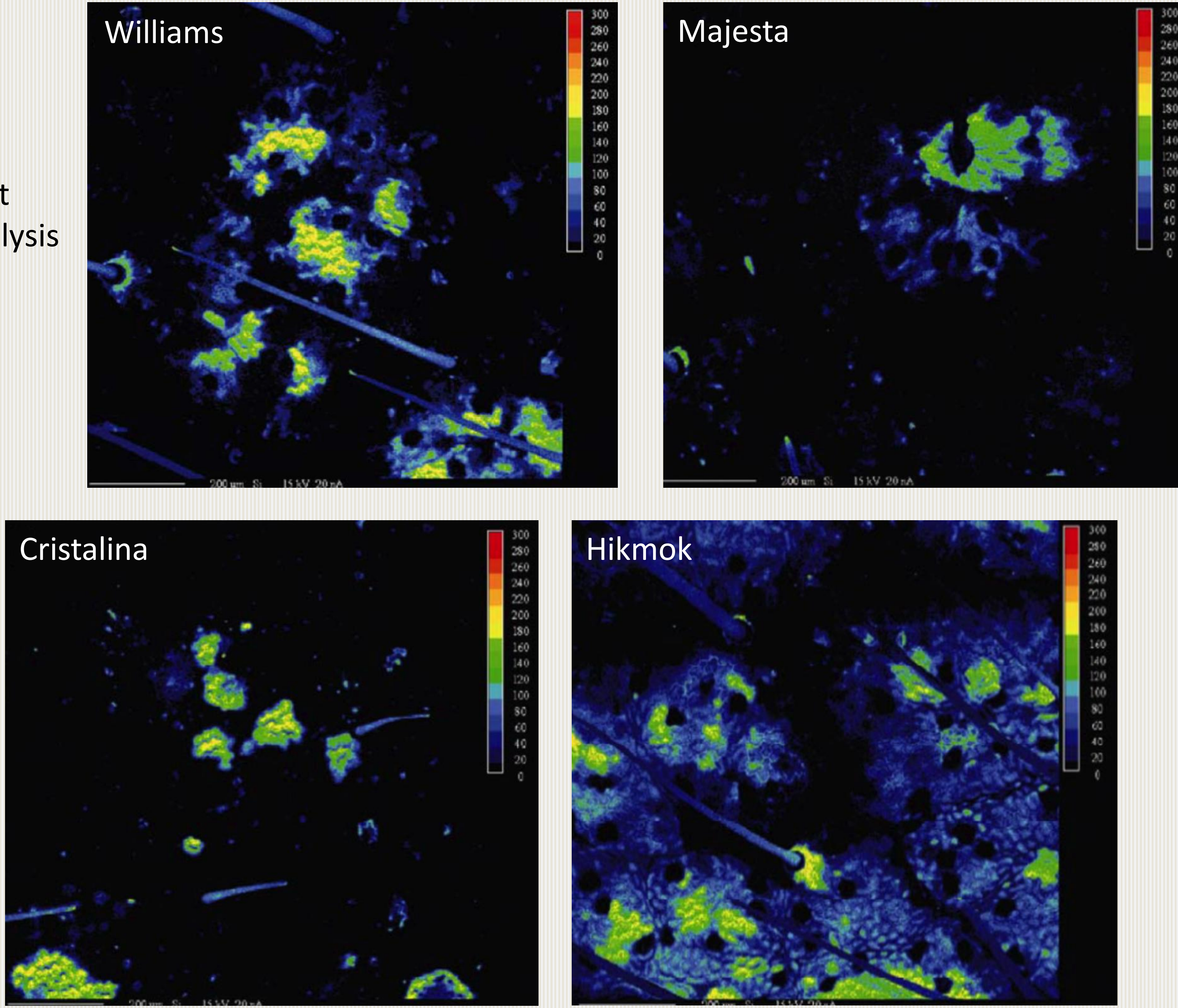
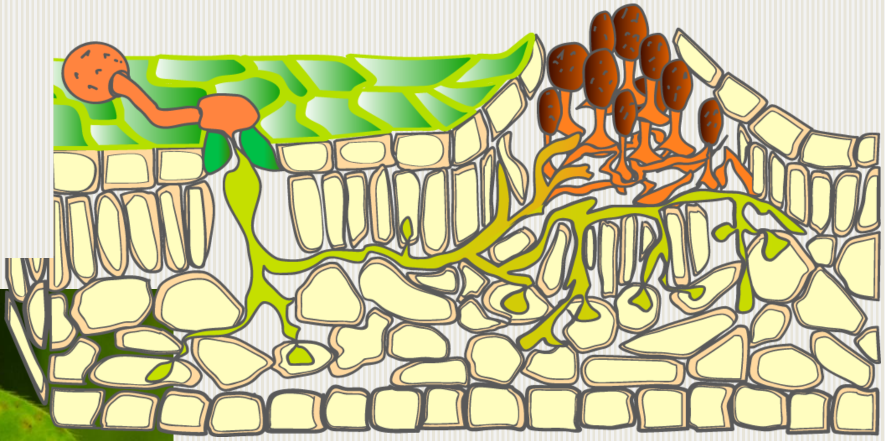
