# Next-Generation Technologies for Tomorrow's Crops: Getting to the Roots of Global Food Security


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# CERC in Global Food Security - Research Areas

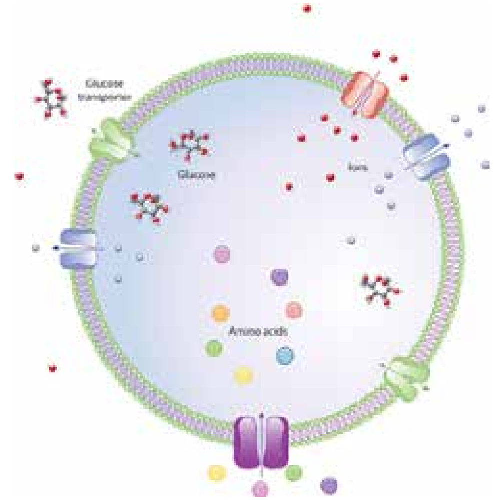
- Crop adaptation to marginal soils
  - How plants deal with too little (insufficient nutrients & water)
  - How plants deal with too much (toxic metals in the soil)
- Role of root system architecture in N, P, K efficiency and drought avoidance (high throughput root phenotyping tools)
- Root Microbiome
- Using discoveries to facilitate the breeding of crops with improved root traits.
- Focus on the key rotation crops for Saskatchewan
  - Canola, lentils and wheat (also soybean)
- Focus on developing country agriculture (Sub-Saharan Africa)
  - Corn and sorghum

60 | NATURE | VOL 497 | 2 MAY 2013
doi:10.1038/nature11909

PERSPECTIVE

# Using membrane transporters to improve crops for sustainable food production

Julian I. Schroeder[1], Emmanuel Delhaize[2], Wolf B. Frommer[3], Mary Lou Guerinot[4], Maria J. Harrison[5], Luis Herrera-Estrella[6], Tomoaki Horie[7], Leon V. Kochian[8], Rana Munns[2,9], Naoko K. Nishizawa[10], Yi-Fang Tsay[11] & Dale Sanders[12]



- A number of important agronomic traits in crop species are conferred by genetic variation (QTL) in membrane transporter genes or in genes that alter root architecture, enabling the plant to strategically place roots where the nutrient is located in the soil horizon.
- These include:
  - Tolerance to salinity stress
  - Aluminum tolerance
  - Pathogen resistance
  - P, N and water acquisition efficiency
  - Enhanced micronutrient uptake and accumulation

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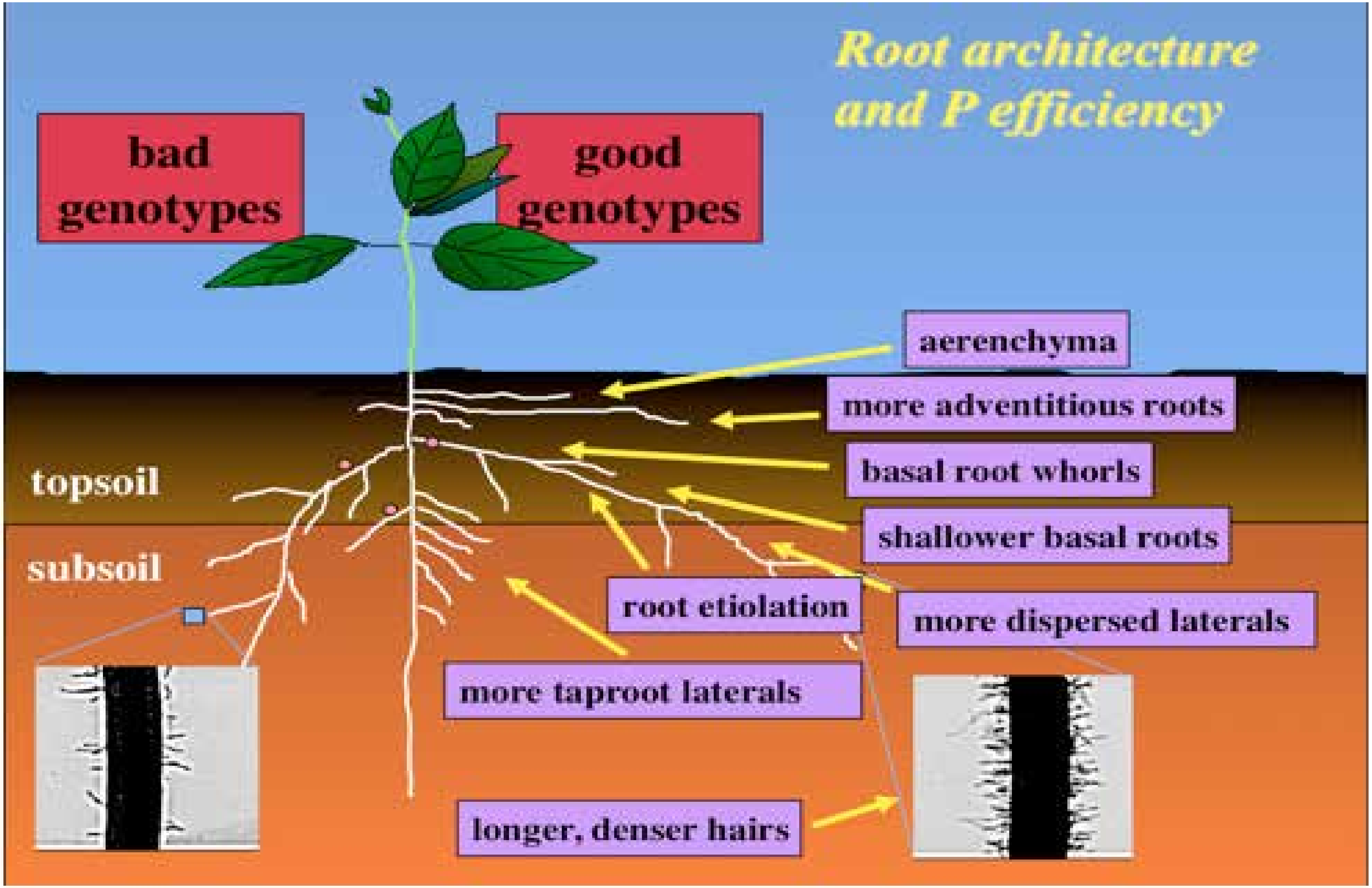
# Root System Architecture and Nutrient Acquisition Efficiency

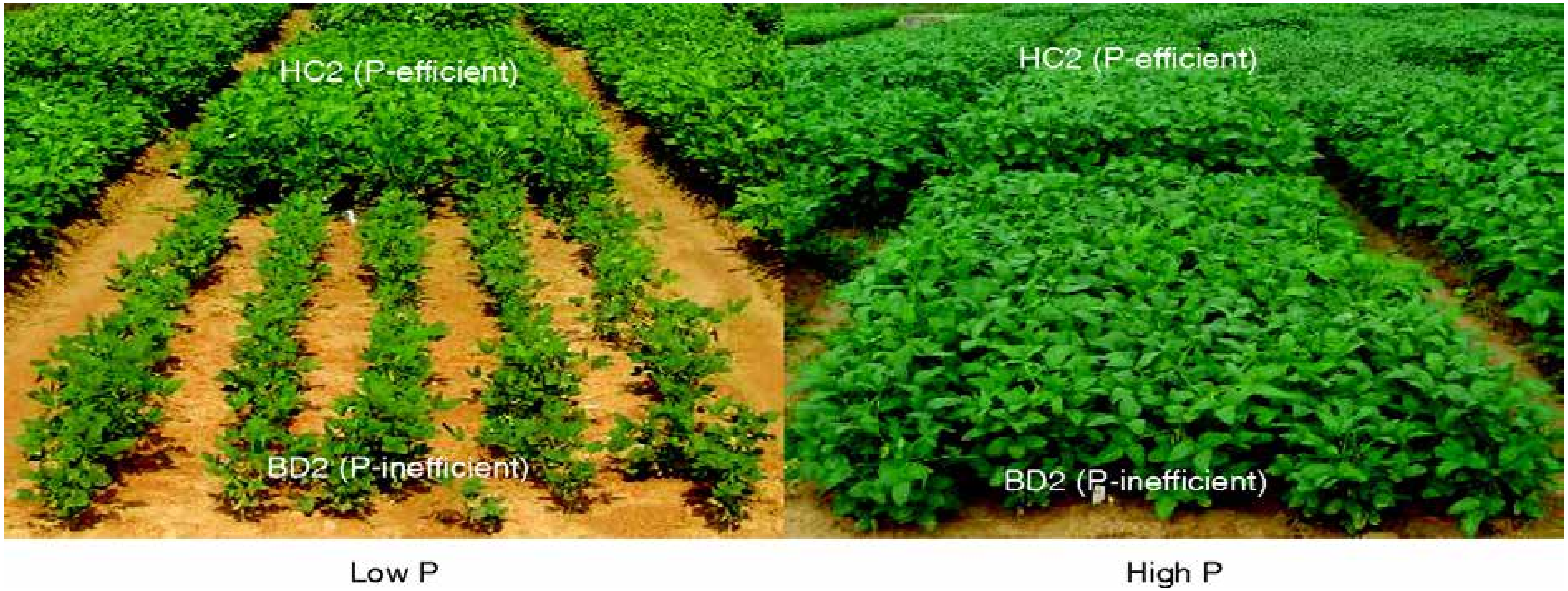
- It is now widely accepted that the spatial distribution of roots in the soil, a trait called root system architecture (RSA), plays an important role in many root-based plant traits such as efficient acquisition of water and mineral nutrients under drought and mineral deficiency in soils.

