Understanding Storage Root Development: classical and molecular

A. Villordon, D. LaBonte, J. Solis, C. McGregor, and N. Firon

Goals….

√ **Background on storage root development**

- **-morphology and anatomy**
- **-environmental factors**
- **-hormonal control and more anatomy**
- **-genes involved in root formation**

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√ **Background on storage root development**

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√ **Where do we go with genomics**

Goals….

√ **Background on storage root development**

-anatomy
-environmental factors
-hormonal control and more anatomy
-genes involved in root formation

√ **Where do we go with genomics**

√ **Basics in a sweetpotato breeding program**

What we don’t want:

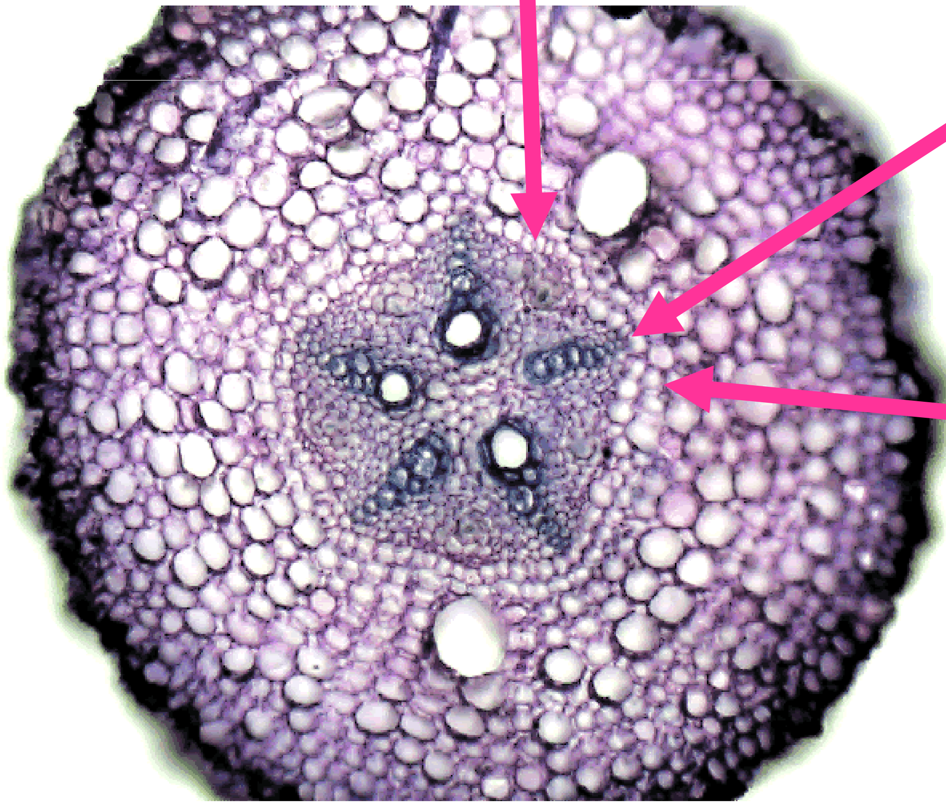
What we want:

Gross morphology: 7 days after planting

Adventitious roots

Terminology: adventitious root cross-section

Stele = vascular cylinder + pericycle



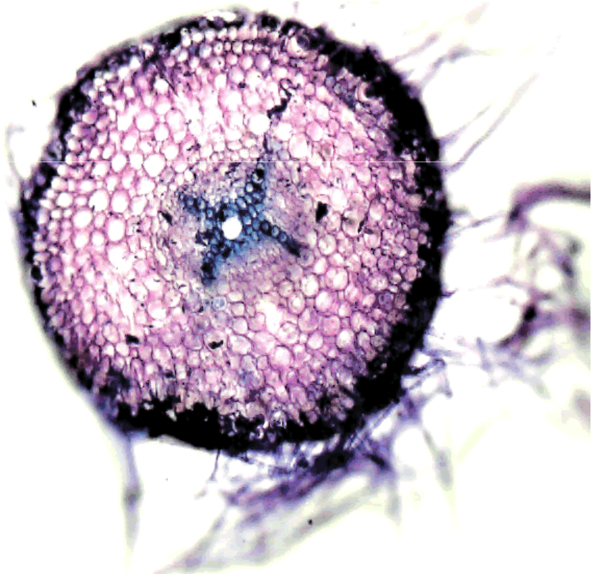
Pericycle - lateral roots, cambial activity

Endodermis – Casparian strip, forces symplastic movement

Fleshy root development – things we know

√ **Storage roots need a polyarch xylem ray patterr**

Pre-requisite for a fleshy root



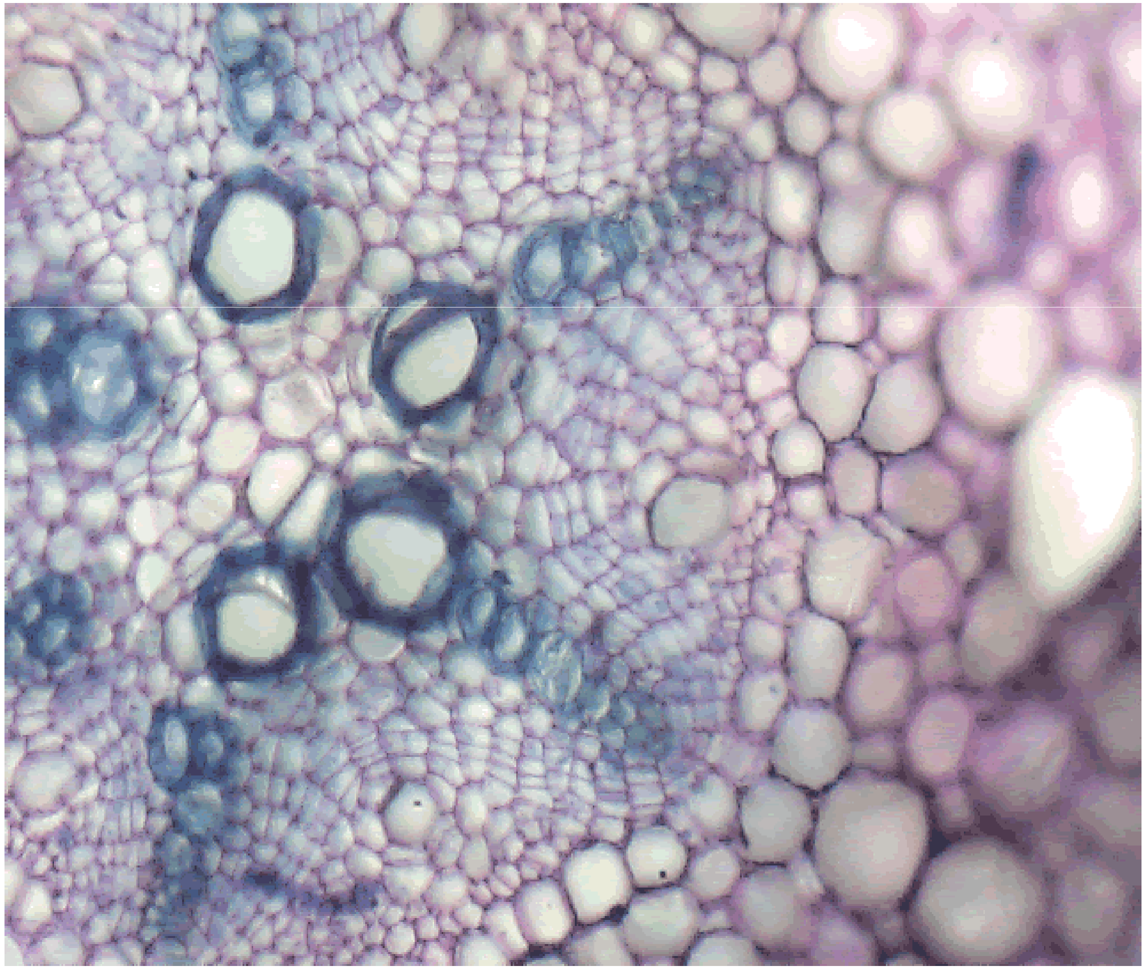
Can't be a fleshy root!

