High Throughput Phenotyping of Roots

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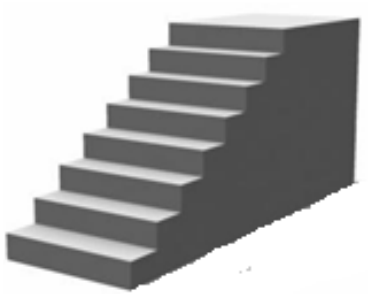
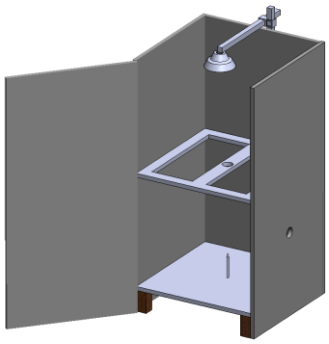
This talk presents a new high-throughput method to estimate maize root complexity and its applications

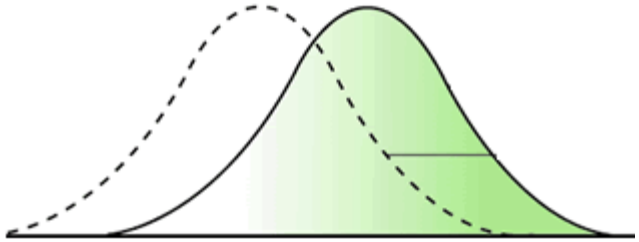
1. Background Information



2. Root Image Phenotyping



3. Applications



The Challenge

" How can we Measure Maize Root Complexity?"

The Challenge

Why Study Root Systems?

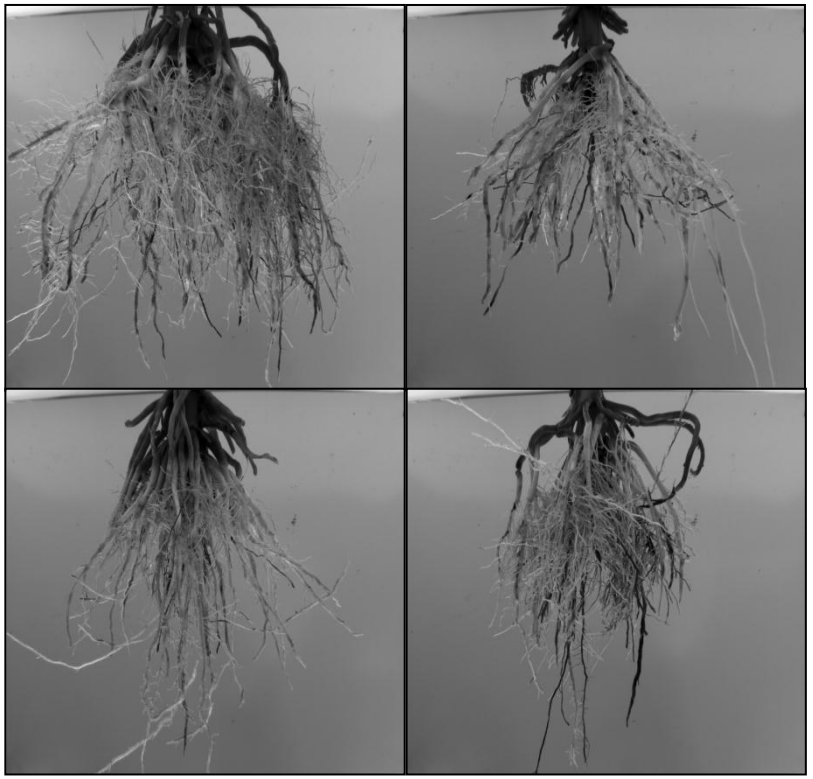
1. Uptake of Water

   Water is one of the most important resources for plant growth and function.

2. Uptake of Nutrients

3. Lodging Capacity

   Economic importance of losses in yield caused by lodging.



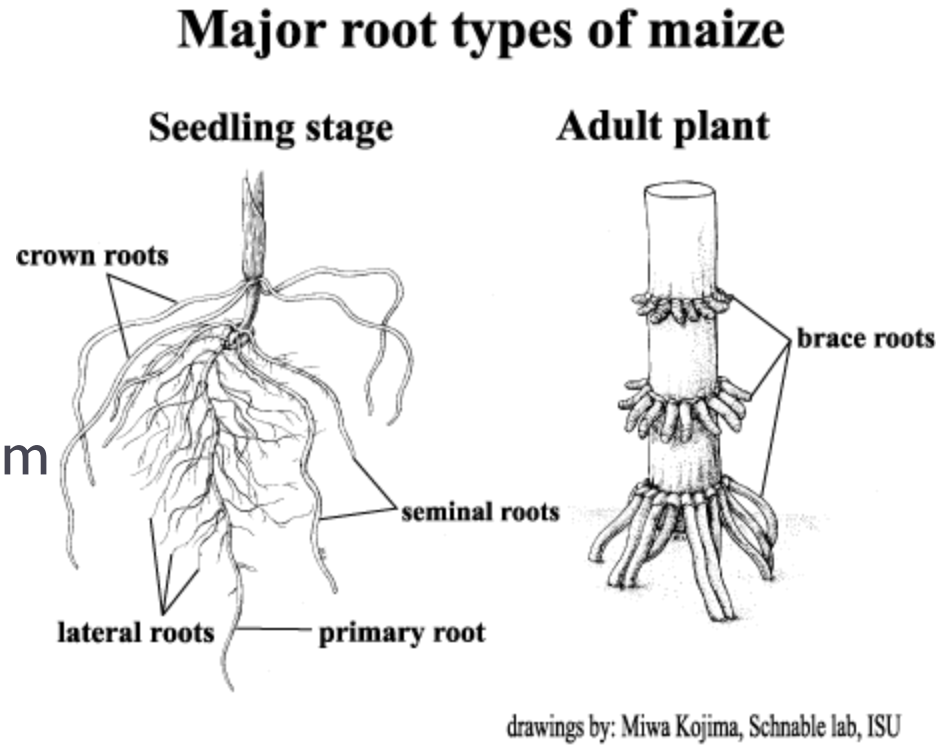
The Challenge

" Major Root Types of Maize"