- Supported by discoveries concerning the role of RSA in P efficiency including surface soil P foraging. Pioneering work from Jonathan Lynch, Xiaolong Yan, and Hong Liao – Lynch & Brown (2001) Plant & Soil; Wang et al (2010) Annals Botany.




RSA traits related to P acquisition. From Lynch 2007.

# Genetic Variation for Soybean Phosphorus Efficiency on Acid Soils



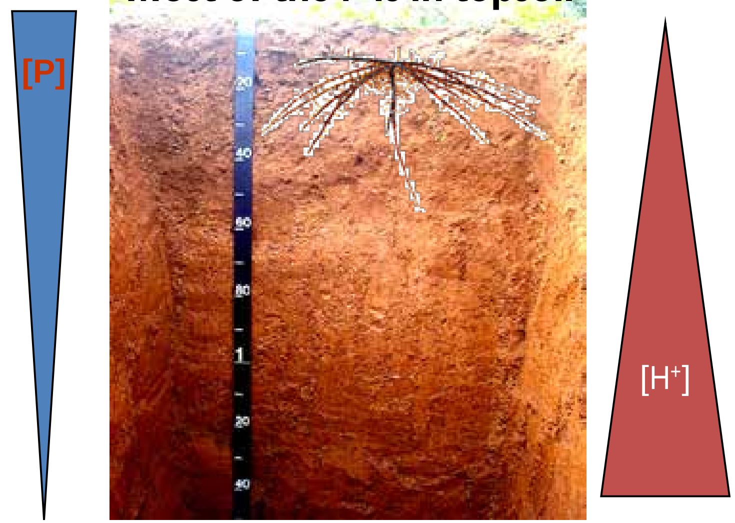

**Wang X et al. Ann Bot (2010)106:215-222:** Two soybean varieties contrasting in P efficiency were grown on a P deficient acid soil with or without P addition (Dr. Hong Liao's group, Root Biology Center, SCAU, Guangzhou)

# What Is Ideal RSA for Soybean in Low P Soils?

Shallow Intermediate Deep


P Inefficient Soybean Line

Most of the P is in topsoil




**Dr. Hong Liao's group, Root Biology Center, Fujian** Agriculture & Forestry University

# Breeding of Root Traits for P Efficiency — Conventional Breeding

**Local Varieties**

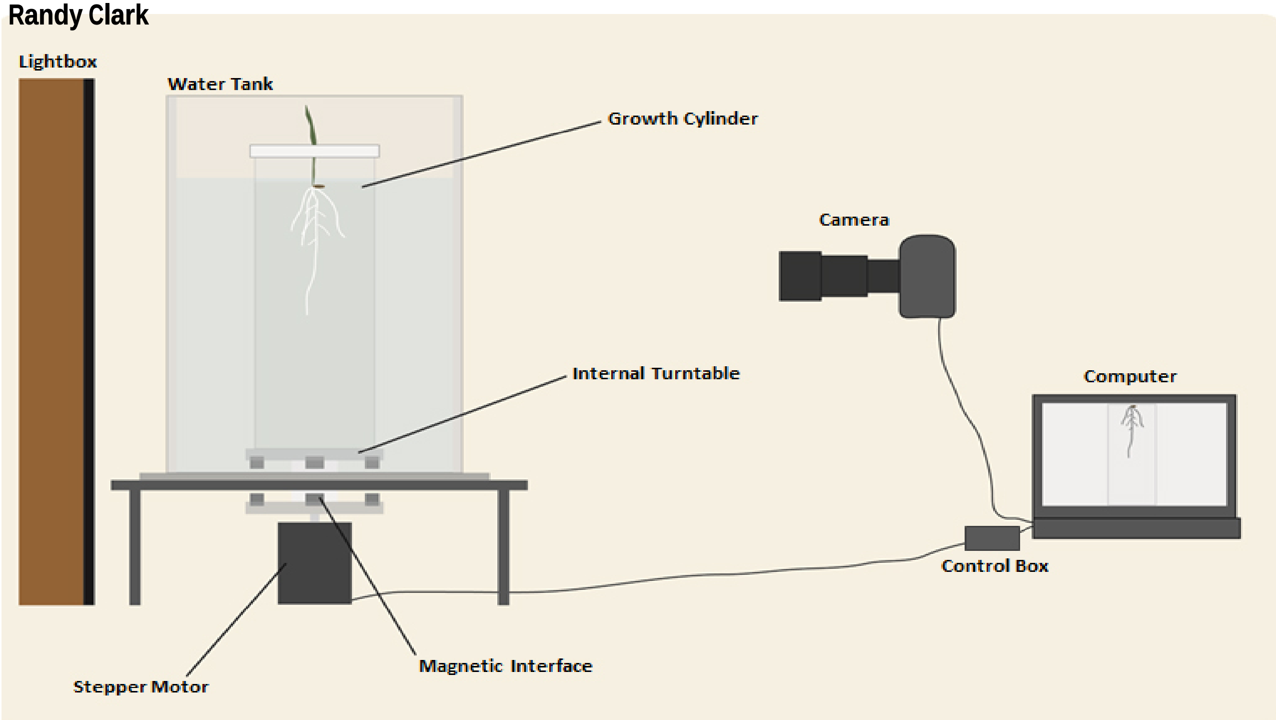
**P-Efficient Lines**

- Backcross breeding & recurrent selection
- Introduced P efficient RSA into locally adapted varieties
- Significant yield increases on low P soils

# High Throughput Phenotyping of Root System Architecture

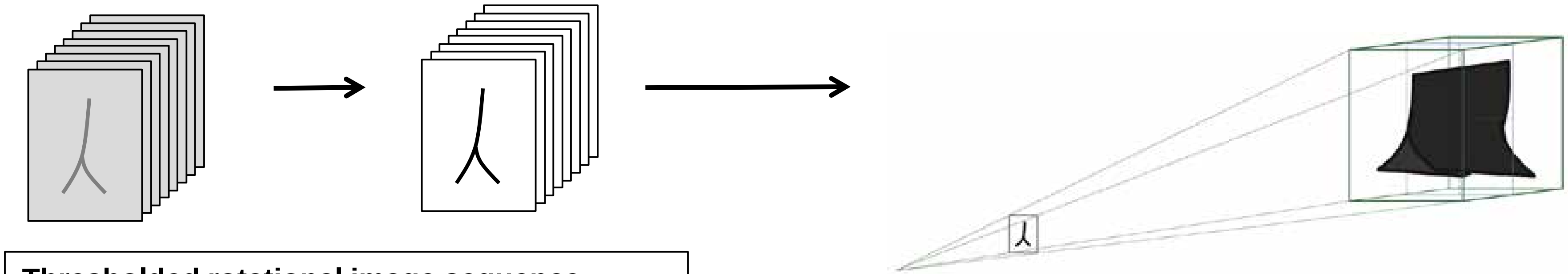
# RootReader 3D: RSA Imaging System

Randy Clark



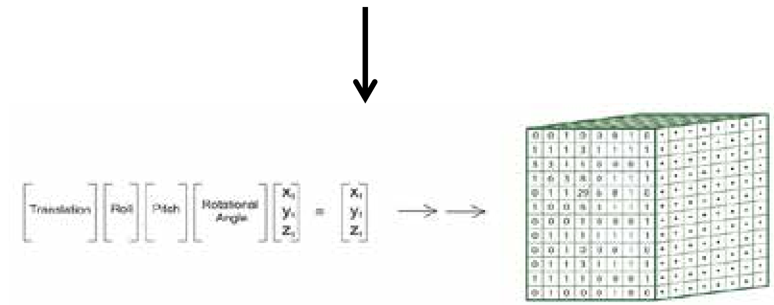

- Stationary camera with fixed capture settings that is synchronized to a turntable via a LabVIEW interface and digital controller
- 100 images captured per root system, 3.6° of rotation between images
- Capture time of approximately 10 minutes per root system

# 3D Reconstruction Process Via RootReader 3D

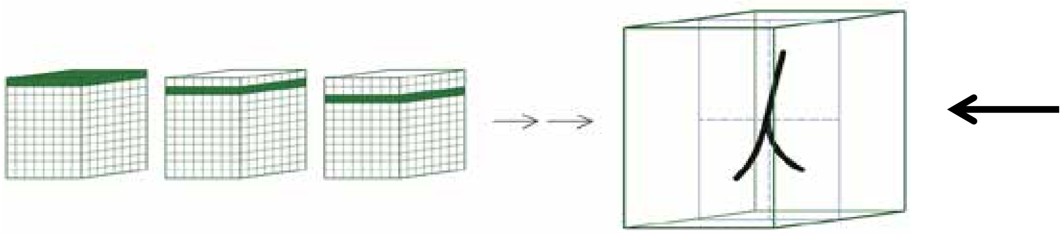


Thresholded rotational image sequence consisting of 40-100 2D images

Perspective back projection of 2D root points from each 2D image into a temporary 3D voxel volume




Transformation of each temporary voxel into a final voxel volume



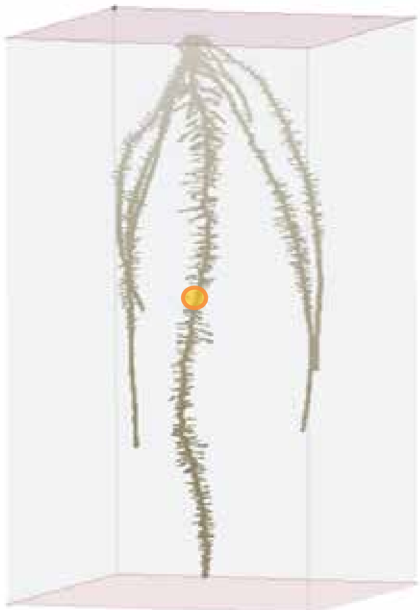
Adaptive thresholding of each horizontal cross section through final voxel volume to generate 3D root model

# Categories of Root Phenotypes

**Global** | Local | Dynamic

--- *Whole root system characteristics* ---

- **Centroid** → Vertical position of the center of mass of the whole root system from the seed.
- Can detect differences in centroid in young seedlings that is maintained in older plants.