Gross morphology: 13 days after planting

Adventitious roots

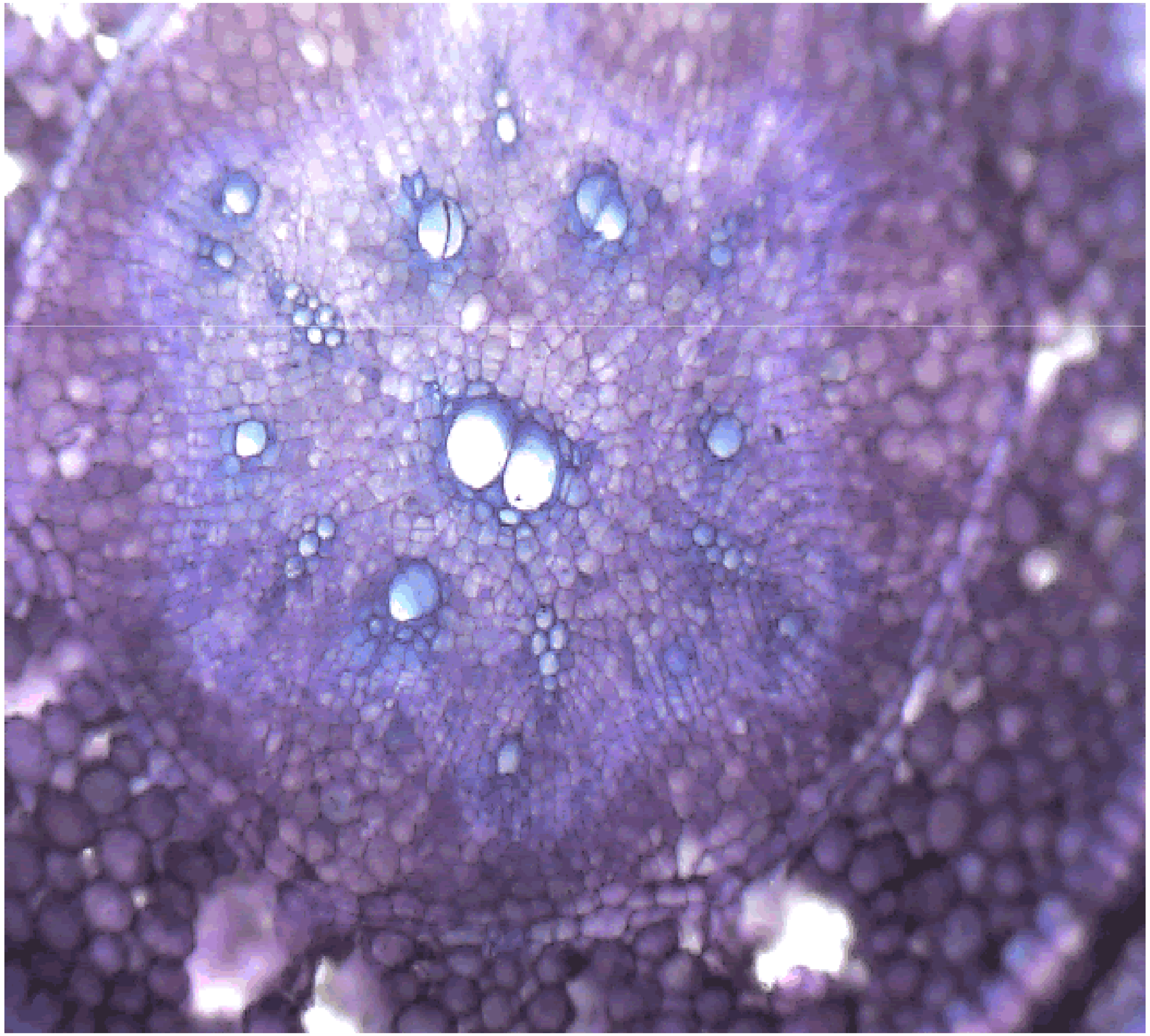
Fleshy root development – things we know

√ Formation of primary vascular cambium



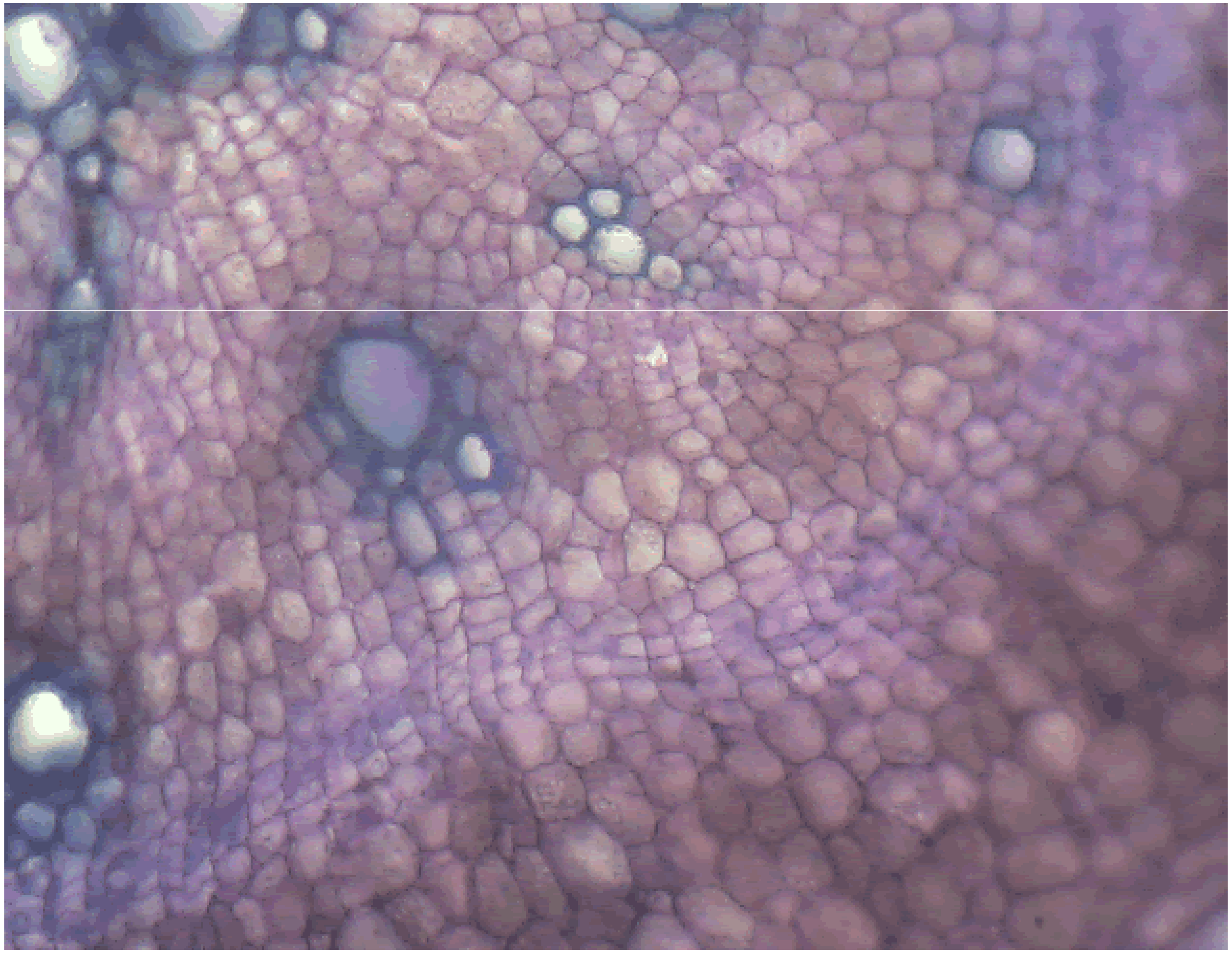
Fleshy root development – things we know