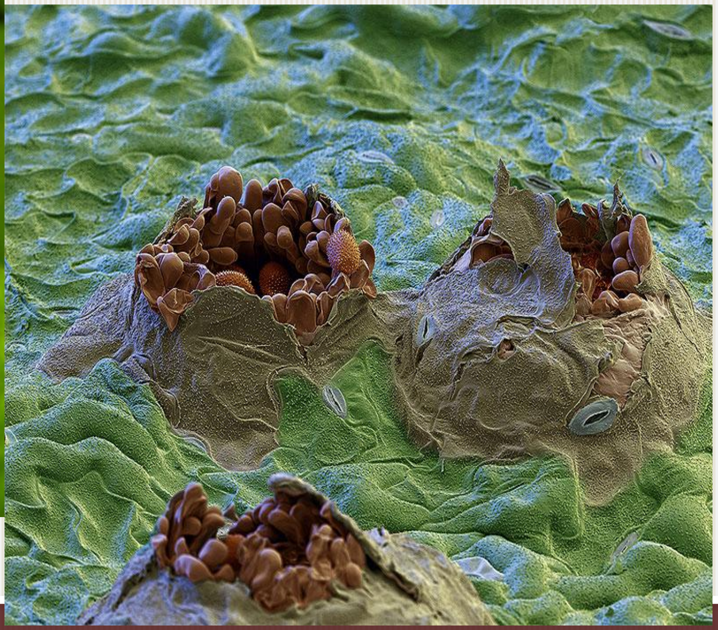
Si content microanalysis