Maize Root System

I. Embryonic Root System
   - Primary Root
   - Seminal Root

II. Postembryonic Root System
   - Lateral Root
   - Crown Root
   - Brace Root

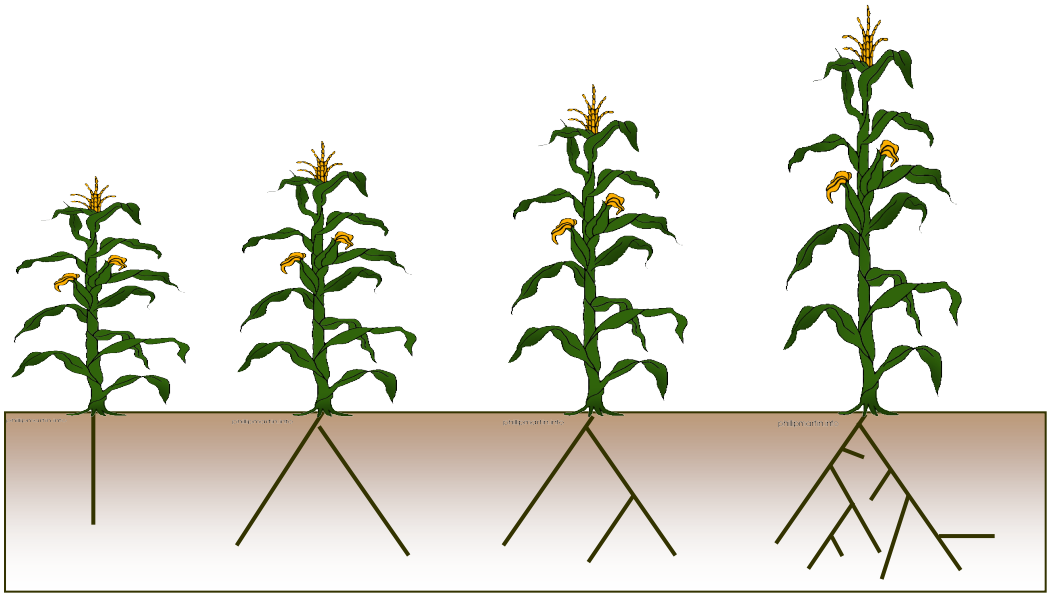


The Challenge

" Lack of studies due to four main factors"

- Root systems usually grow underground and recovering roots from field experiments is labor intensive
- Lack of reliable phenotyping methods
- Size of the root system
- Abiotic and biotic factors, which unpredictably influence root architecture and development

The Challenge
Assumptions



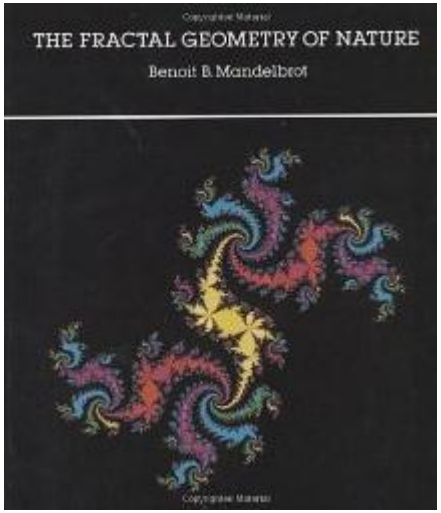
Branch. points 0 1 2 8

Complexity

Soil face explored

The Challenge
Assumptions

The complexity of root systems can be determined by applying fractal dimensions.



"A fractal is an object or quantity that displays self-similarity, in a somewhat technical sense, on all scales. The object need not exhibit *exactly* the same structure at all scales, but the same "type" of structures must appear on all scales."

http://mathworld.wolfram.com

“a rough or fragmented geometric shape that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole”






- http://www.google.com/images

The Opportunity

Example of methodologies used to study root systems

1. Early Growth Stages,
   i. Germination paper towel
   ii. Distilled water
   iii. Plastic growth pouches
   iv. Hydroponics
2. Later Growth Stages,
   i. Columns or boxes
   ii. Pots
   iii. Simulation
   iv. Field

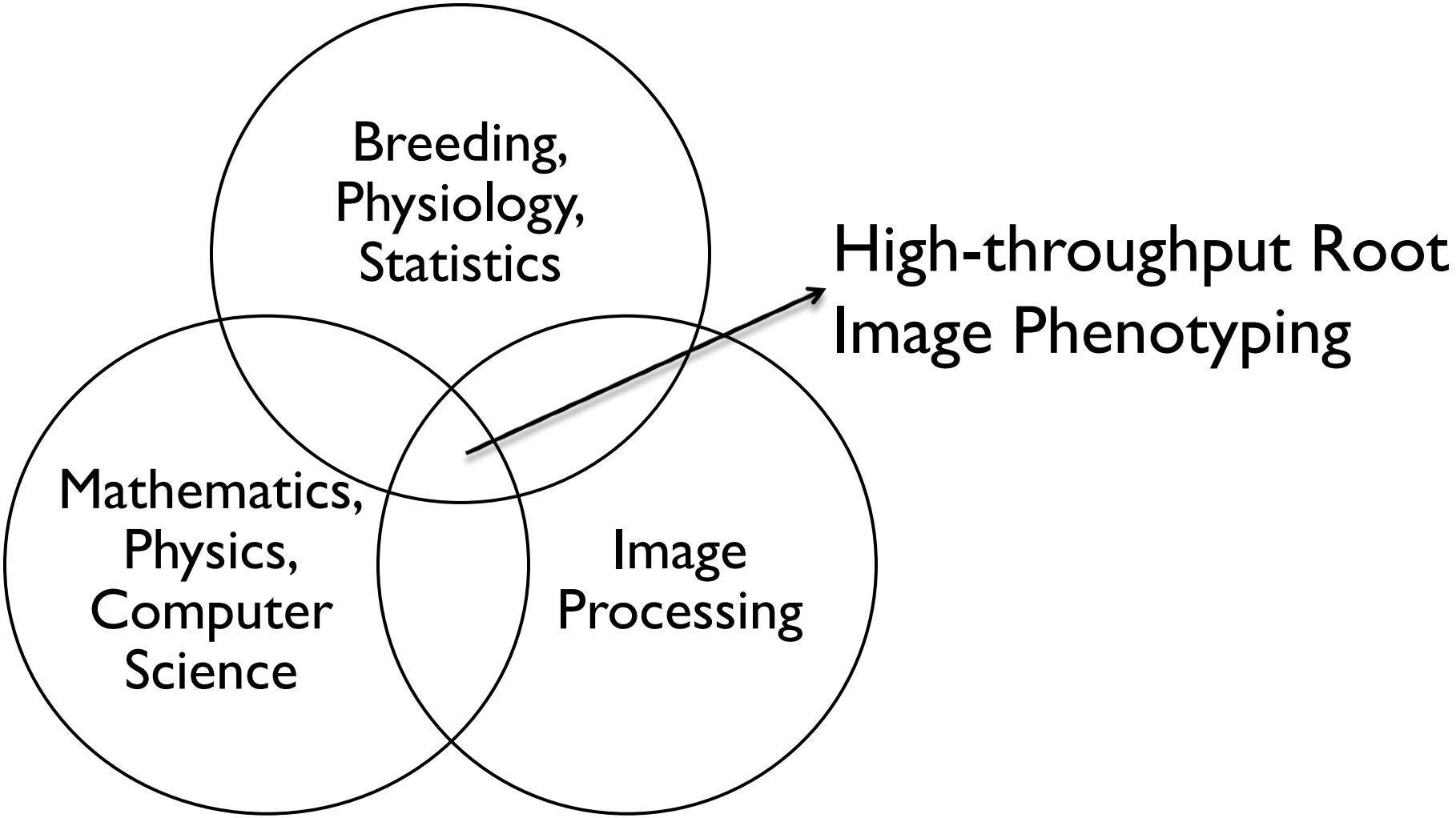
The Opportunity

Development of a New Phenotypic Method

Goal

To develop a high throughput root imaging and analysis method to accommodate a growing need to measure root complexity in an unbiased way.