√ Complete vascular cambium



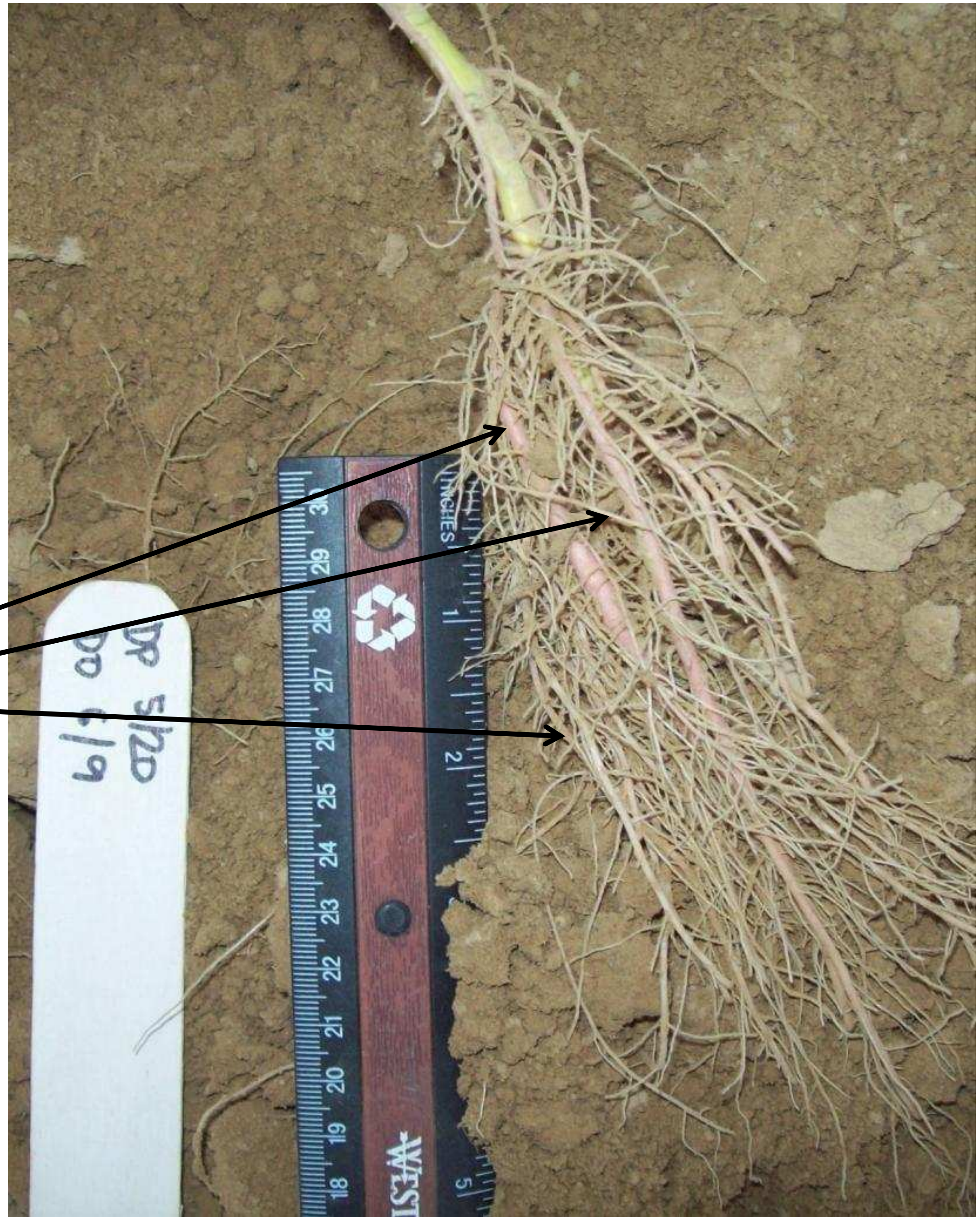
Fleshy root development – things we know

√ Formation of anomalous cambia



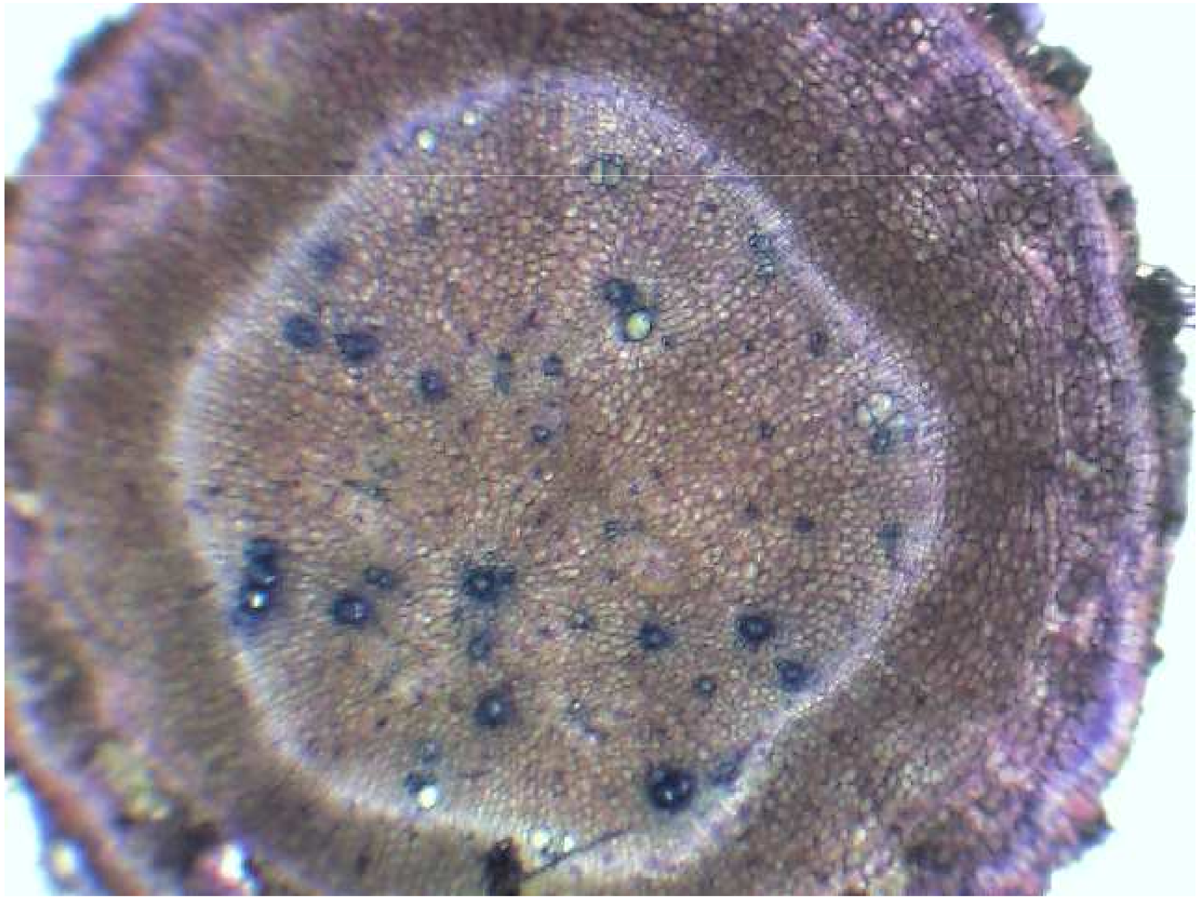
Gross morphology: 20 days after planting