**Azucena**

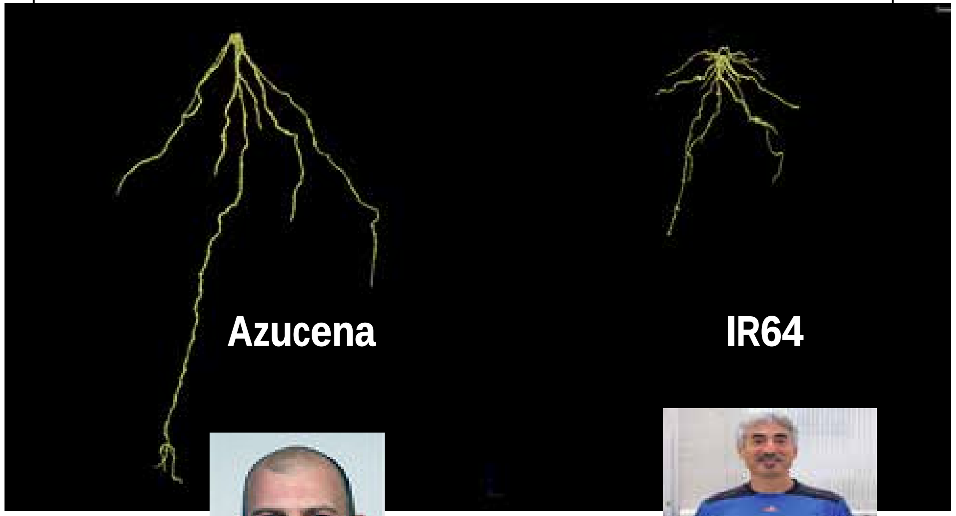
**Upland Rice**
***(aus)***

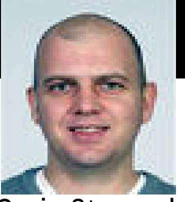
IR64

**Paddy Rice**
***(indica)***

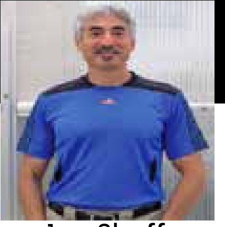
**X-Ray CT Scan of Azucena & IR64 Roots in Soil**

**Collaboration w/ Malcolm Bennett's Lab, University of Nottingham**



Craig Sturrock

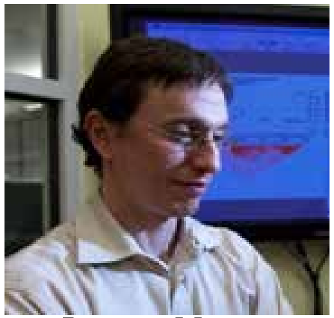


Jon Shaff

# Genome-Wide Genetic Mapping of Rice RSA

Project is a collaborative effort with Susan McCouch's and Jason Mezey's Labs at Cornell University

**Susan McCouch**



**Jason Mezey**

Focus on 13 core traits derived from a set of
<u>19 global root system architecture (RSA) traits</u>

- Centroid
- ConvexHull
- MaxDepth
- MaximumRoots
- MaxWidth
- MinWidth
- mmRtpcC
- Solidity
- SRL
- TipCount
- TRSLVpcC
- TRSVSApcC
- VolumeDistribution

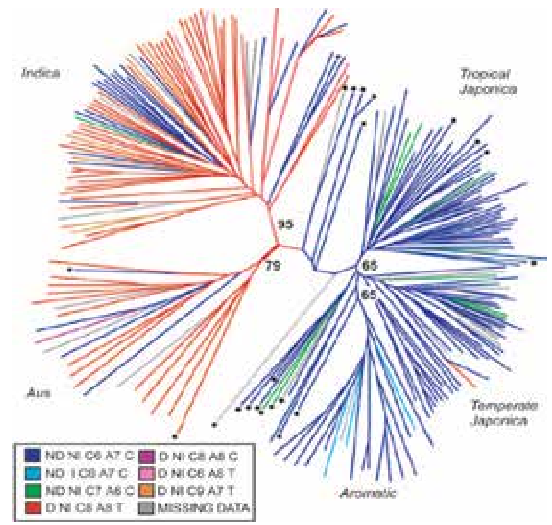
**Randy Clark**

**Rice Diversity Panel 1 (RDP1)**

400 *O. sativa* accessions chosen for:
- Genetic and geographic diversity.
- Genomic and agricultural significance

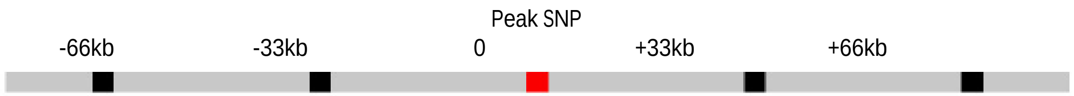
100 *O. rufipogon* accessions chosen for:
- Genetic diversity
- Country of origin – natural range
- Vegetative & reproductive trait variation

**Genotyped with 700,000 SNPs**

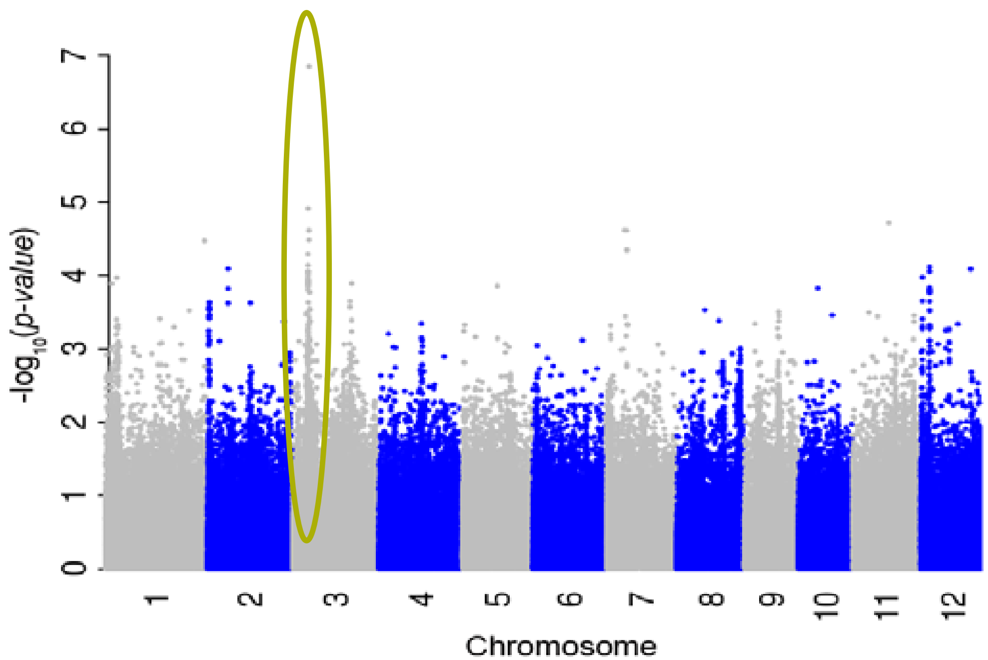


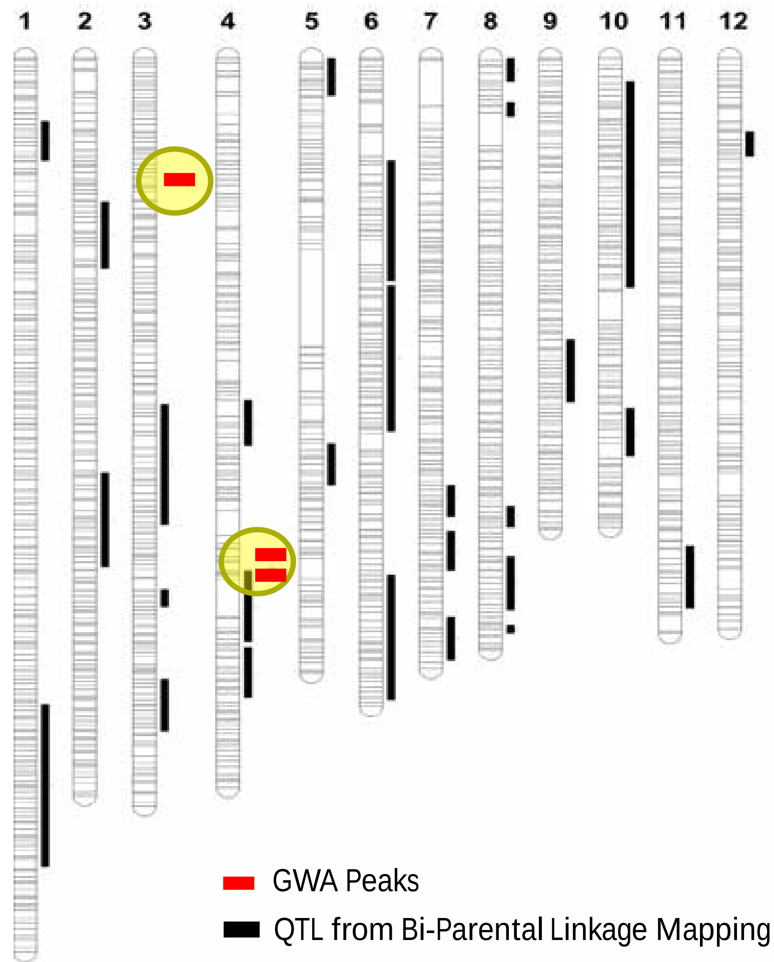
# Genetic Mapping of RSA – 3 Interesting GWA Peaks