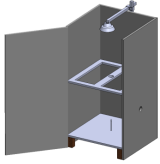
Tools necessary to achieve our goal



Benefits of the Root Image Phenotyping

- High-throughput system
- Unbiased estimates of thousands of roots
- Reliable phenotypic tool
- Easy to transport
- Data set of thousands of images that can be used to estimate new traits

Root Image Phenotyping First Generation



Problems:

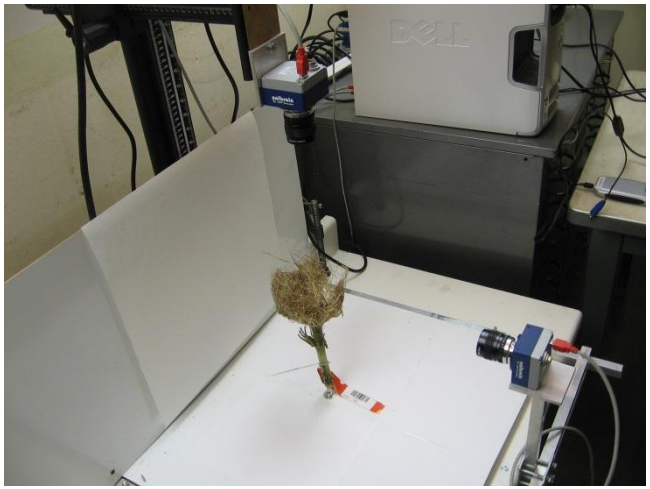
I. Box illumination
II. Box color
III. Box size
IV. Not be able to see the image when is taken

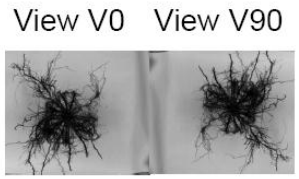
Front view

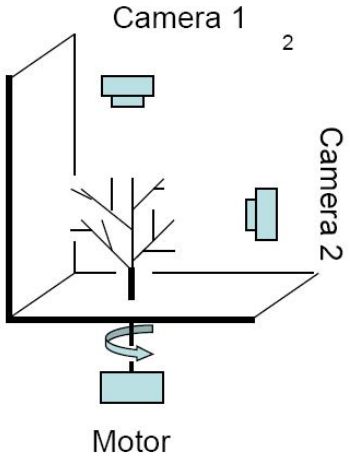
Left view

Right view

Root Image Phenotyping Second Generation







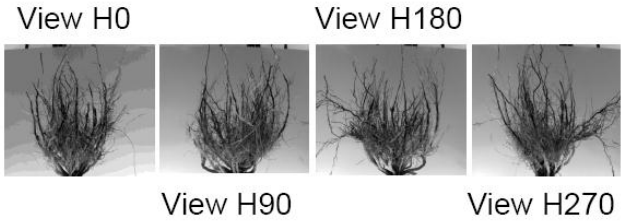
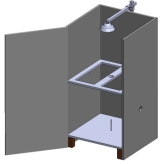