Effects of Si on soybean rust



Adapted from Hahn, 2000



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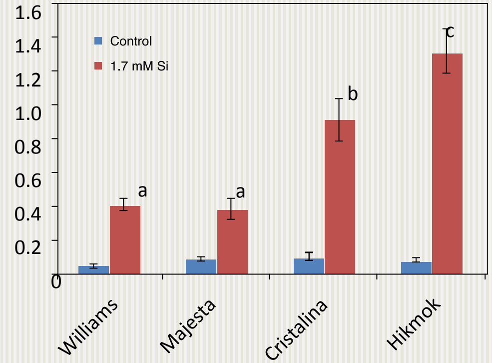
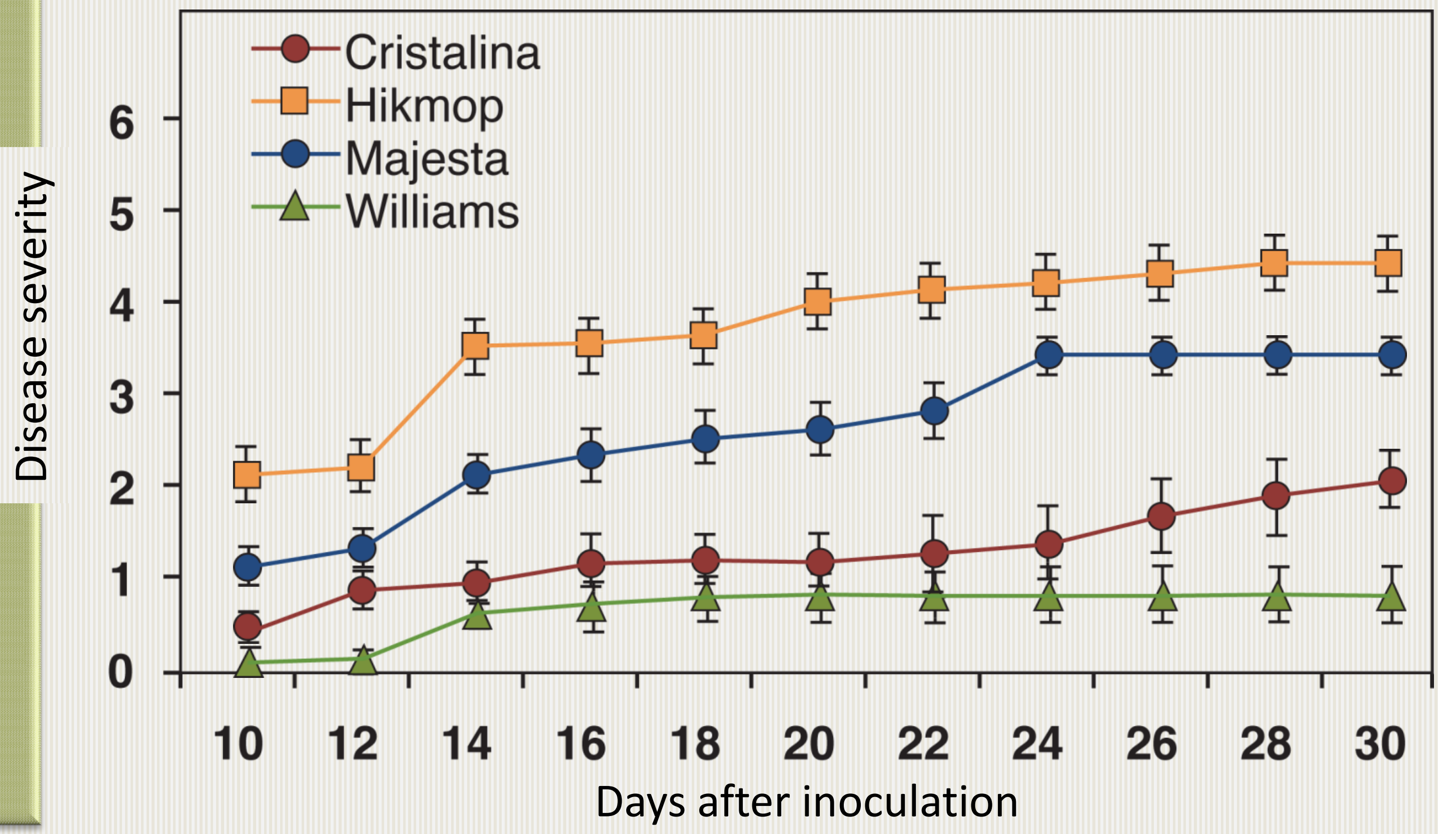
ASR Evaluation