Germplasm and Screening | **Mapping Results**



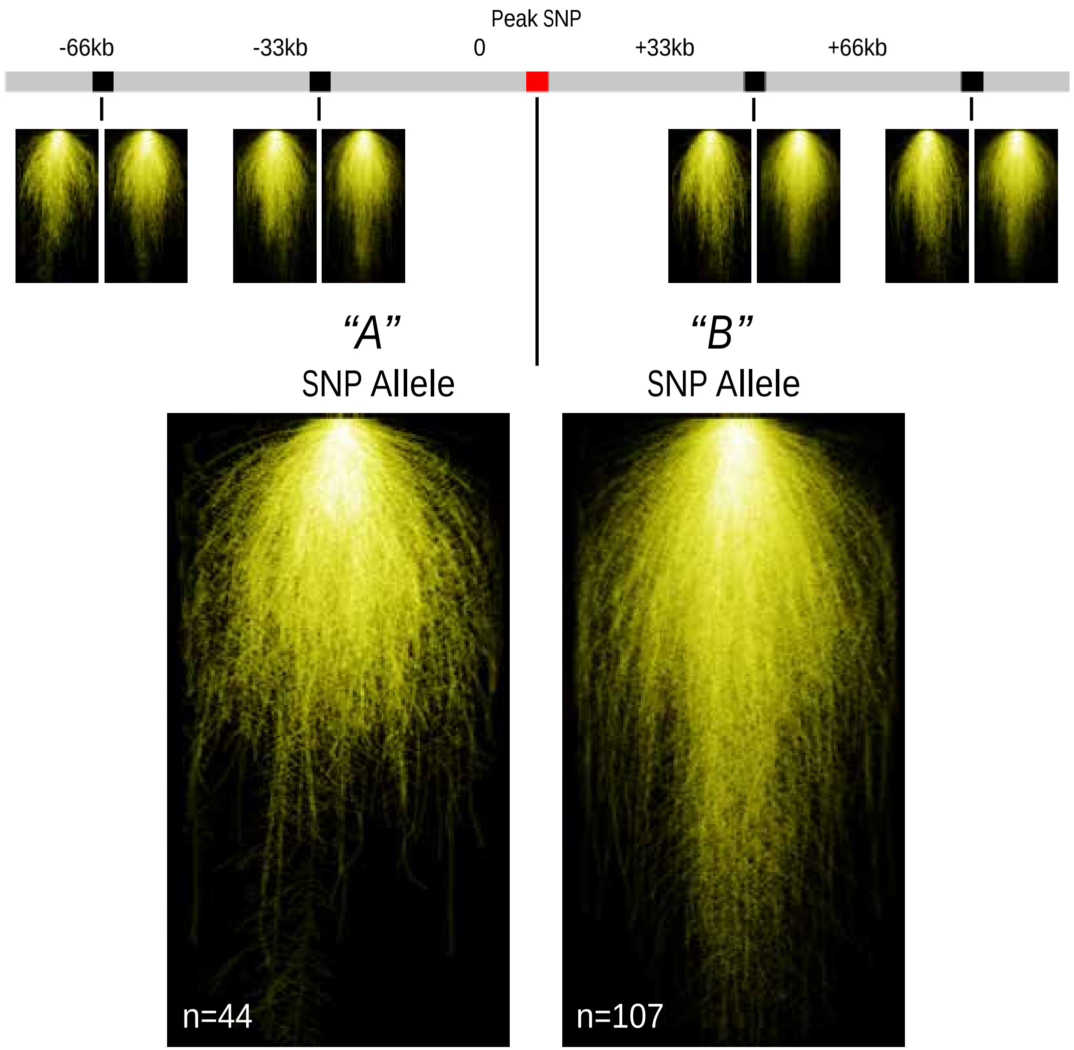

- Region significantly correlated with rooting depth traits in the *indica* subpopulation:
  - Centroid, Maximum Depth, Maximum Width, and Volume Distribution

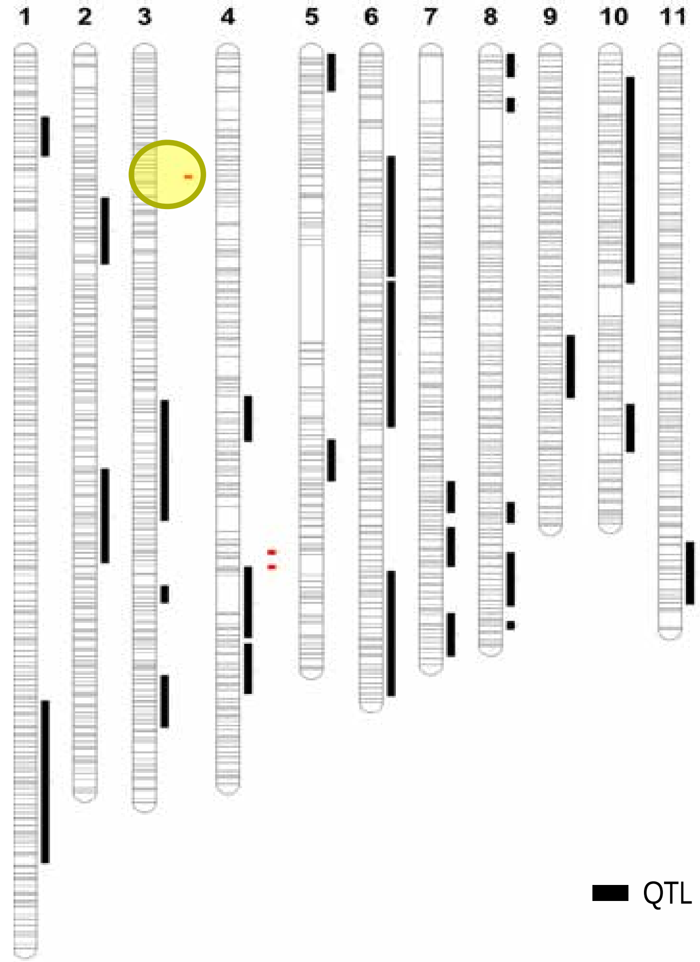


# Genetic Mapping of RSA – 1st GWA Peak

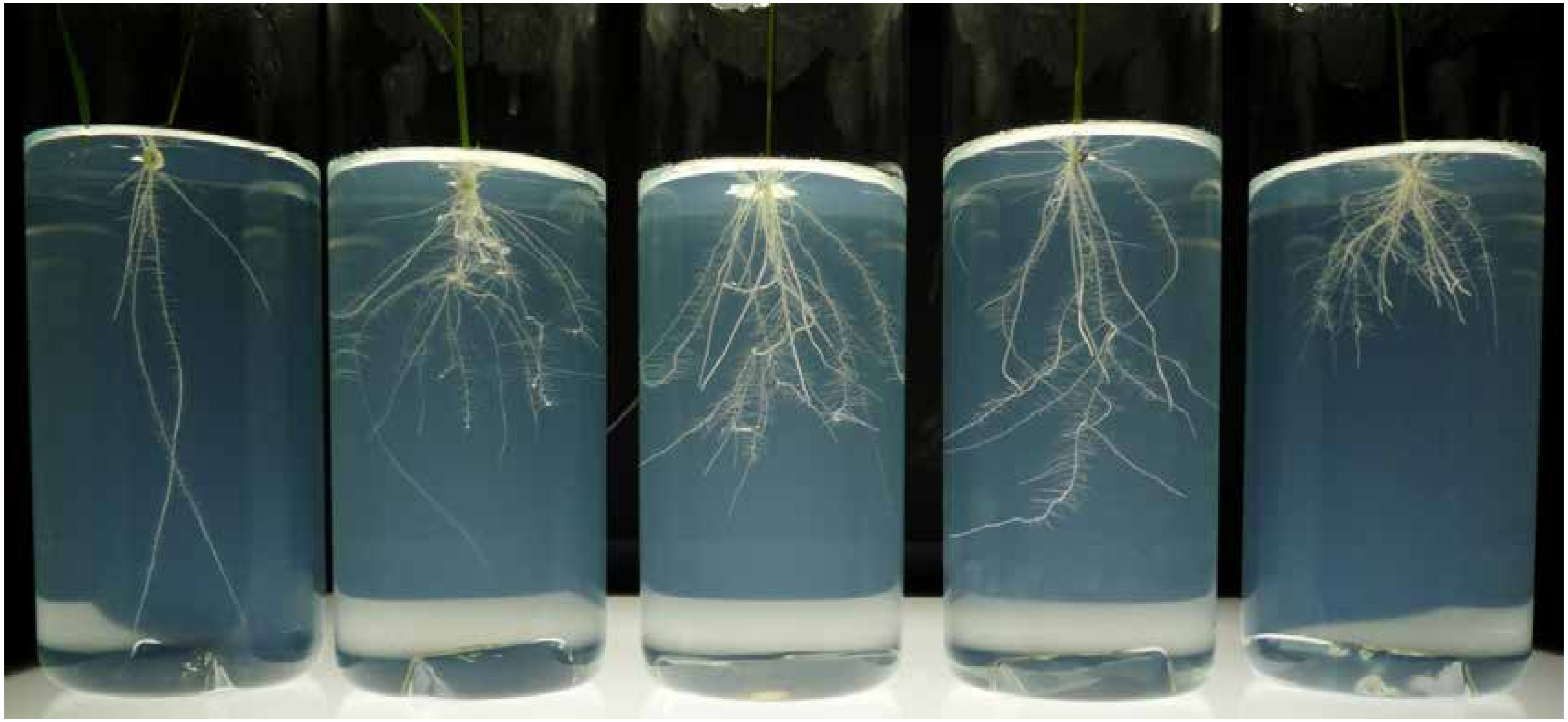
Germplasm and Screening | **Mapping Results**