Adventitious roots



Fleshy root development – things we know

√ Further development of primary and Anomalous cambia



Gross morphology:
115 days after planting:

genetic potential
w/ optimal
growing environment

Storage root

Gross morphology:
115 days after planting:

Similar genetic component, poor growing nvironment

What we want:

Knowledge that leads to optimum results

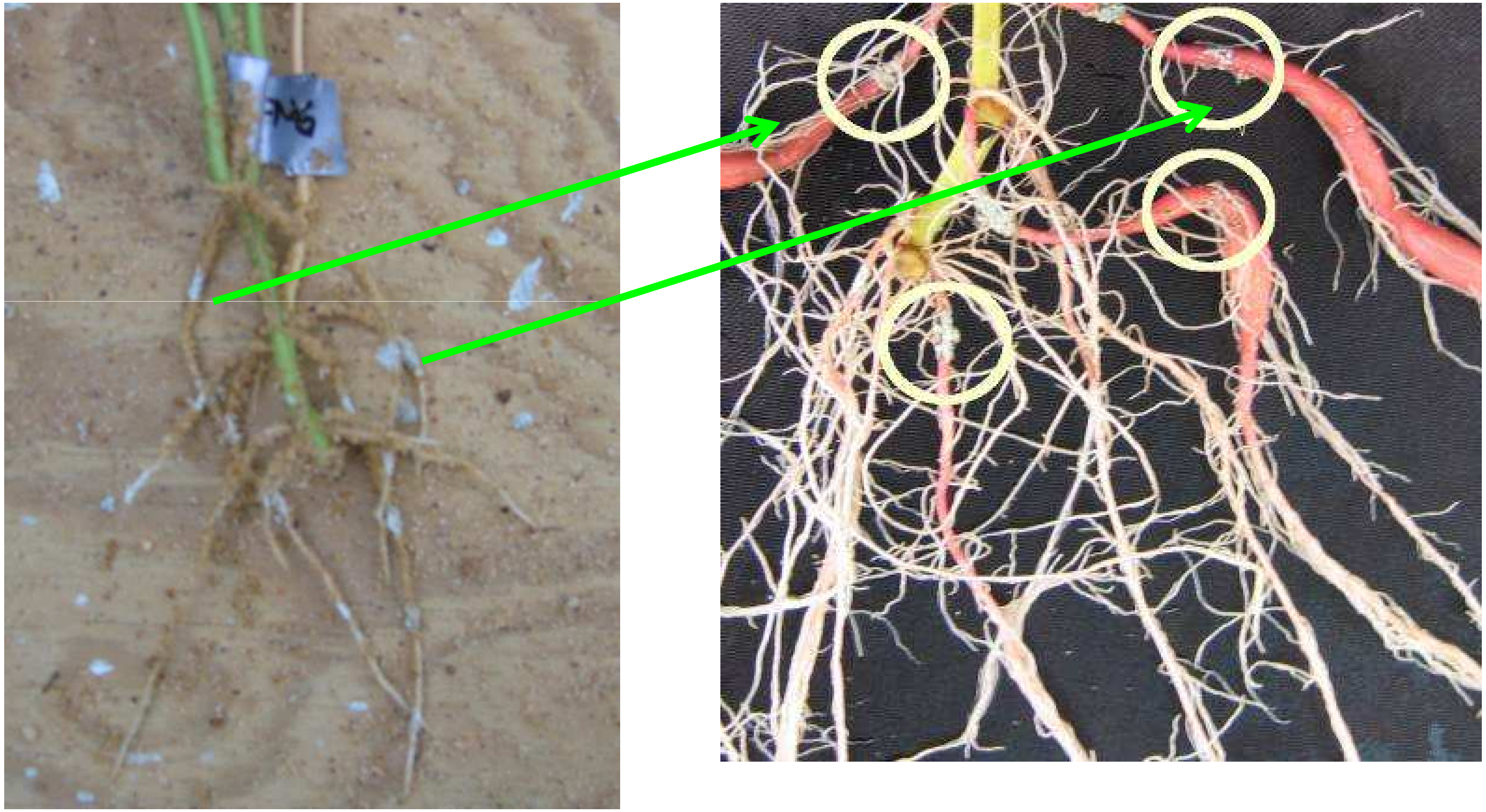
What happens to adventitious roots between emergence-storage root formation and harvest?

?

?

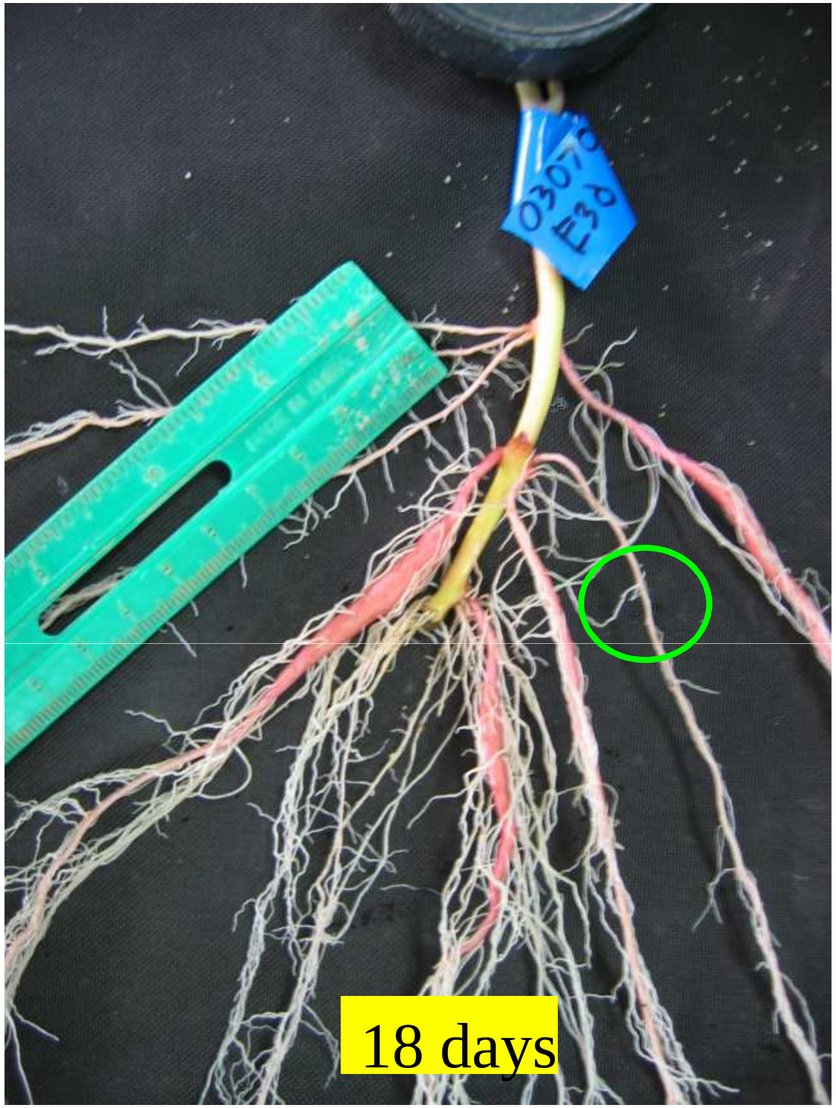
Where do storage roots come from?