WITHOUT…



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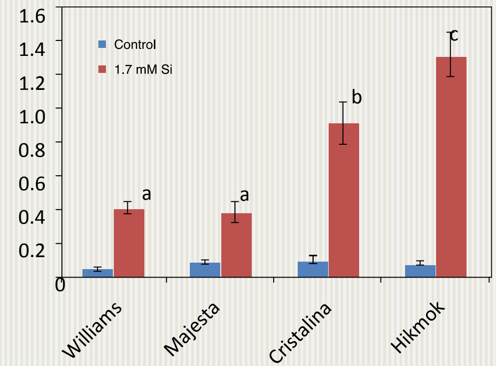
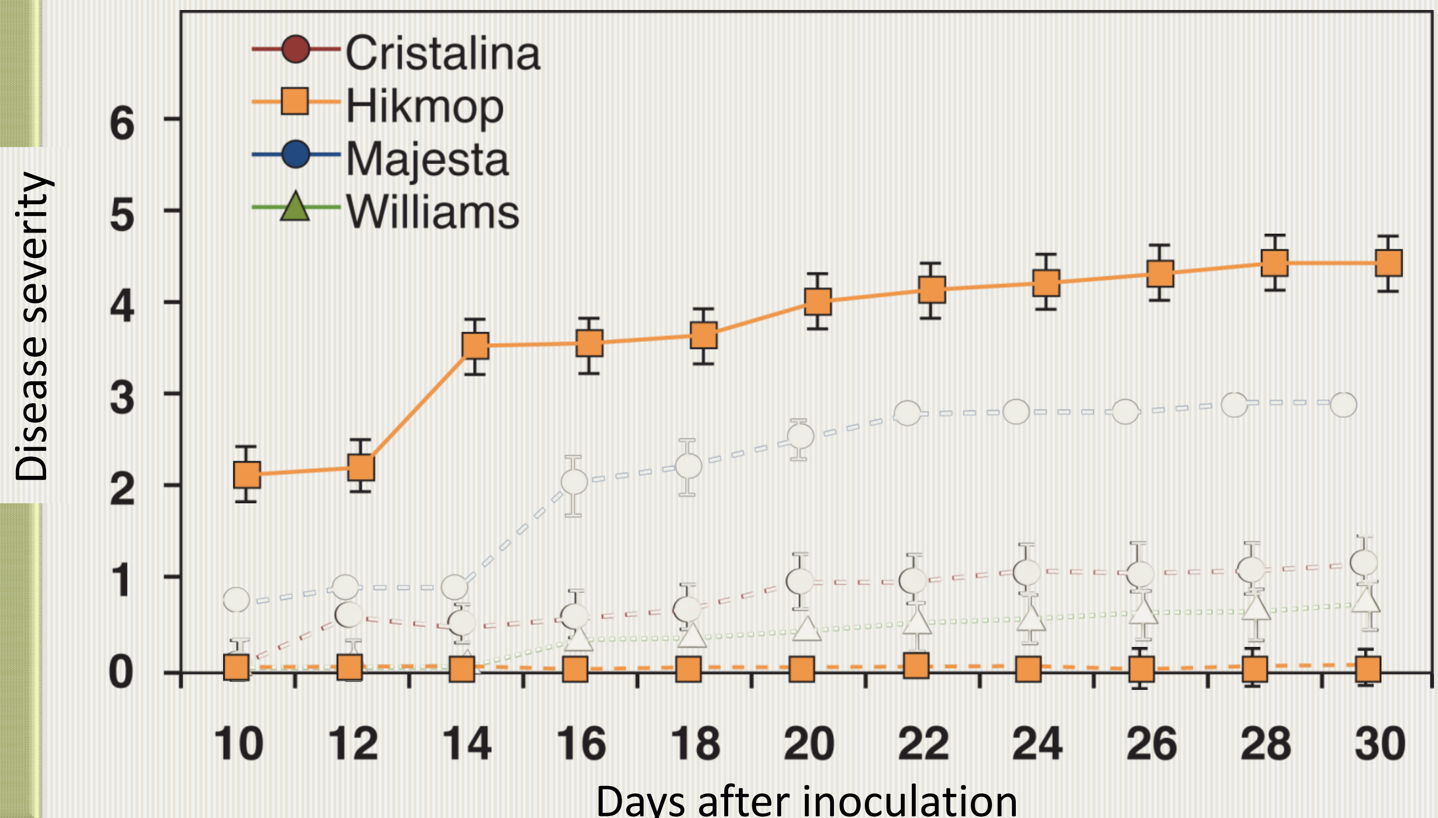
ASR Evaluation

… OR WITH SI





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DISEASE ASSESSMENT :ASR

HIKMOK Si-

HIKMOK Si+

Effect of Silicon Absorption on Soybean Resistance to *Phakopsora pachyrhizi* in Different Cultivars

Geneviève Arsenault-Labrecque, Département de Phytologie, Centre de Recherche en Horticulture, Université Laval, Québec, Canada G1V 0A6; **James G. Menzies,** Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB, Canada R3T 2M9; and **Richard R. Bélanger,** Département de Phytologie, Centre de Recherche en Horticulture, Université Laval, Québec, Canada G1V 0A6

Abstract

Arsenault-Labrecque, G., Menzies, J. G., and Bélanger, R. R. 2012. Effect of silicon absorption on soybean resistance to *Phakopsora pachyrhizi* in different cultivars. Plant Dis. 96:37-42.

Silicon (Si) is recognized for its prophylactic role in alleviating diseases when absorbed by plants and has been proposed as a possible solution against soybean rust, caused by *Phakopsora pachyrhizi*. However, little is known about its potential effects on soybean (*Glycine max*) because the plant's ability to absorb Si is poorly defined. In this work, our objectives were to evaluate and quantify the absorption of Si in leaves of different soybean cultivars and to determine if such absorption was able to enhance resistance to soybean rust. In a first set of experiments with cv. Williams 82, hydroponic plants were supplied or not with Si and inoculated with urediniospores of *P. pachyrhizi*. Chemical analyses revealed no significant differences in the plants' Si content regardless of the treatment, which translated into no effect on rust incidence. However, in a second set of experiments with different cultivars, plants of Korean cultivar Hikmok sorip absorbed nearly four times more Si than those of Williams 82. At the same time, plants from this cultivar exhibited a near absence of disease symptoms when supplied with Si. This resistance appeared to be the result of hypersensitive (HR) reactions that were triggered when plants were fed with Si. These results support the concept that a plant's innate ability to absorb Si will dictate the benefits conferred by a treatment with Si and provide evidence that Si can protect soybean plants against soybean rust through mediated resistance.