Fig. 1 Schematic representation of the images acquisition

Root Image Phenotyping Second Generation



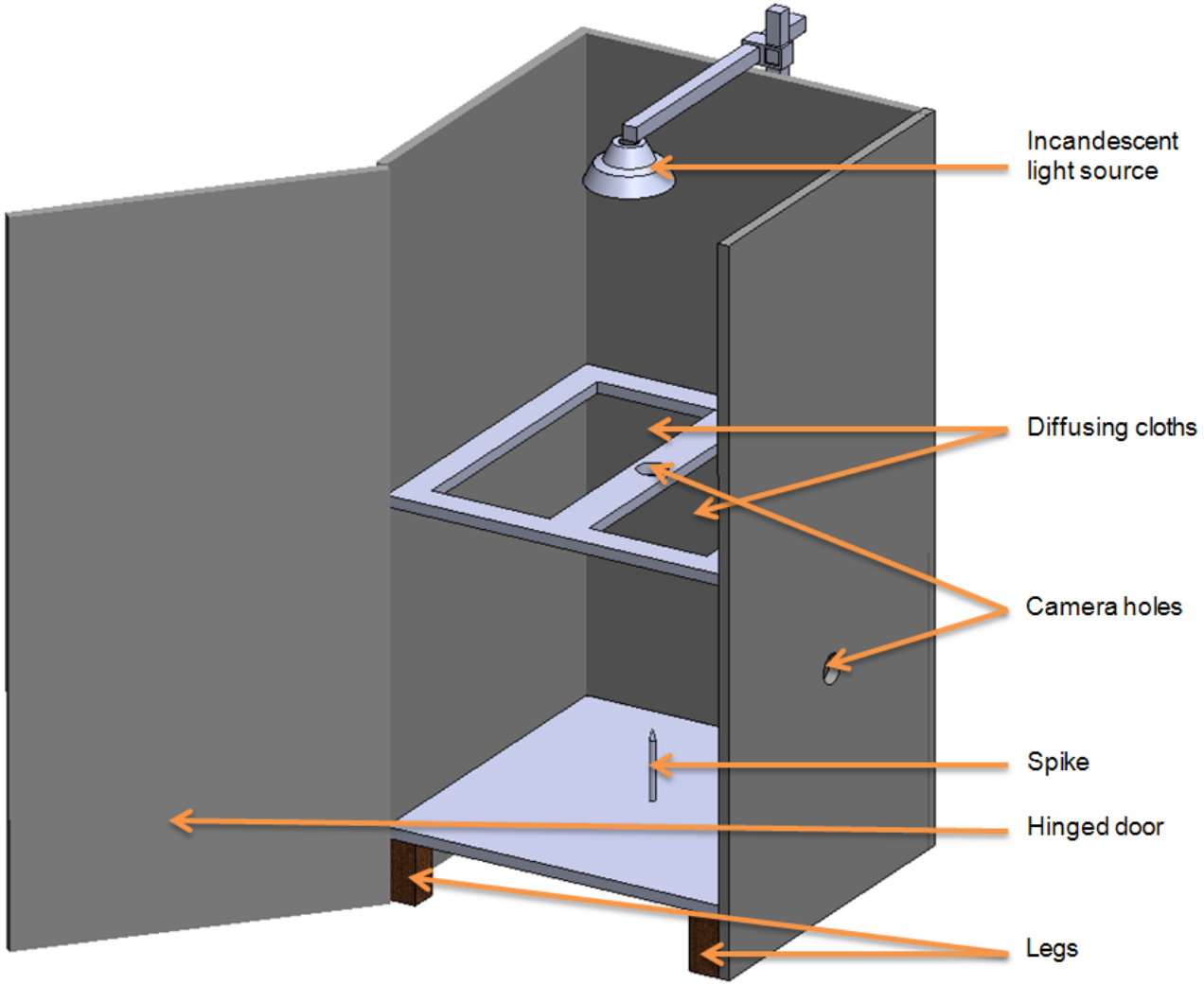
Problems

I. Even distribution of the light
II. Size
III. Not practical, needed a lot of space to assemble and a dark room

Traits measure for each image

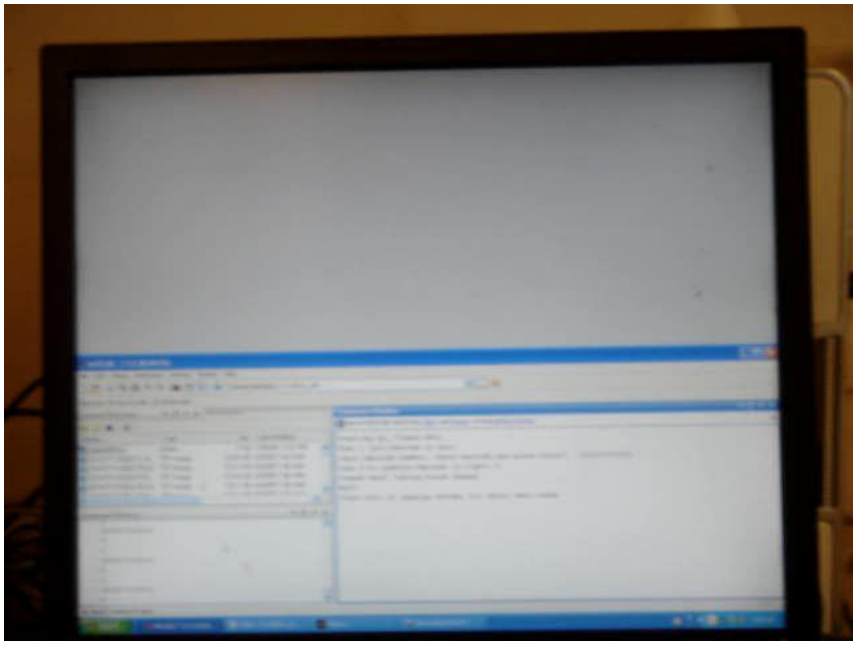
I. Fractal dimension
II. Entropy
III. Number of white pixels

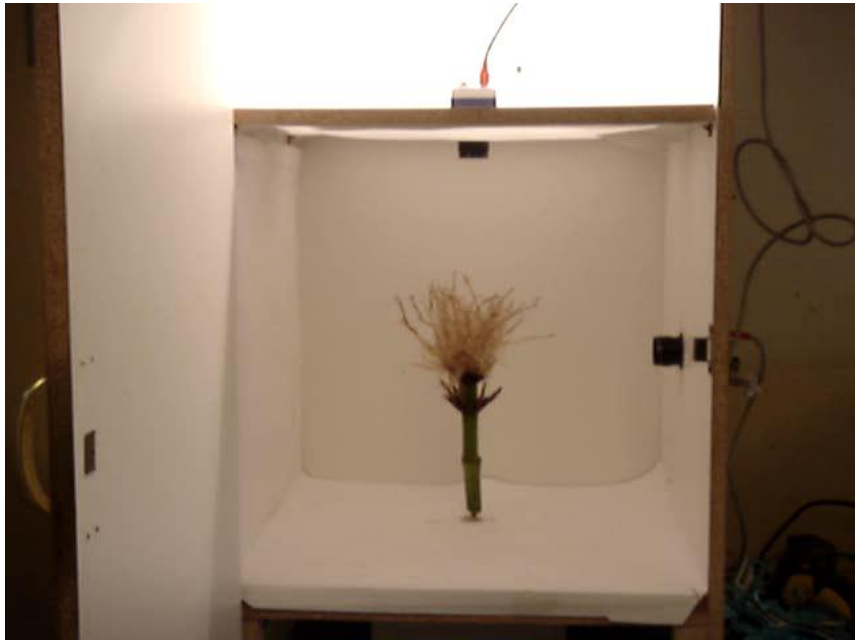
Root Image Phenotyping Third Generation



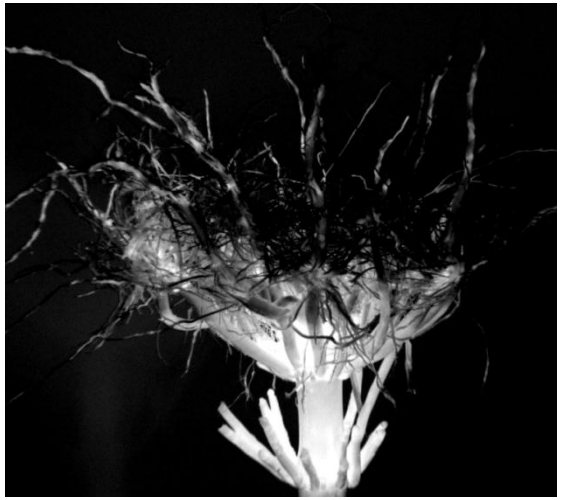
- Open: Imaging box sample.pdf

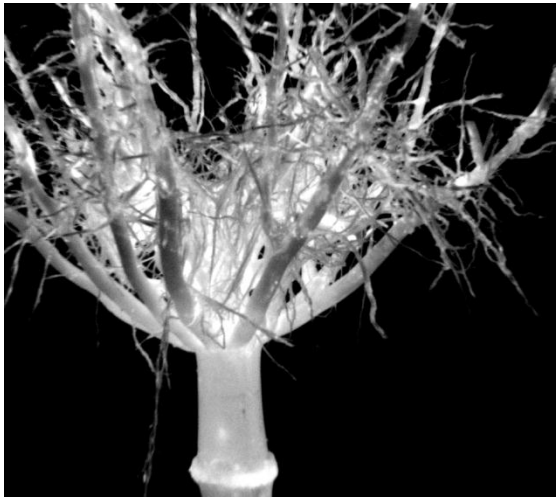
Root Image Phenotyping
Third Generation





The new images taken in the soft box (right) show much more even illumination than the older ones (left)





The root angles were measured automatically using MatLab® code. What exactly is the root angle here?

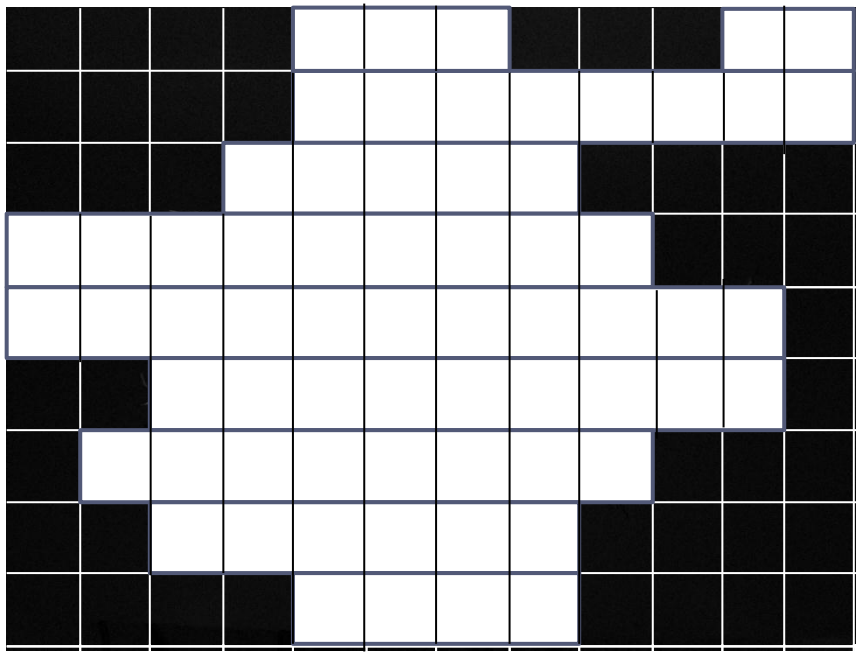
Improve Root Image Phenotyping

Traits measure for each root image:

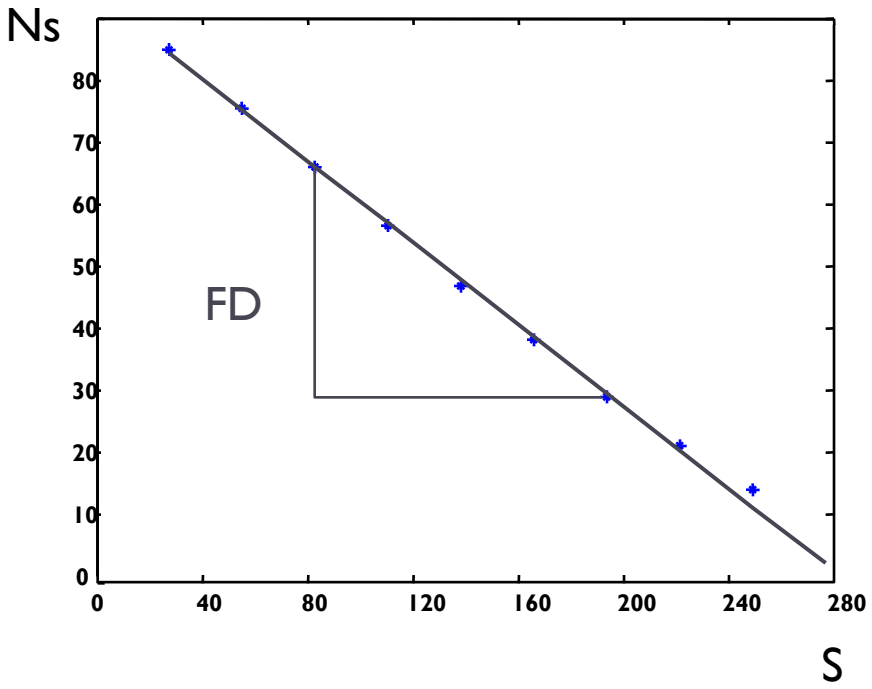
I. Fractal dimension
II. Right angle
III. Left angle
IV. Stem diameter

The Root Movie

Trait Evaluation- Fractal Dimension (**Box counting method**)