90% of storage roots harvested between 100-120 days can be traced directly to adventitious roots at 3-7 days

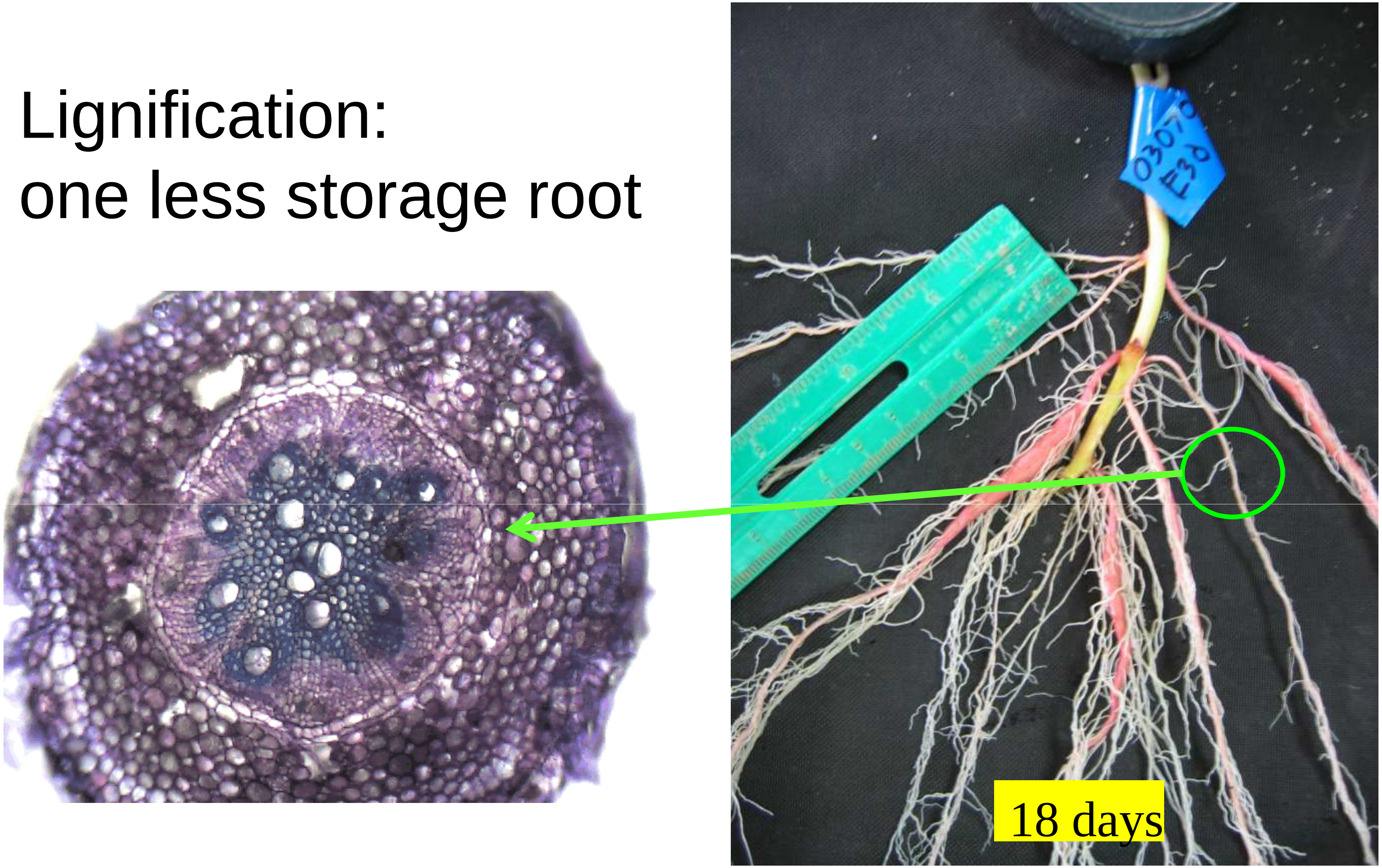


Villordon et al., 2009

Lignification:
one less storage root



Lignification:
one less storage root



Lignification:
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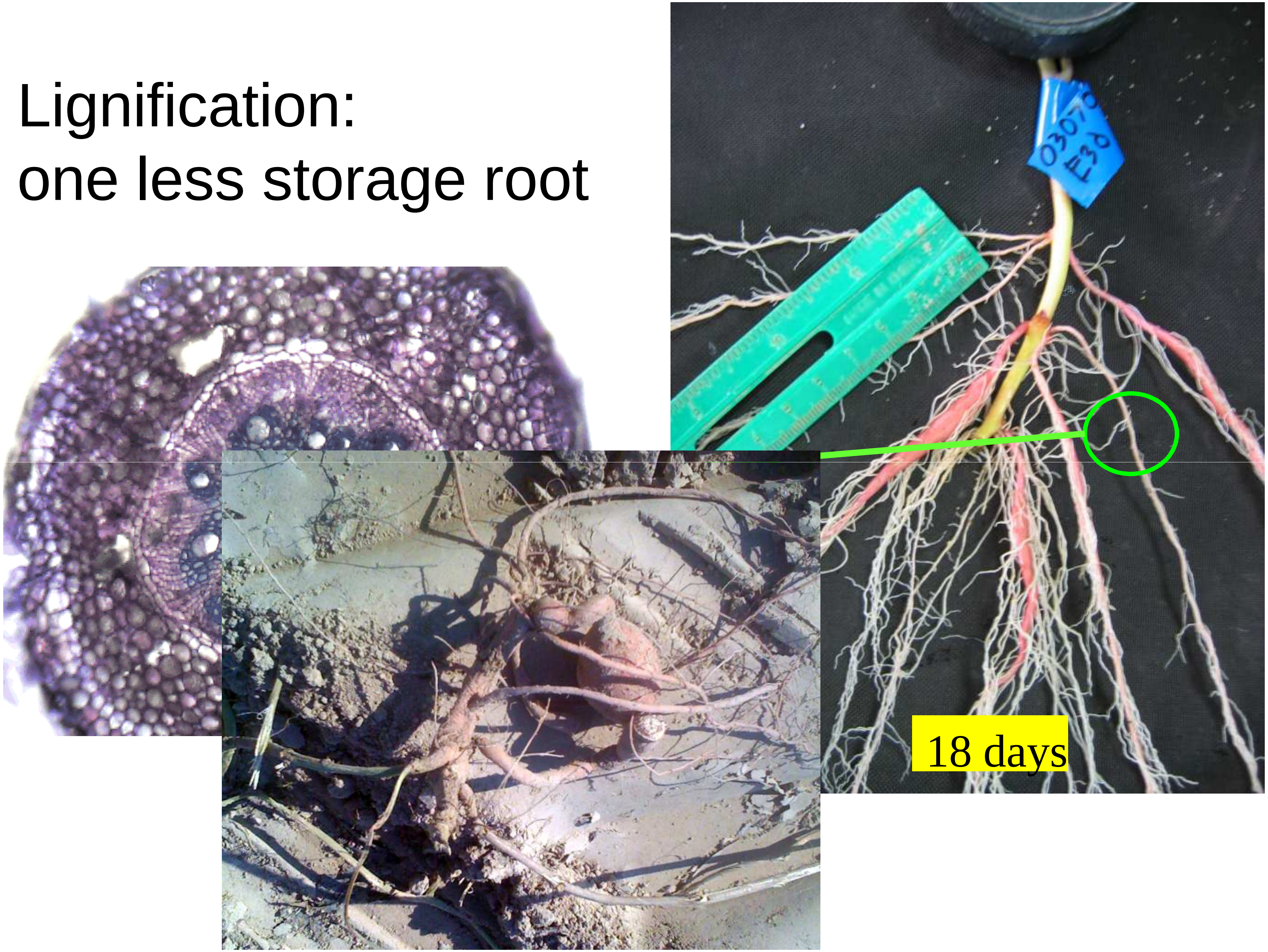