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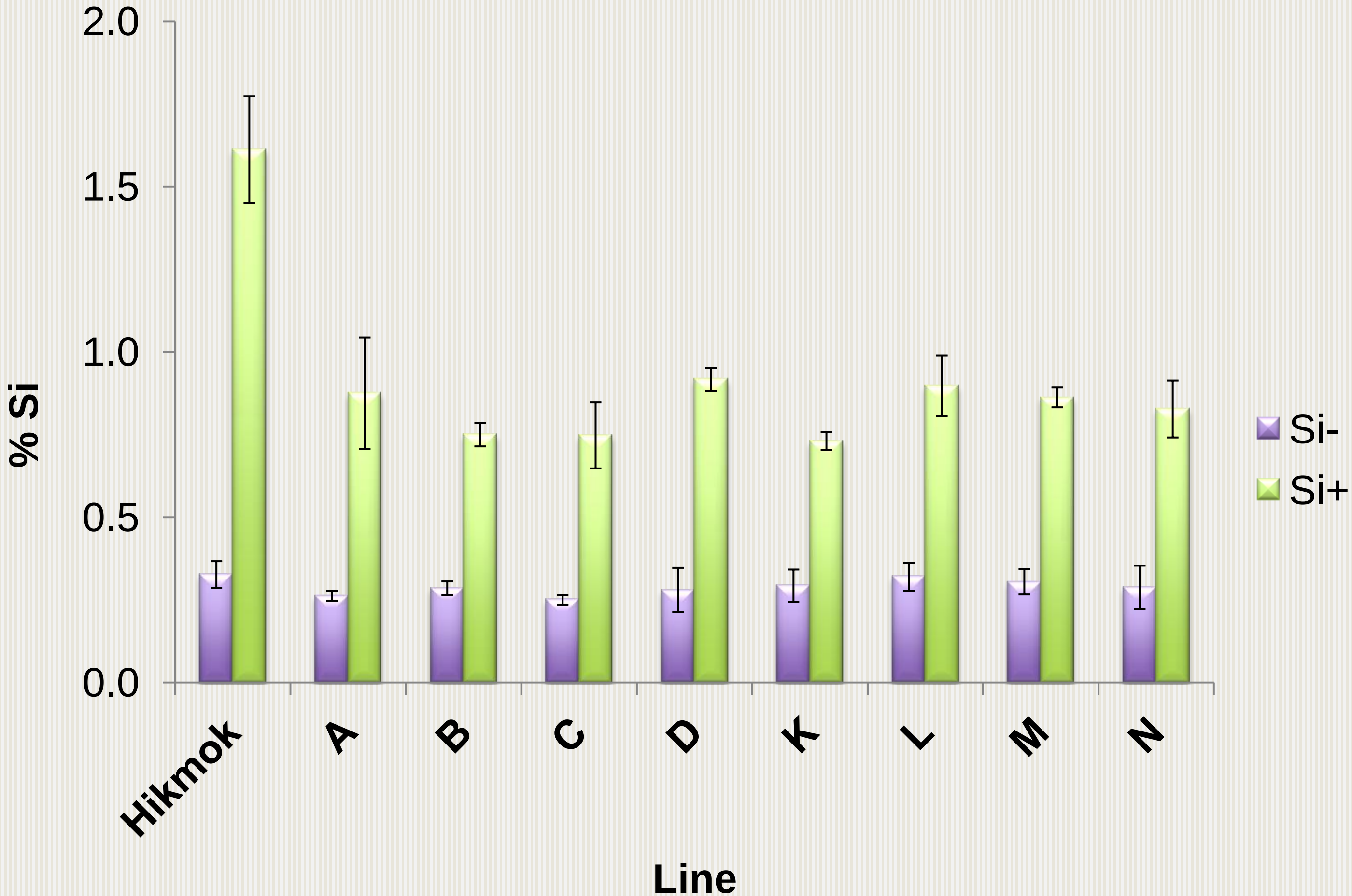
Effect of Si uptake against *Phytophthora sojae* in soybean

Stem and root rot
(*Phytophthora sojae*)

❑ Evaluate Si-competent soybean plants' tolerance to PRSR

Plant	• Soybean ○ Hikmok ○ UL-soybean lines
Disease	• Stem and root rot (*Phytophthora sojae*)