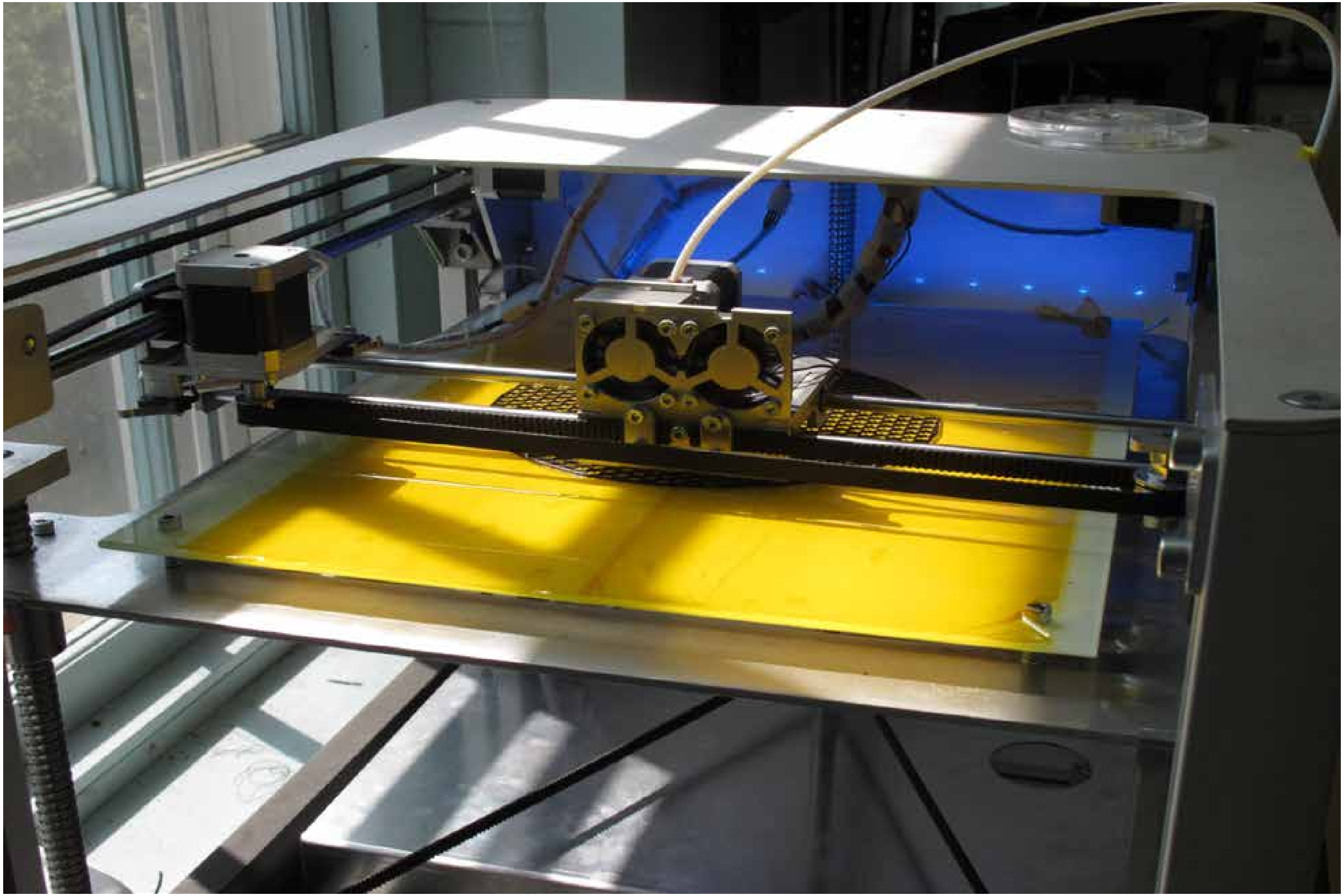
Jon Shaff

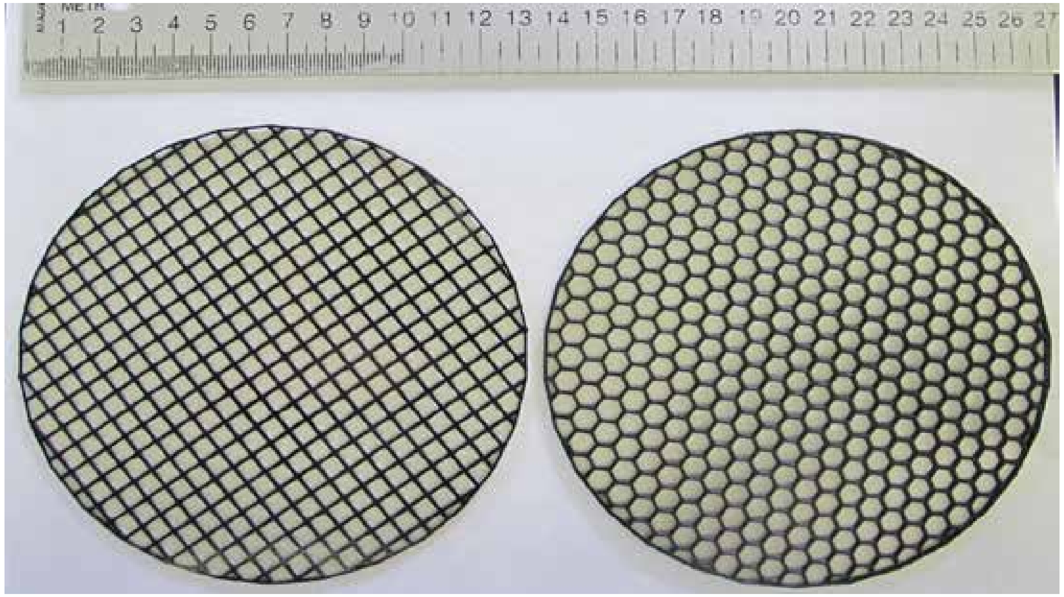
# Improving Plant Growth and Root Imaging Systems



- Rice roots grow well in the gellan gum media but root systems of many other plant species do not (maize, sorghum, etc).
- The problem is not reduced [$O_2$] deeper in the gellan gum cylinder (checked with $O_2$ minielectrode). Possibly due to elevated ethylene at near root tip?

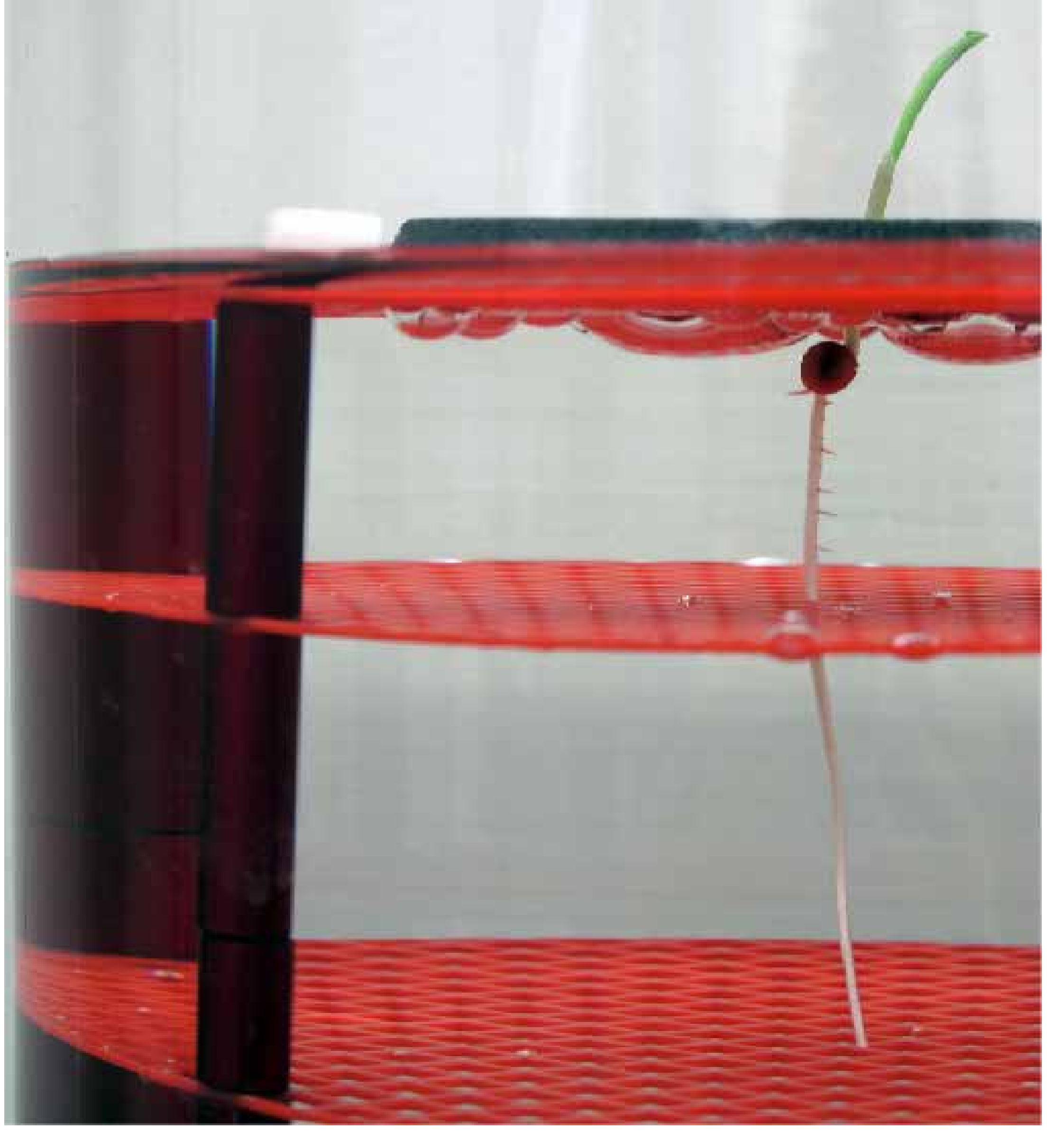
# 3-D Mesh Printer Fabricating 15cm Diameter Hexagonal Mesh