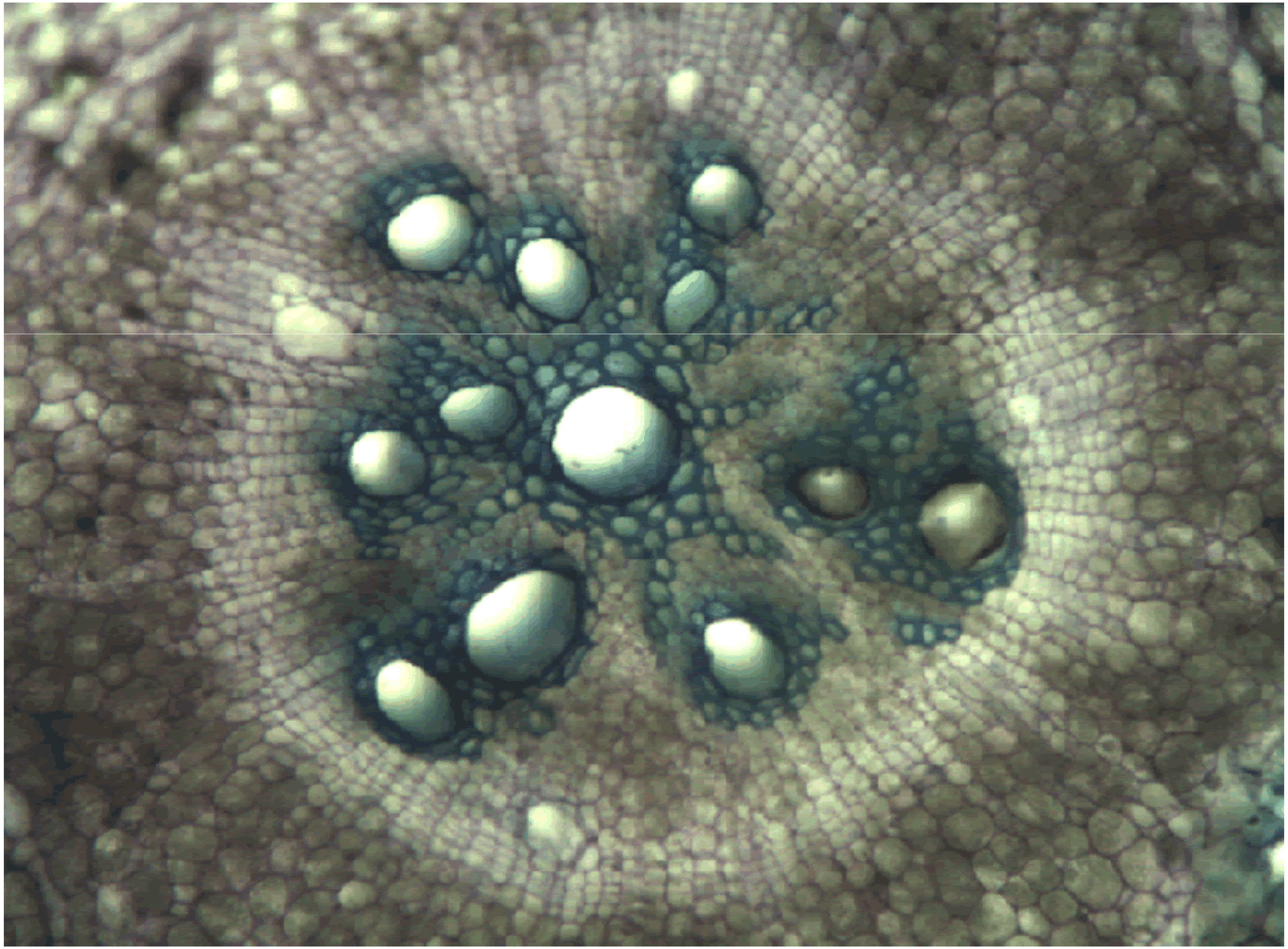
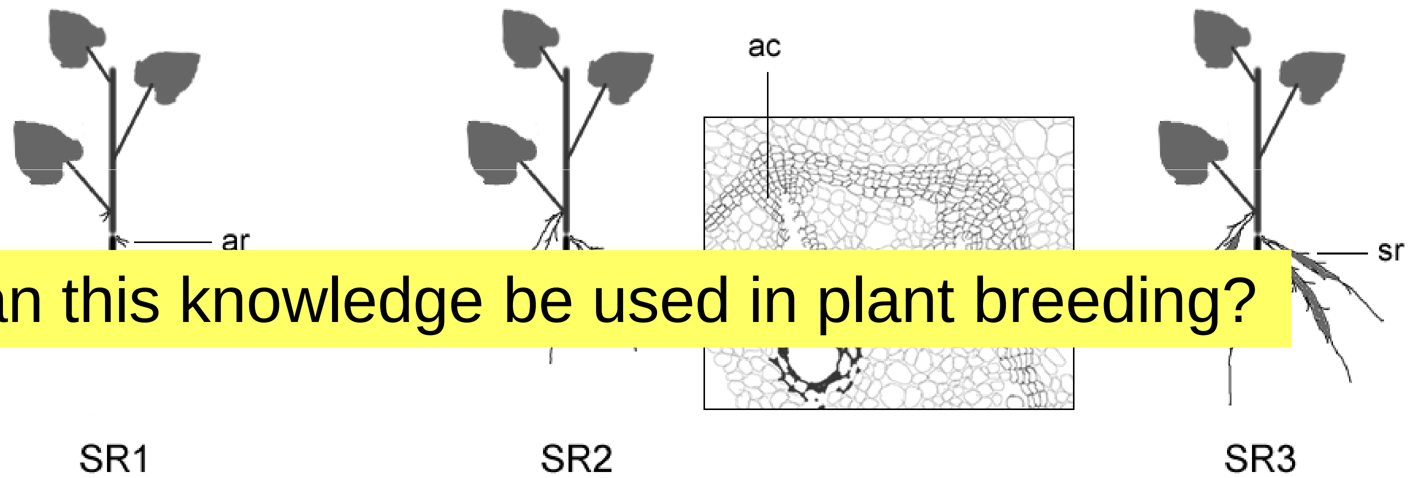


Fleshy root development – things we know

√ Lignification stops the process



Critical phenological stages leading to storage root yield determination



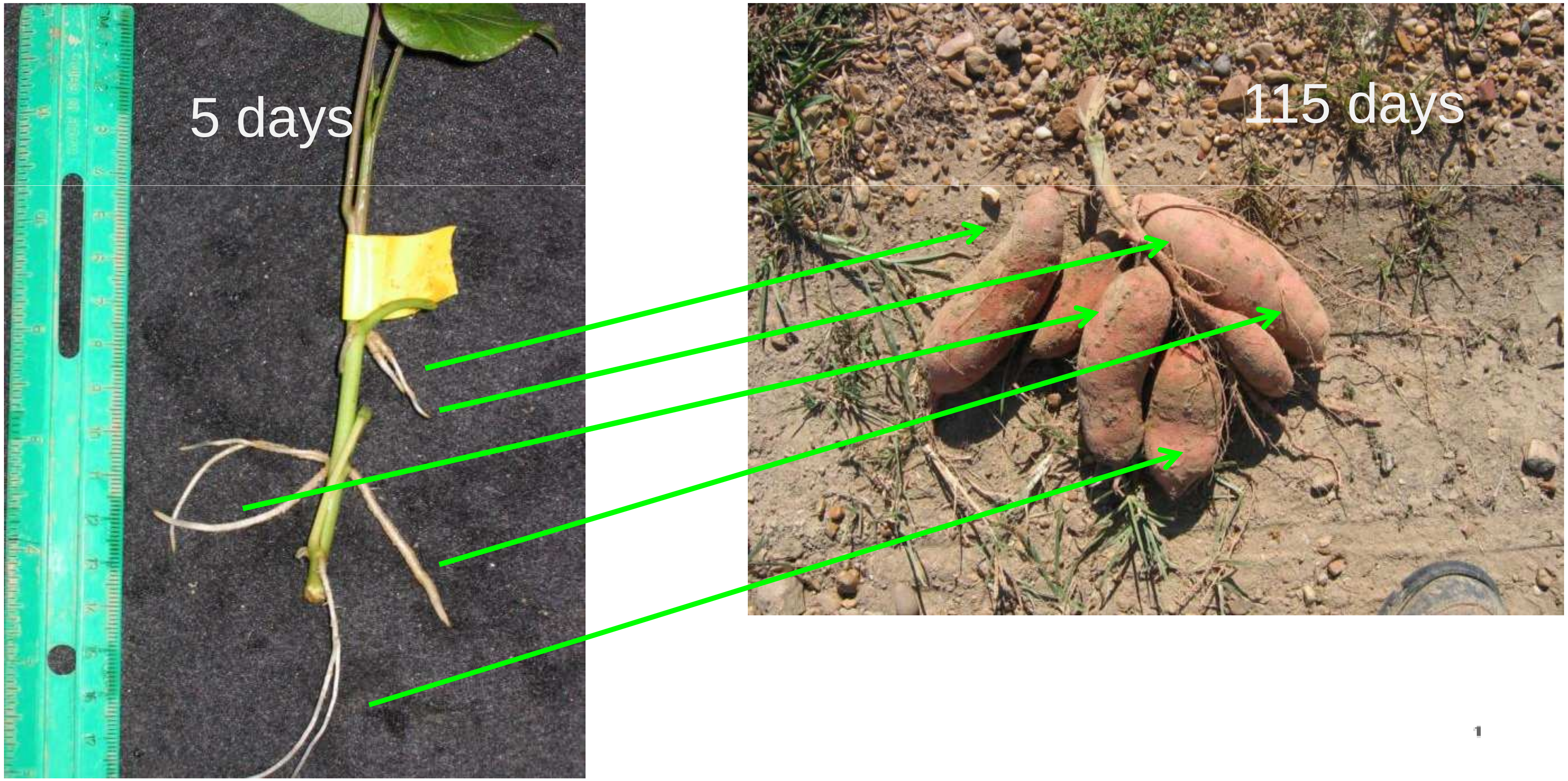
Can this knowledge be used in plant breeding?