❑ Evaluation of Si content in different soybean lines



Evaluation of Si-competent soybean plants' tolerance to PRSR

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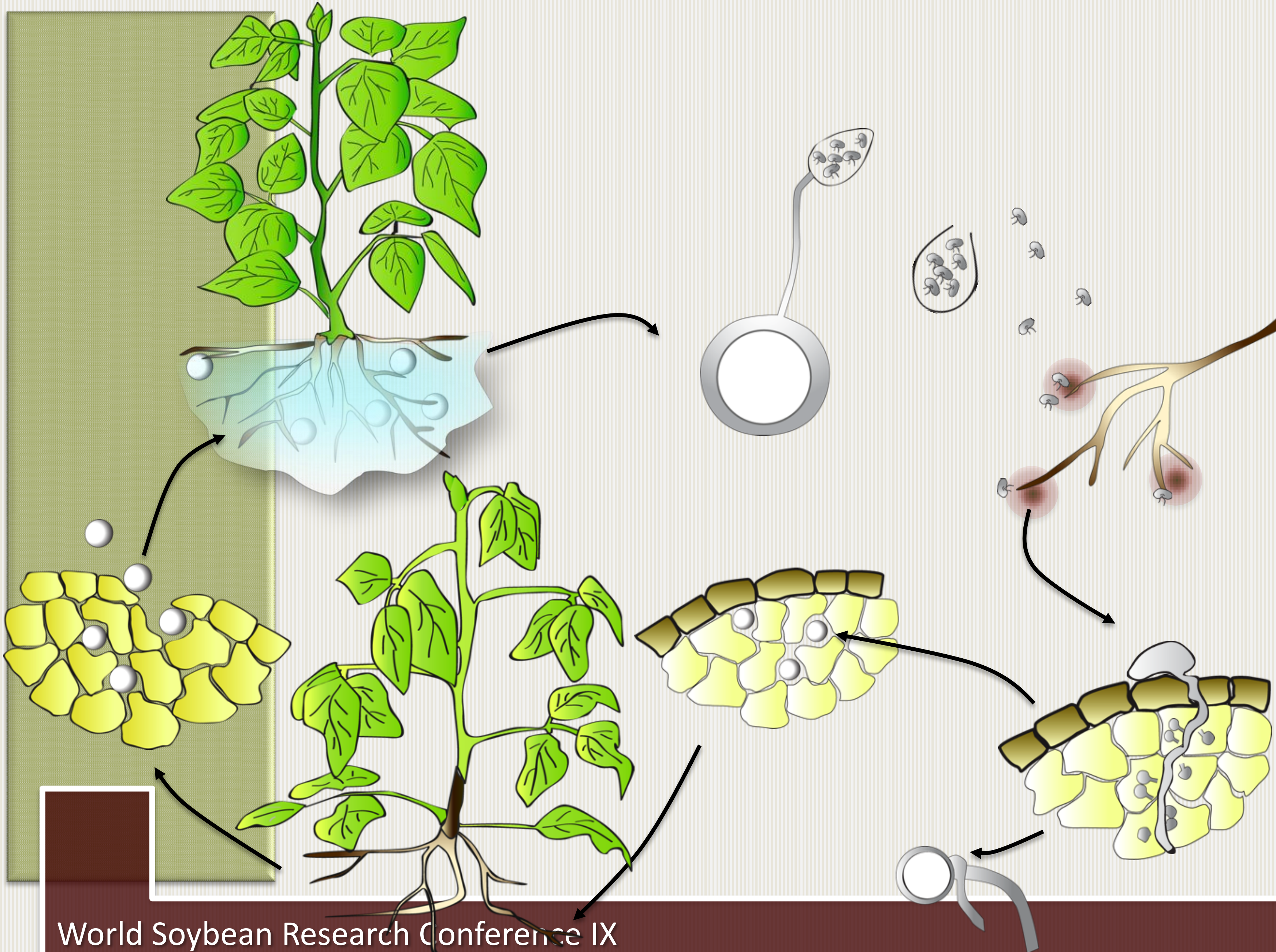
Usual methodology: Inoculation of hypocotyls with a syringe (Dorrance *et al.* 2008. doi:10.1094/PHP-2008-0118-01-DG)

Inconvenients

- Tedious
  (plants inoculated one by one)
- Inconsistent
- Bias caused by injury



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Adapted from Dorrance et al. 2007