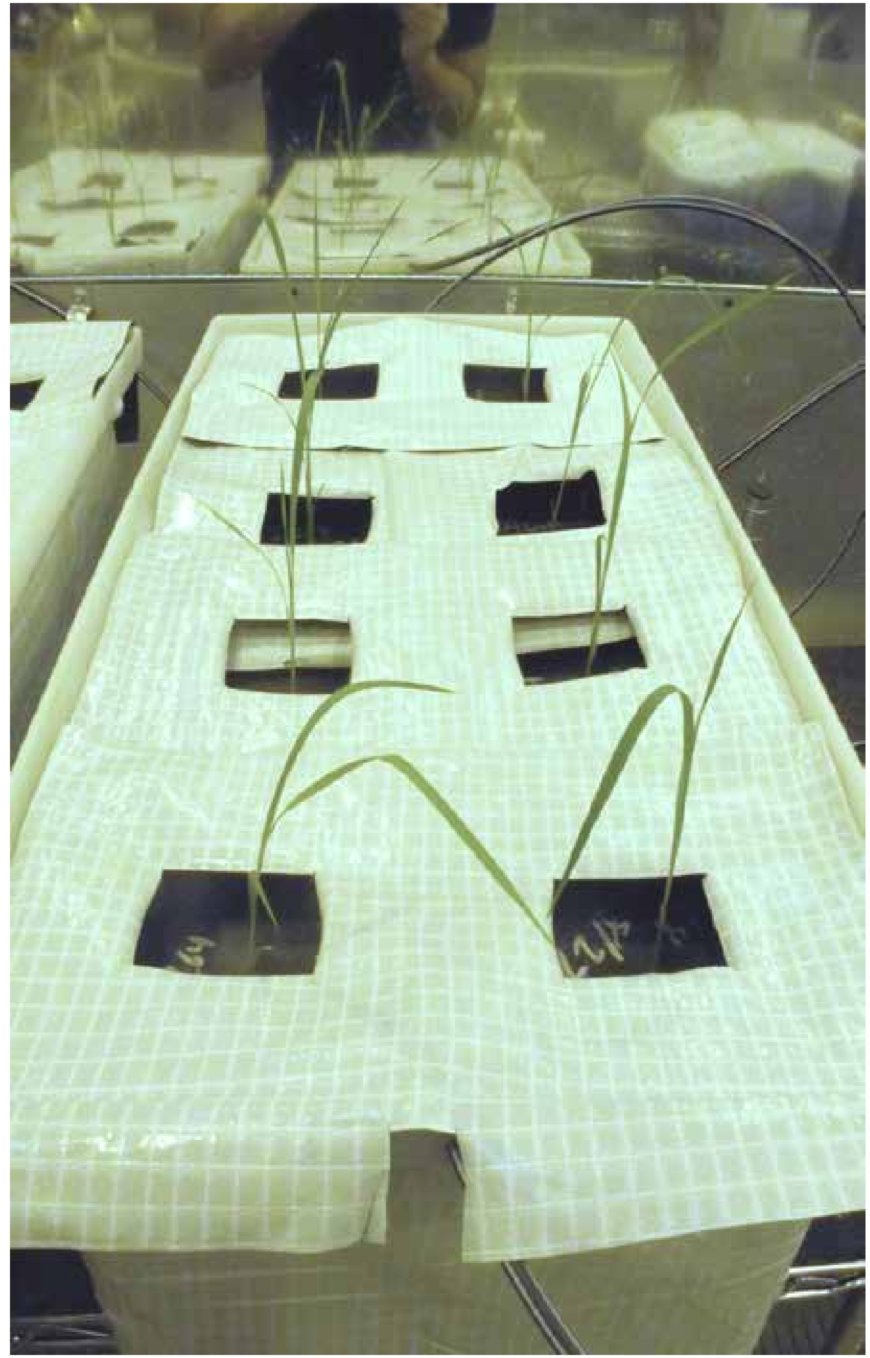


The growth system is created from ABS plastic mesh circles made with a 3-D printer.

The mesh system serves to constrain the roots, but does not impede their growth.



Side view - a young sorghum plant held with black foam.

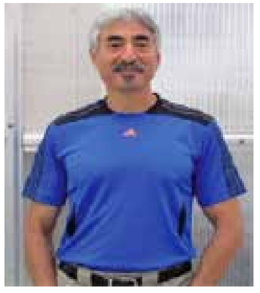


# 3D Imaging/Analysis of RSA in Hydroponic Culture

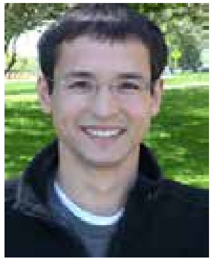
Root system on 15 day old sorghum plant

- Developed hydroponic system using plastic mesh to maintain RSA.
- New thresholding software removes image of everything that is not root (including plastic mesh)
- Then reconstruct 2D image series into a 3D root system.
- Can phenotype a wide range of plant species (sorghum, maize, canola, cucumber, wheat).
- Can image older plants and larger root system species such as maize and sorghum with more developed crown root systems.
- Can more easily modify composition of growth media in hydroponics.

Dave Schneider



Jon Shaff

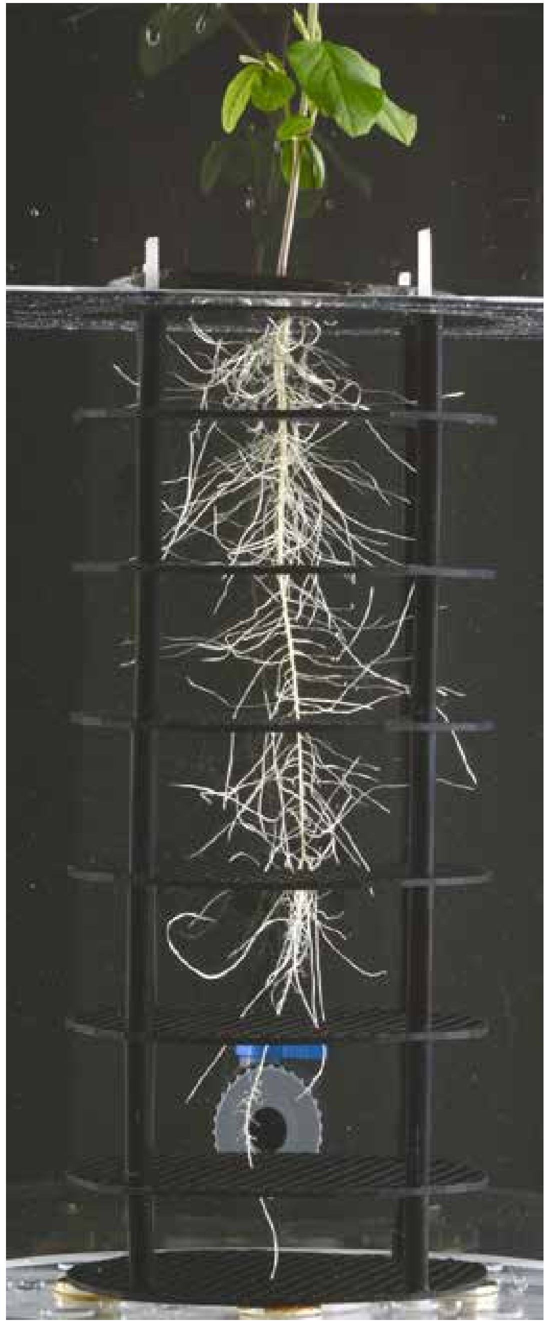


Randy Clark

3D reconstruction of 100 2D images of sorghum root system

Rice grown for 9 days

Soybean 12 days



Sorghum 10 days

# Canola Roots on Hydroponically Grown Plants

5 DAT

16 DAT

60 DAT

# 3D RSA Imaging System

Side-lighting for plant imaging

Calibration Rod

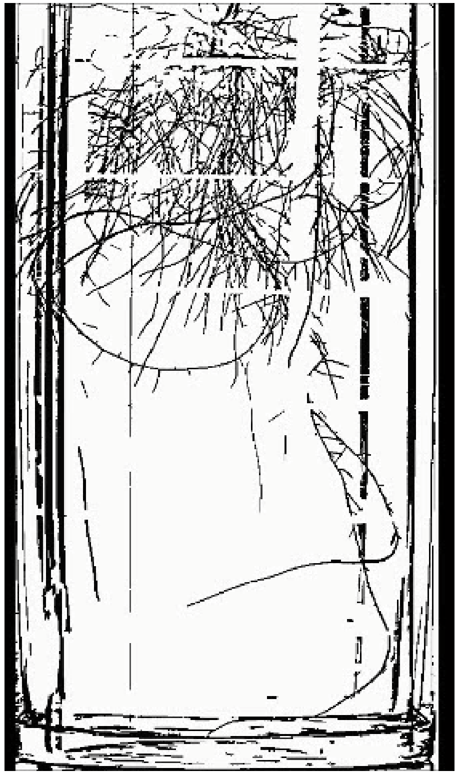
Magnetic turntable

Stepper motor

- 100 images per root system, 3.6 degree of rotation between images
- About 30 min per root system (including imaging calibration rod)