3-7 days   13-18 days   26-32 days

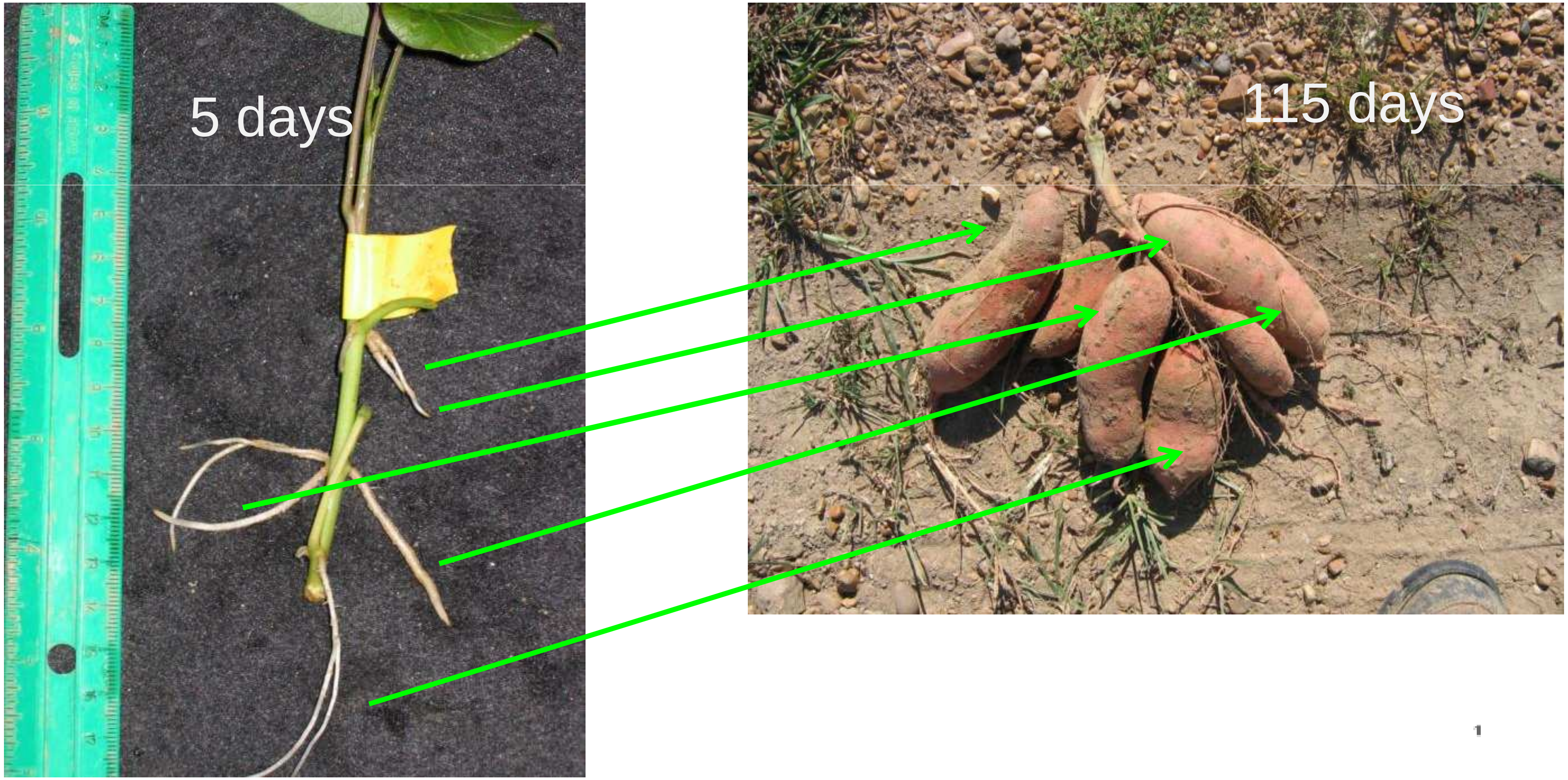
Villordon et al., 2009b

Could it be as simple as this?

Yes and No



Plant breeder – optimize genotype to ensure that phenology of the crop is well matched to the resources and constraints of the production environment