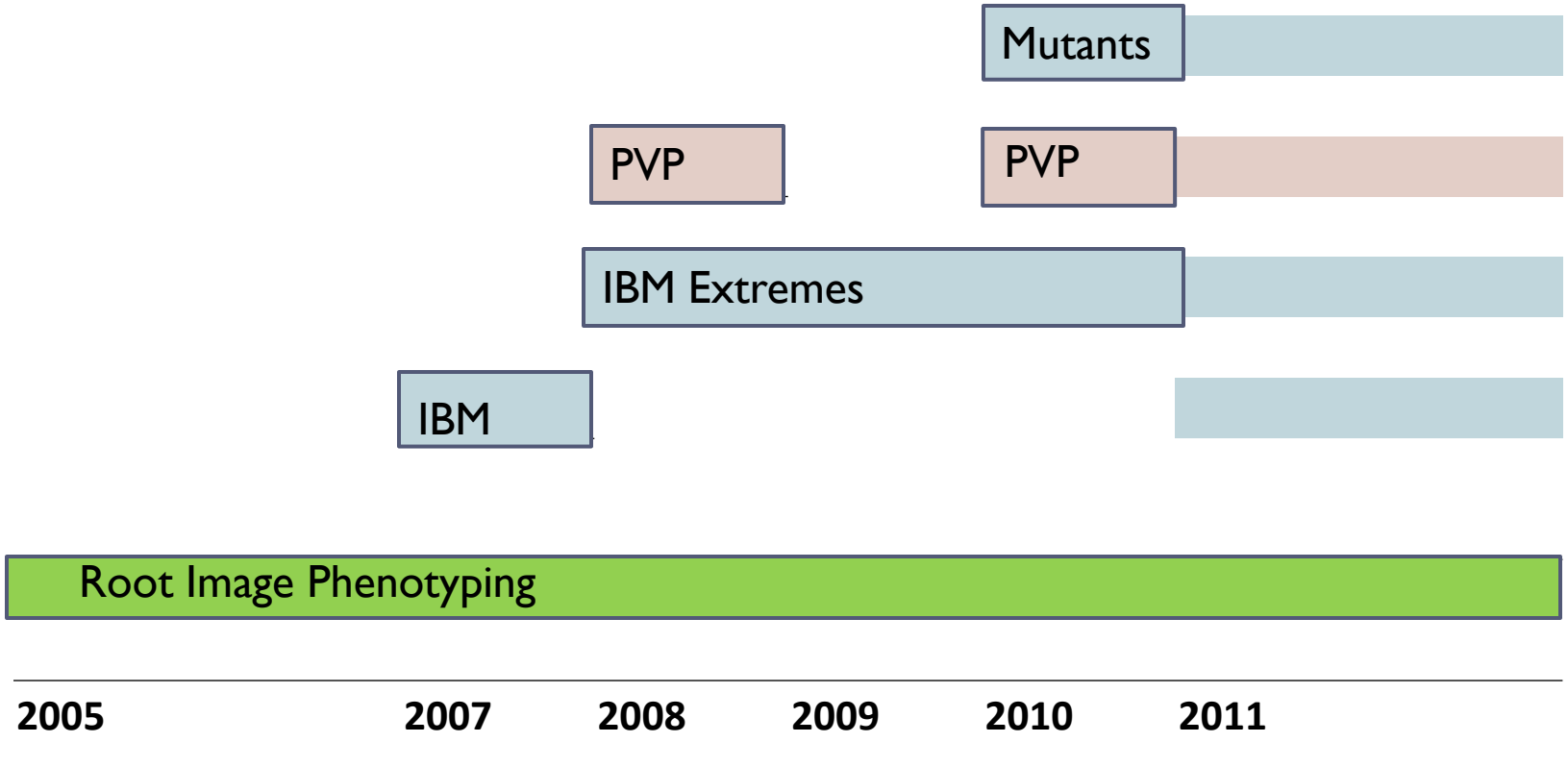
N=65



S = Side length of grid squares

N_s = Number of squares intersected by image

Experiments



Sampling

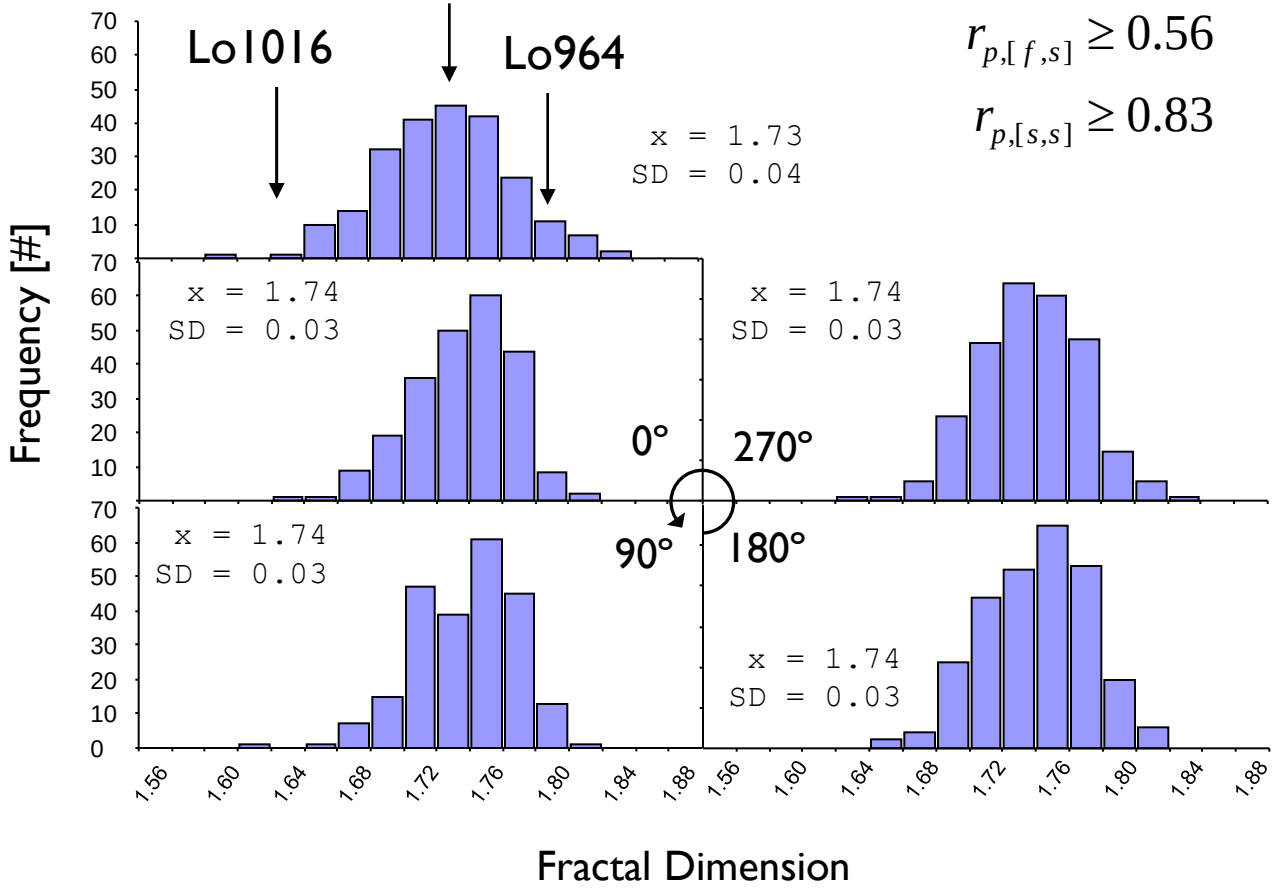
IBM – Postembryonic Root System

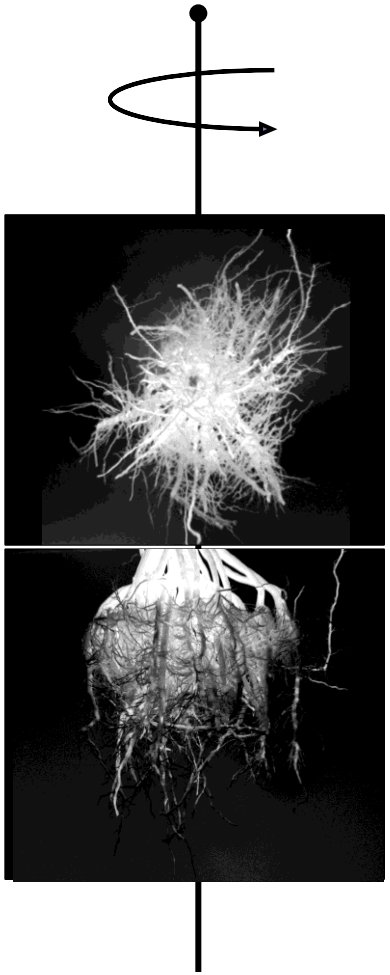
$$\text{Rep} \geq 0.57$$

$$r_{p,[f,f]} = 0.91$$

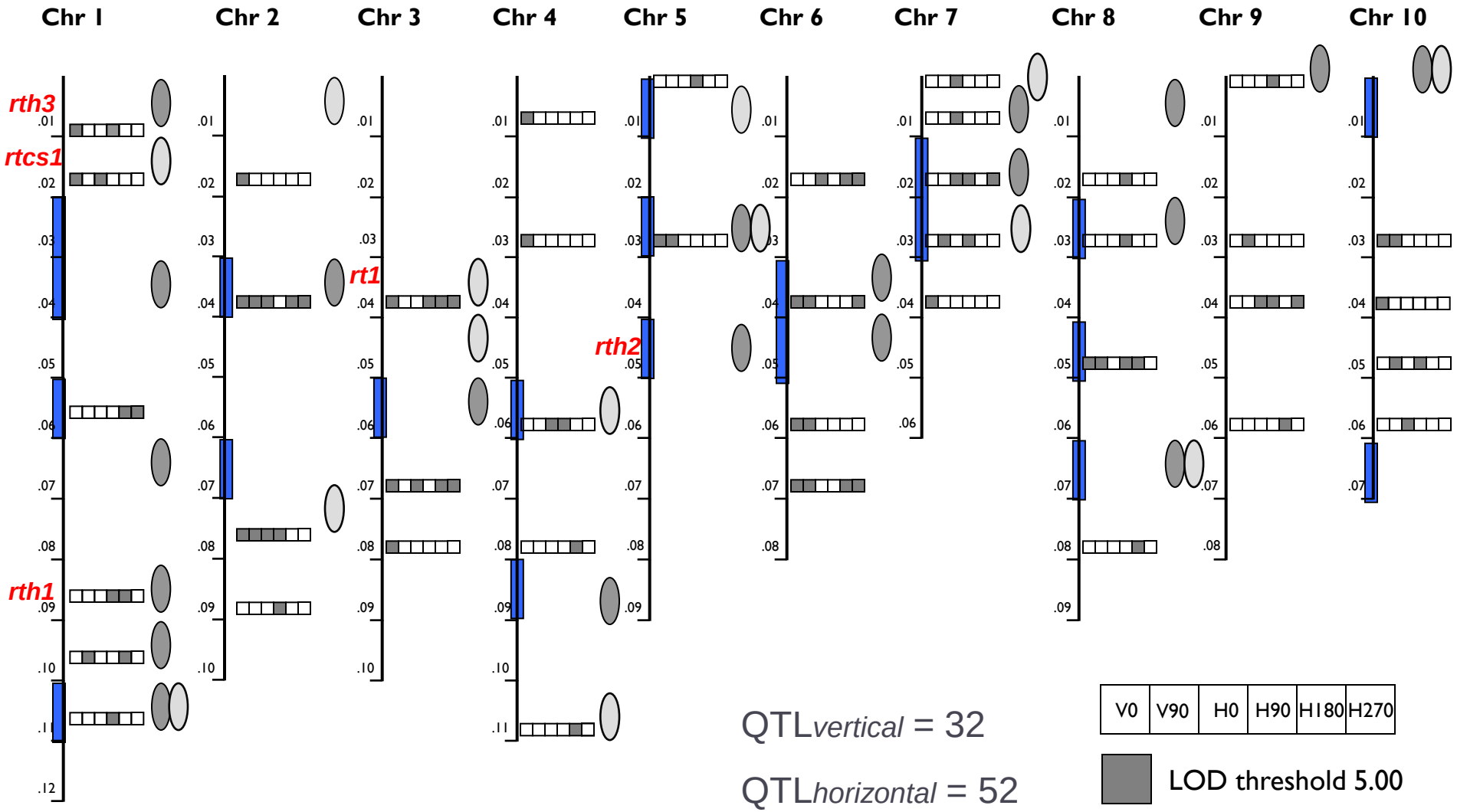
$$r_{p,[f,s]} \geq 0.56$$