# Needed to Improve Previous System to Properly Grow and Image Canola Root System Architecture

Original image



Processed image

3D-reconstructed image

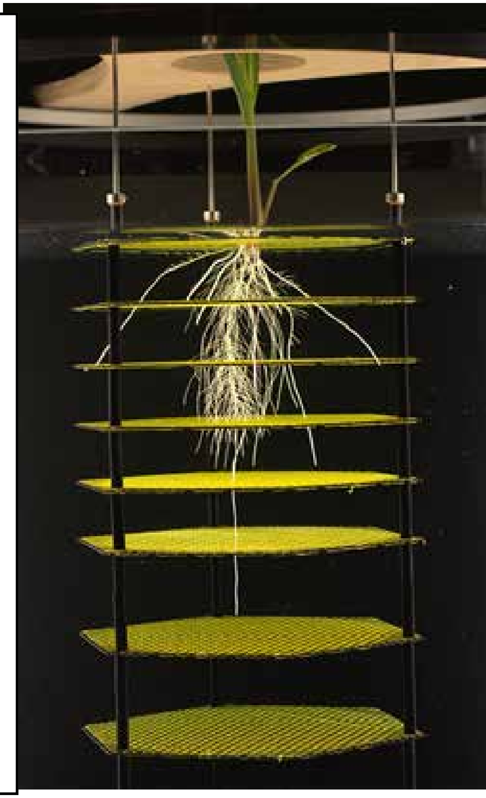
**22 Day Old Plant**

Tyler Davis

# Improved RootReader 3D Platform

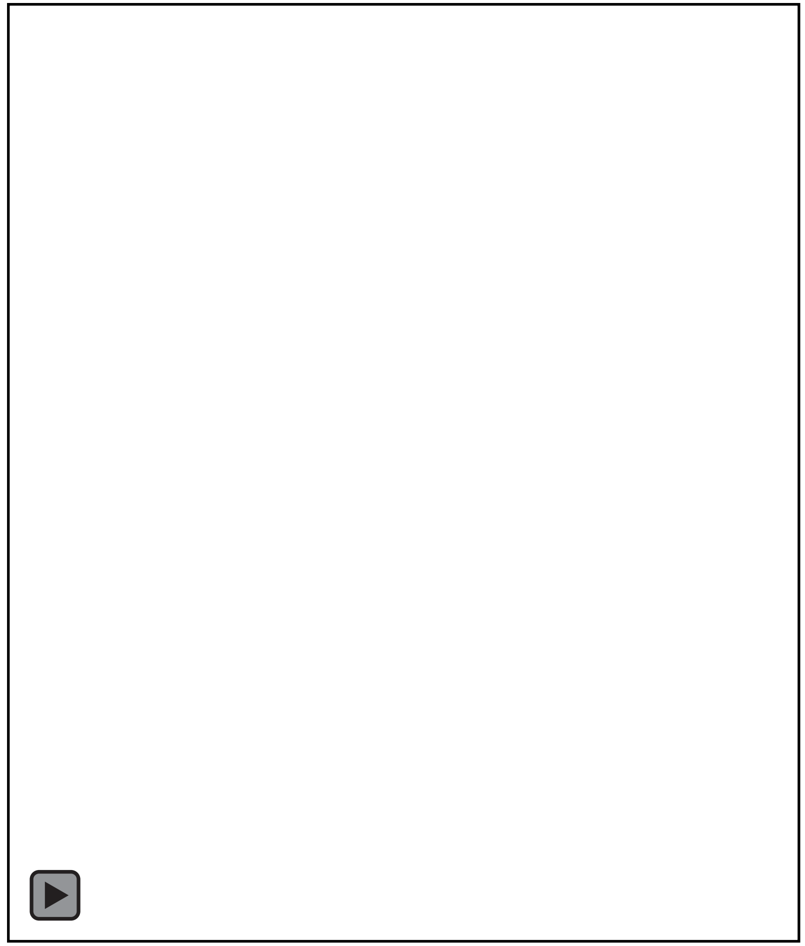
Dave Schneider

- -Does not require a dark room
- -Uses a Raspberry Pi 2 computer
- -Smaller footprint
- -Can use a simpler mesh tower
- -Precision motor and bearings
- -Better automation



# New Thresholding Software Improves Image Analysis

Dave Schneider



- New thresholding removes all of image that is not root – including mesh
- Improved consistency and sensitivity
- Less image fragmentation
- Faster processing time
- More automated image processing

Sorghum grown for 10d in nutrient solution with 2.5µM P

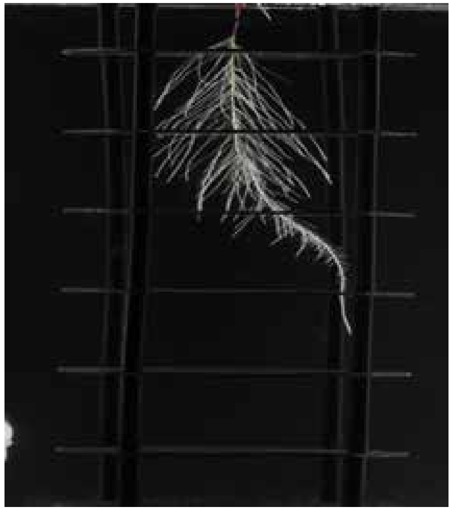
Jurandir Magalhaes

# Used RootReader 3D System to Identify Sorghum Genes that Confer Tolerance to Low P Soil Conditions

Hufnagel et al. 2014. Duplicate and conquer: Multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils. Plant Physiology 166: 159-167

Barbara Hufnagel

- Collaboration with Embrapa Maize and Sorghum in Brazil
- Quantified RSA traits for 250 diverse sorghum lines in the lab using RootReader 2D and 3D platforms.
- Measured grain yield, shoot biomass, and shoot P uptake in the same lines *in the field* on low and sufficient P acidic soils in Brazil.
- Combined this with genomic and molecular genetics approaches to identify sorghum homologs of the rice *Pstol1* gene that confers phosphorous starvation tolerance.
- Superior alleles of several *Pstol1* genes are associated with sorghum P efficiency and the root system architecture on the left, which allows the plants to more effectively forage the topsoil for P fixed to surface of clay minerals.



**3D RSA for P efficient sorghum genotype**

**3D RSA for P inefficient sorghum genotype**

- Dr. Magalhaes has identified genetic markers that are linked to superior (more P efficient) *SbPstol1* genes and these markers will be tested in the field with sorghum breeders in Brazil and then Africa.


Global Institute for Food Security
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Saskatoon, Canada

# GIFS Founding Facts

*GIFS is public-private partnership that operates as an Institute at the U of S, with its own Board of Directors*

PotashCorp

University of Saskatchewan

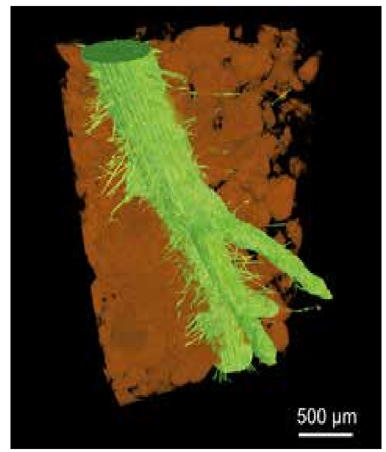
Government of Saskatchewan

GIFSs research focus is to perform fundamental research generating information that can be used to deliver transformative innovation to agriculture in both the developed and developing world.

# Canada Excellence Research Chair: Establish a World Class Center for Root Phenotyping at GIFS

Continue to advance current optical imaging and 3D reconstruction technologies.

The Canadian National Light Source will be a fantastic resource for X-ray imaging of roots and shoots in soil-grown plants.




**Synchrotron imaging of wheat roots grown in soil.**

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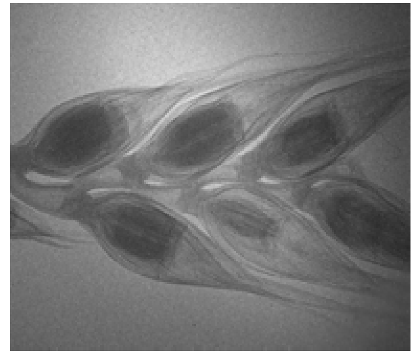
# Major Goal is to Establish a World Class Center for Root Phenotyping at GIFS & UofS

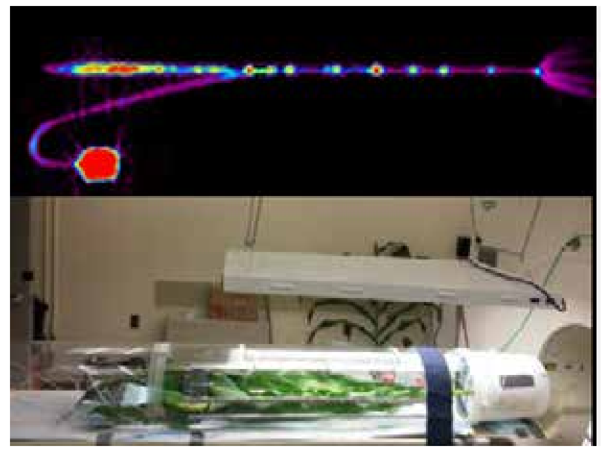
- Other more portable devices can produce synchrotron-quality X-rays in the lab for imaging shoots and roots in soil.