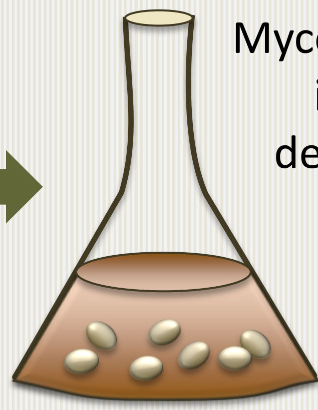
__New method__: Hydroponic system

Inoculation with zoospores

Production of zoospores in vitro

(Li et al., 2009. Journal of Phytopathology)

7 day old *P. sojae* culture (V8 Agar)



Mycelial plugs in soil decoction

Incubation time	T˚C	Light conditions
5 days	25	Continuous light
2 days	15	Complete darkness
2 days	25	Continuous light
1 day	15	Complete darkness
1 h	4	
1 h	25	

Zoospore observation and cellular count

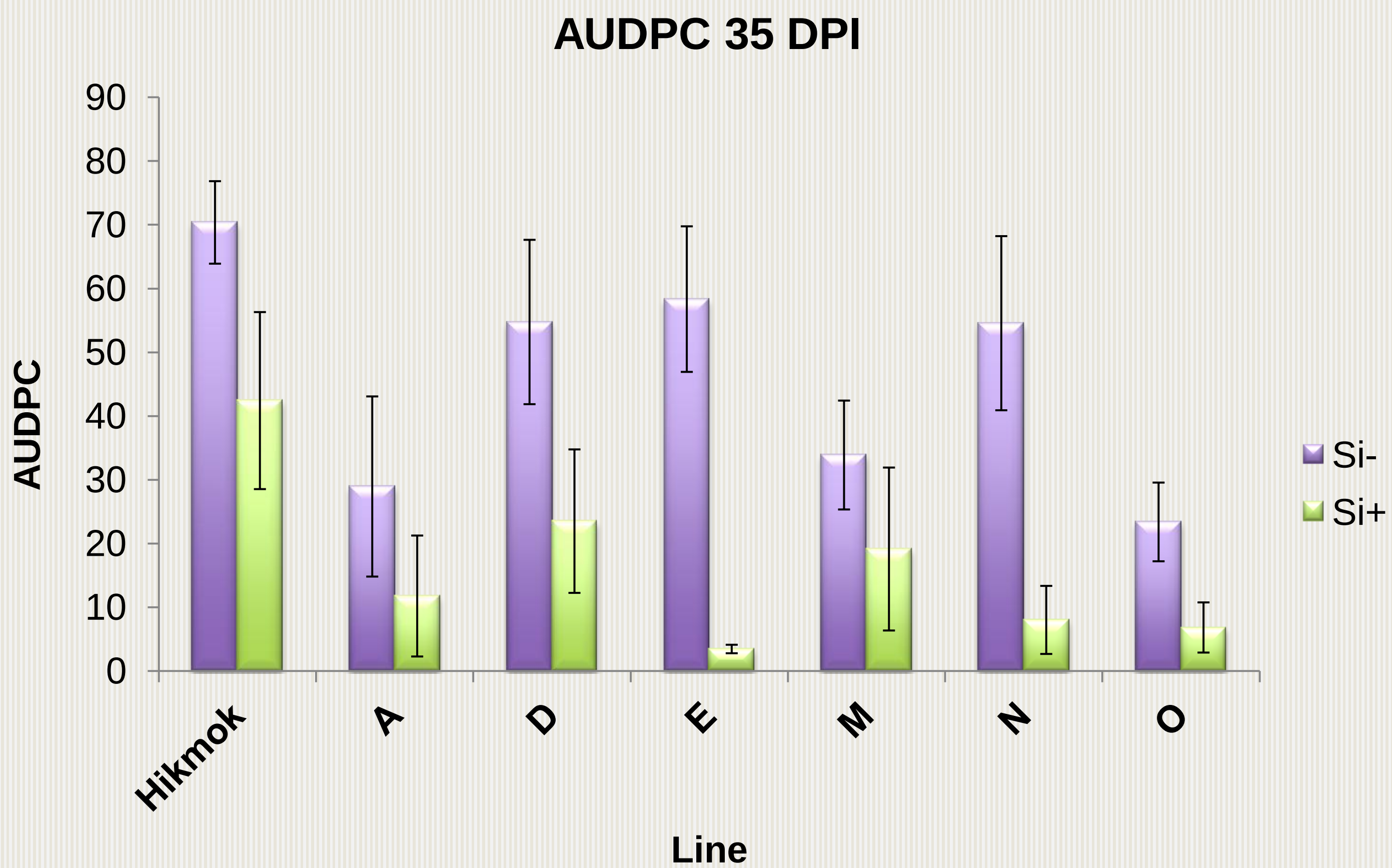
inoculation of nutrient solution


Inoculation

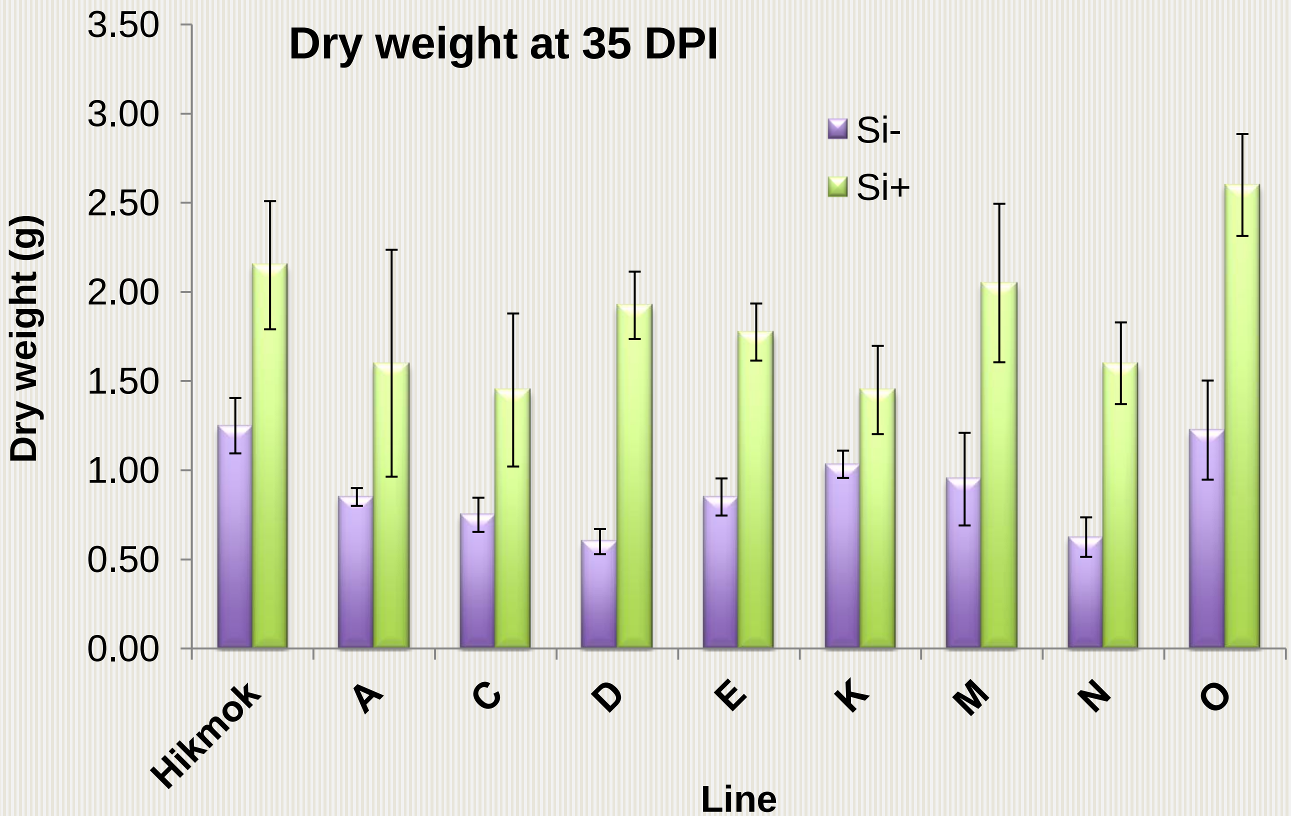
❑ Evaluation of Si-competent soybean plants' tolerance to PRSR

0.5 (no canker; root browning) **1** **2** **3** **4** (death)

❑ Evaluation of Si-competent soybean plants' tolerance to PRSR



❑ Evaluation of Si-competent soybean plants' tolerance to PRSR



Phytophthora sojae: 24 days post inoculation


Hikmok Si-
Hikmok Si+


Line E
Si+
Si-
Line O
Si+
Si-

Conclusion

- Silicon can protect soybean against rust and *Phytophthora*
- Protection is linked to the natural ability of soybean cvs to absorb Si
- There is natural variation within soybean germplasm for Si absorption
- Selection of Si competent cvs is an approach compatible with all other genetic, chemical and cultural methods

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