$$r_{p,[s,s]} \geq 0.83$$

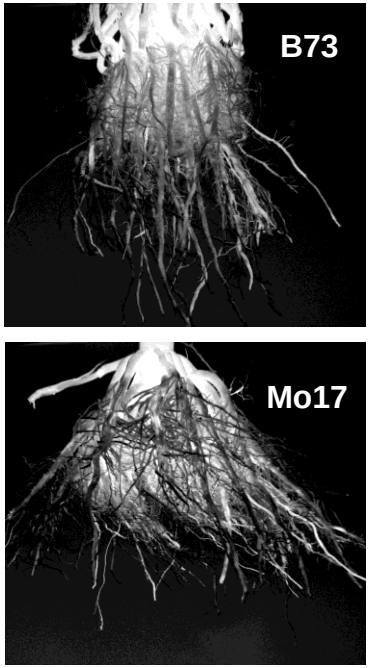




IBM - QTL Mapping Results



IBM – Root Angle



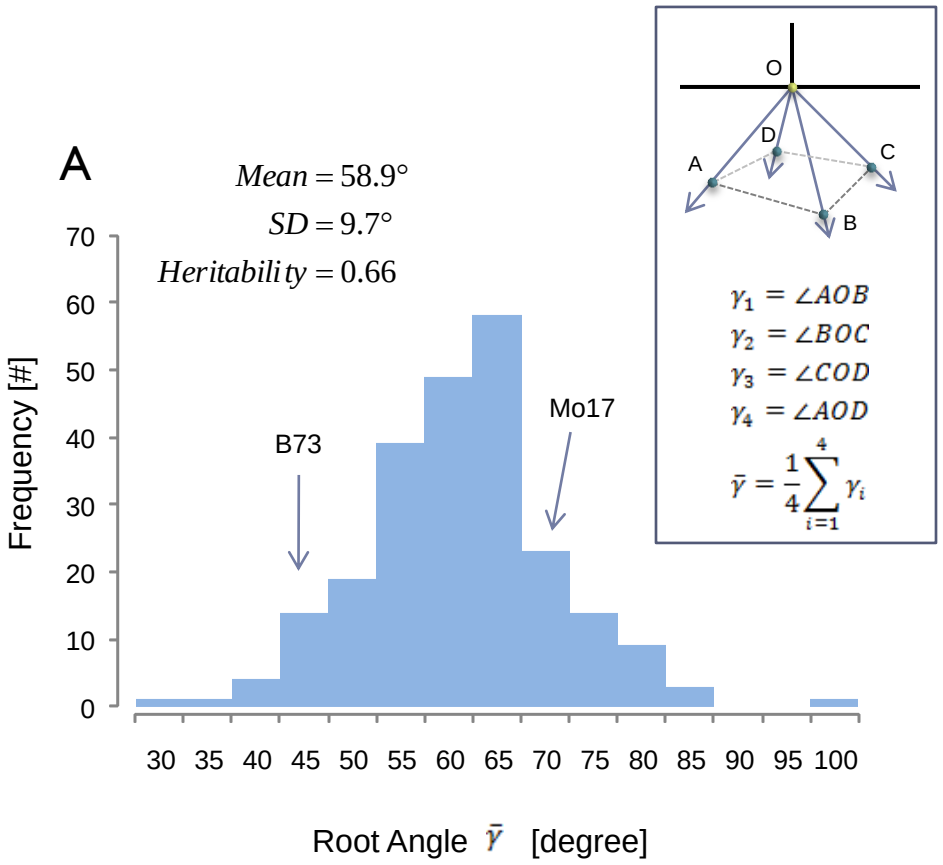


Fig. 2 A. Frequency distribution for the average root angle of adult maize roots obtained from 230 RILs of the IBM(B73×Mo17) population. **B.** Schematic explaining which root angles were used to determine the average root angle.