**(Dr. Emil Hallen (GIFS) in Collaboration with Advanced Laser Light Source, Varennes, Quebec)**



Disease in wheat seeds using Advanced Laser Light Source imaging.

- UofS has a world class cyclotron facility which generates positron emitting isotopes. In collaboration with Univ of Regina, Canada's first plant radioisotope imaging system, PHYTOPET, is now operating at the UofS.
- Isotopes for plant research include $^{11}CO_2$, $^{13}N_2$ (as well as $^{13}NO^-_3$ & $^{13}NH^+_4$), $H_2{}^{15}O$, $^{52}Fe$, and $^{62}Zn$.

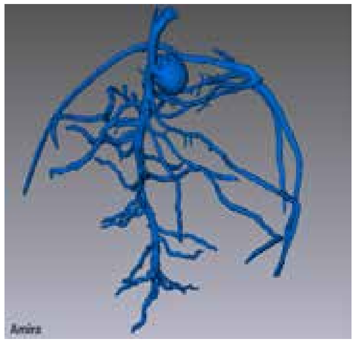


**$^{11}CO_2$ PET imaging of sugar fluxes from sorghum source leaf to sinks**

# Major Goal is to Establish a World Class Center for Root Phenotyping at GIFS & UofS (con't)

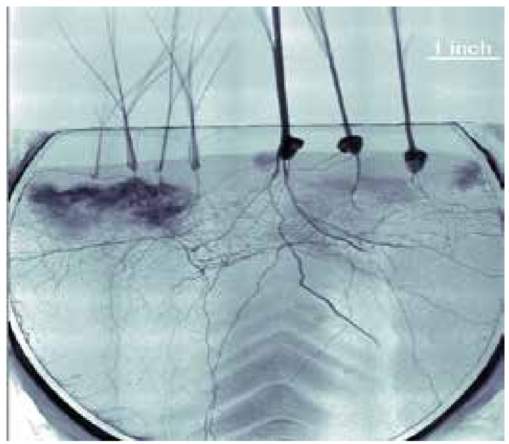
- **Neutron imaging of root systems of intact plants in soil.**

(Dr. Emil Hallen (GIFS) & Canadian Nuclear Laboratory in Chalk River, Ontario)



**3D reconstruction of pea root system in soil imaged with neutrons**

**Roots of two wheat seedlings and one pea seedling in soil**




**Neutron imaging of roots of wheat (left) and corn seedlings in soil (note mycorrhiza around wheat roots)**

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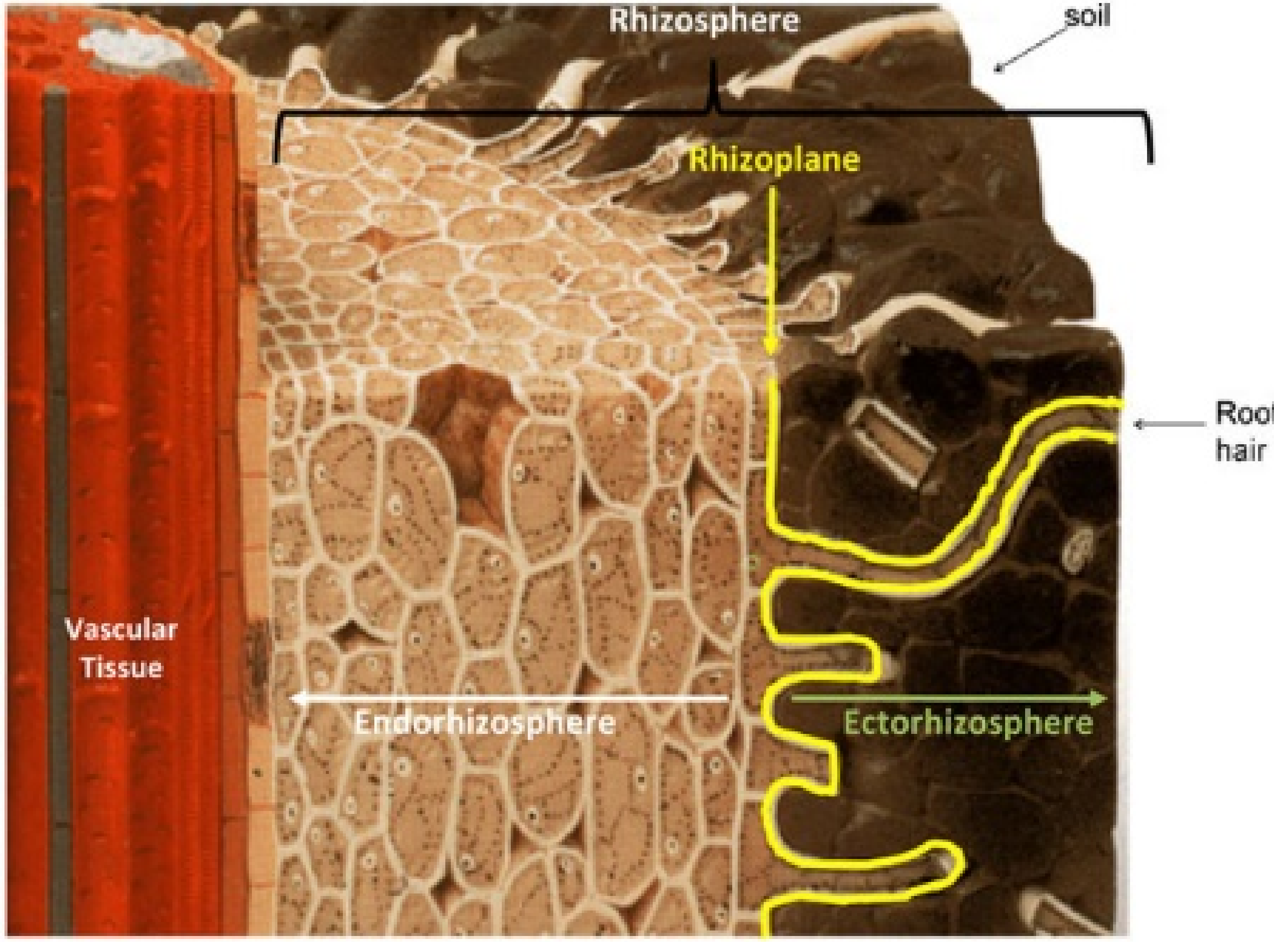
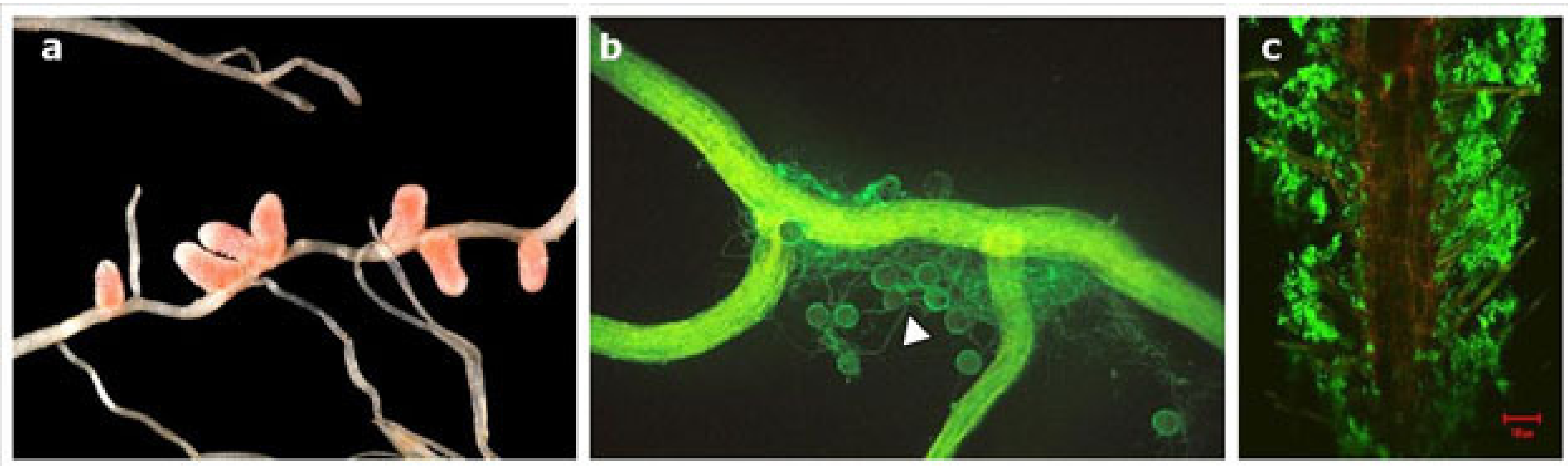
# The Rhizosphere: Where a Second Plant Genome Resides – the Root Microbiome



# The Root Microbiome: A New Frontier for Agricultural Research




$N_2$ fixing bacteria living in nodules on alfalfa root

A corn root colonized with mycorrhizal fungi

Beneficial bacteria forming biofilm on root surface

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# What Will be the Outcomes of Root Research For Global Food Security?

- Breed for increased yields with more strategic use of fertilizer (NPK).
- Breed for increased yields using less water.
- Breed for crops with healthier and more active root microbiomes,
- Crops with bigger root systems and "better" root microbiomes can significantly decrease agriculture's carbon footprint..

Increased agricultural yields have, since 1961, already "stopped" 590 billion tons of global $CO_2$ emissions via carbon storage in roots and the root microbiome.

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