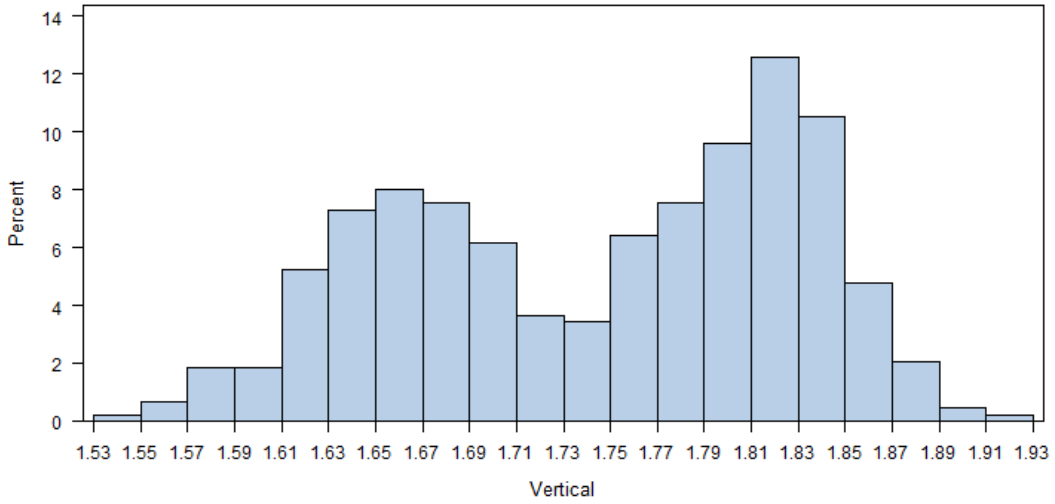
Experiment
Mutants







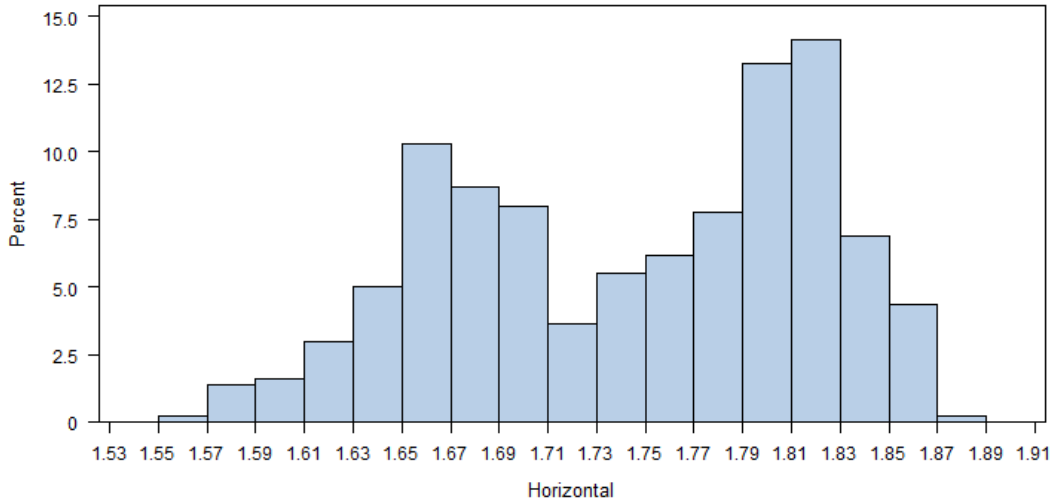

IBM Extremes
Root complexity - 2008 and 2009



$\bar{x} = 1.74$

$\sigma = 0.08$

Repeatability= 0.69



$r_{(V,H)} = 0.94$

$\bar{x} = 1.74$

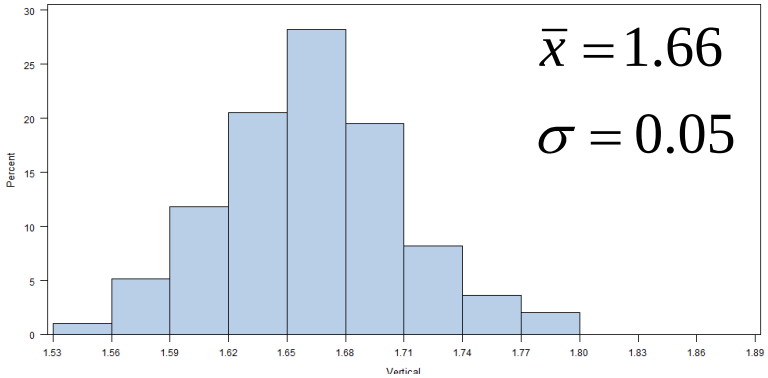
$\sigma = 0.07$

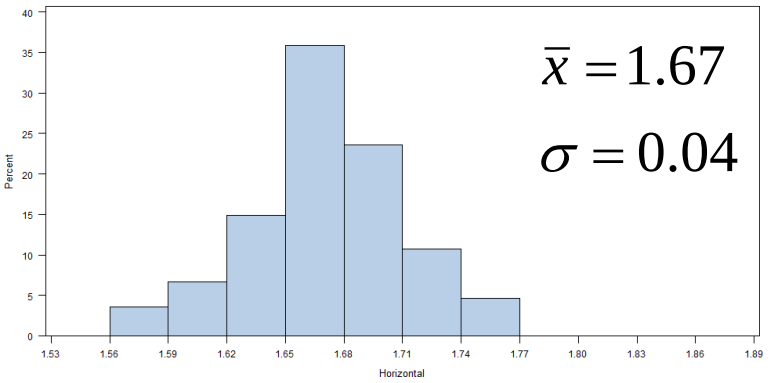
Repeatability= 0.65

Statistical Analysis -2008 and 2009 What causes the distribution?

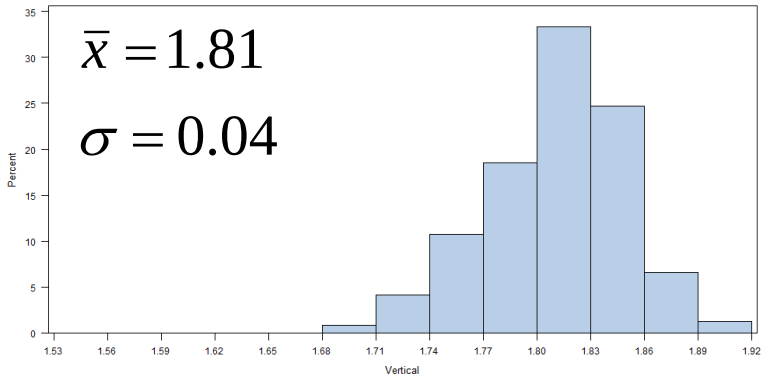
- Pedigree (p value <.0001)
- Year (p value <.0002)

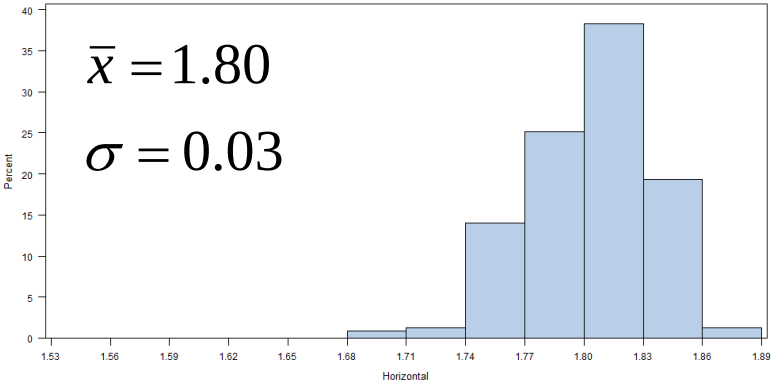
2008



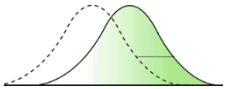


2009





Statistical Analysis - 2008 and 2009
What causes the distribution?



Year	Month	Precip. (inches)	Rain days	High temp	low temp	Mean temp	Daily max precip.	Days > 90F	Days > 32F
2008	May	6.07	15	68.8	48	58.4	1.4	0	0
2008	Jun.	6.4	11	83.4	63	73.2	2.46	0	0
2008	Jul.	7.89	15	83.8	63.7	73.8	1.88	0	0
2008	Aug.	0.79	9	82.4	61.7	72.1	0.34	1	0
2009	May	5.71	14	73.9	52.6	63.3	1.44	0	0
2009	Jun.	4.42	14	84.5	64.6	74.6	1.13	10	0
2009	Jul.	6.3	12	79.2	60.8	70	1.67	0	0
2009	Aug.	5.62	12	80.5	59.8	70.2	2.67	3	0

Precipitation - Amount of precipitation recorded, in inches.
Rain Days - Number of days that rain occurred.
High Temp - Average high temperature recorded, in degrees Fahrenheit.
Low Temp - Average low temperature recorded, in degrees Fahrenheit.
Mean Temp - Average mean temperature recorded, in degrees Fahrenheit.
Daily Max Precipitation - Highest amount of precipitation recorded during one day, in inches.
Days 90 - Number of days that the high temperature was at or above 90 degrees.
Days 32 - Number of days that the low temperature was at or below 32 degrees.

- http://www.isws.illinois.edu/data/climatedb/dataex.asp

Summary

- Characteristics of the Improved Methodology
  - Field based
  - Time efficient
  - Repeatable
  - Unbiased measurements
  - Image-based (database)
  - Applicable to other crops

  - Correlate root complexity with agronomic performance

Summary

- The high-throughput Root Image Phenotyping provides the technical basis for a systematic investigation of the maize root complexity.
- The tight correlations between the fractal dimension values determined for non-overlapping views provide circumstantial evidence of self-similarity.
- Root angle, independent from fractal dimension.

Summary

- Applying QTL mapping approach we were able to identify:

  - chromosome regions in the maize genome carrying putative genes for root complexity

  - QTL regions associated with root mutants

- Increase in knowledge about the genetic relationship between root complexity and agronomic characters could accelerate the development of improved maize cultivars.

Acknowledgements

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  - Dr Martin Bohn
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ACES
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MONSANTO

Questions?