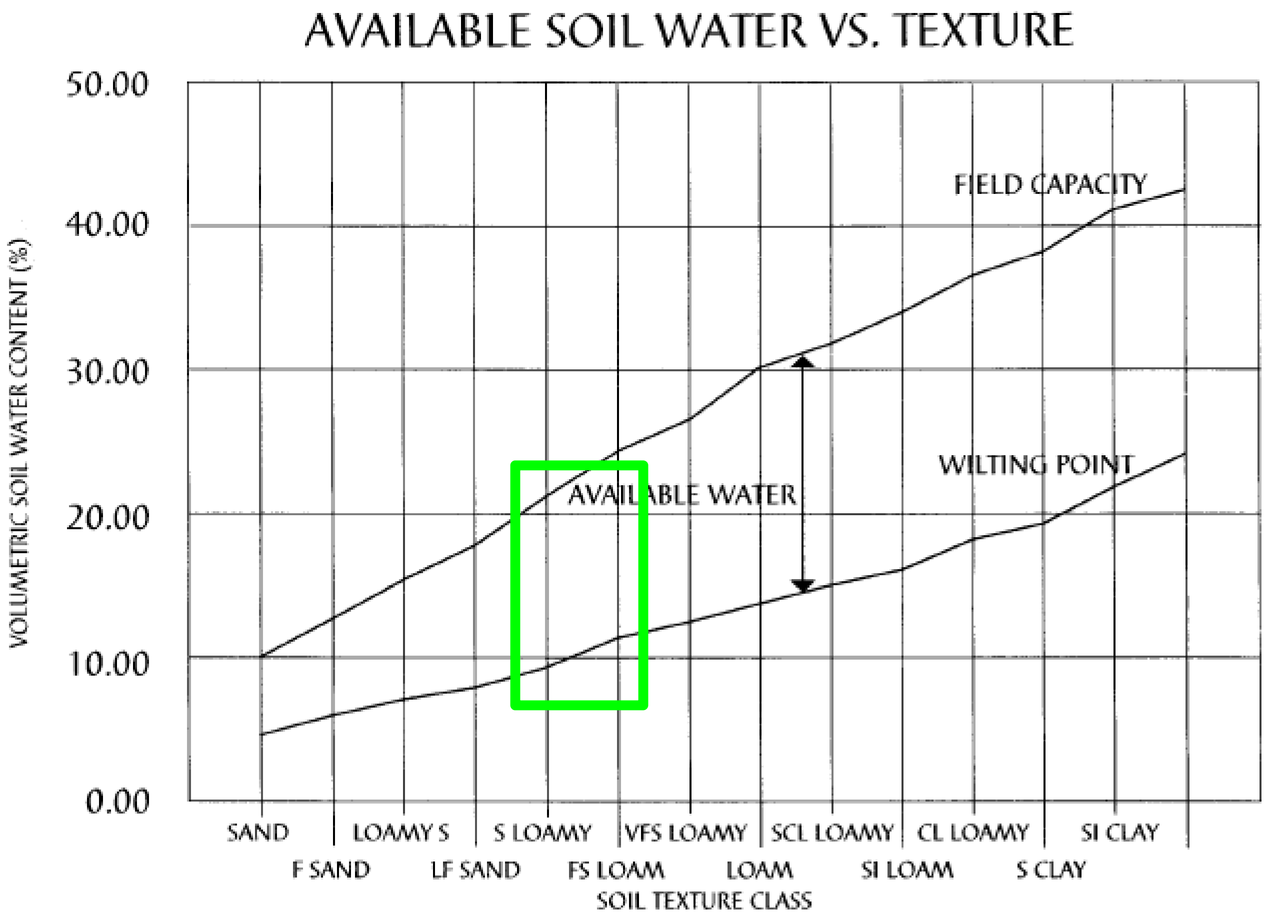


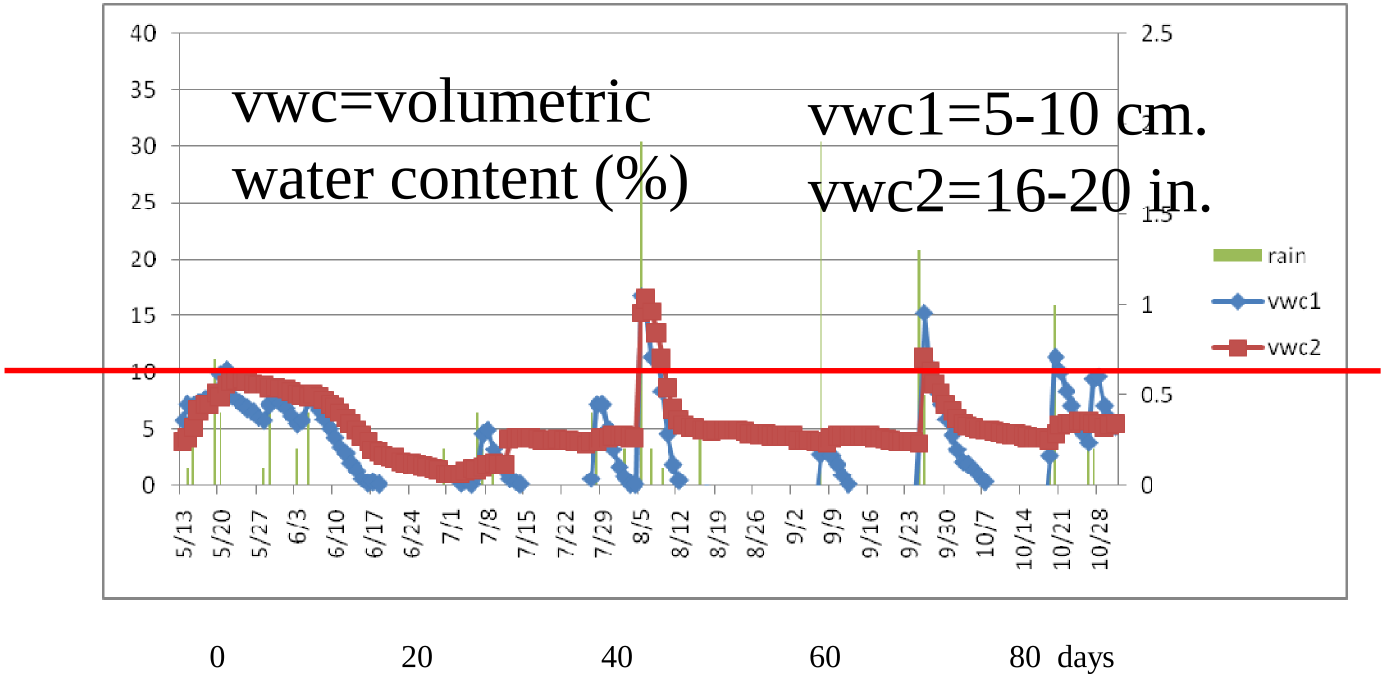
What factors are important?

storage root initiation
vs. lignification against
a background of extreme
agroclimatic variability
(drought stress,
above-average temperatures)

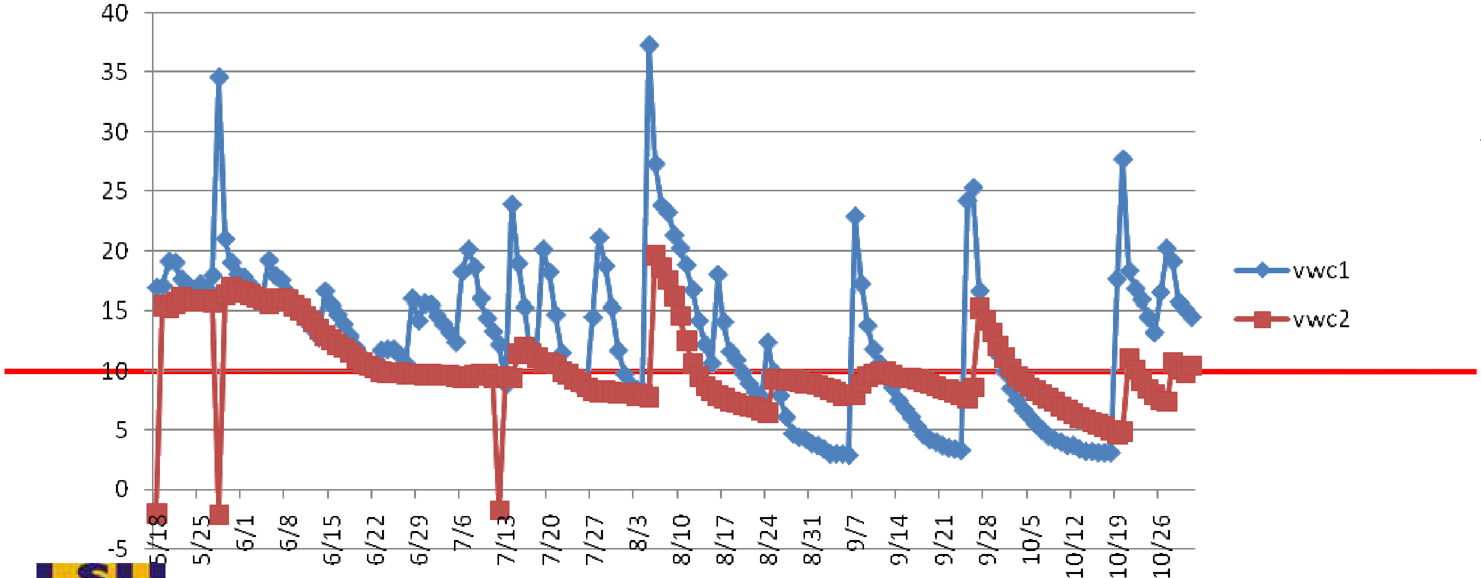
Agricultural drought:

Soil drought occurs if an average soil water content is below the soil water characterized by permanent wilting point (Sutor et al. 2005)



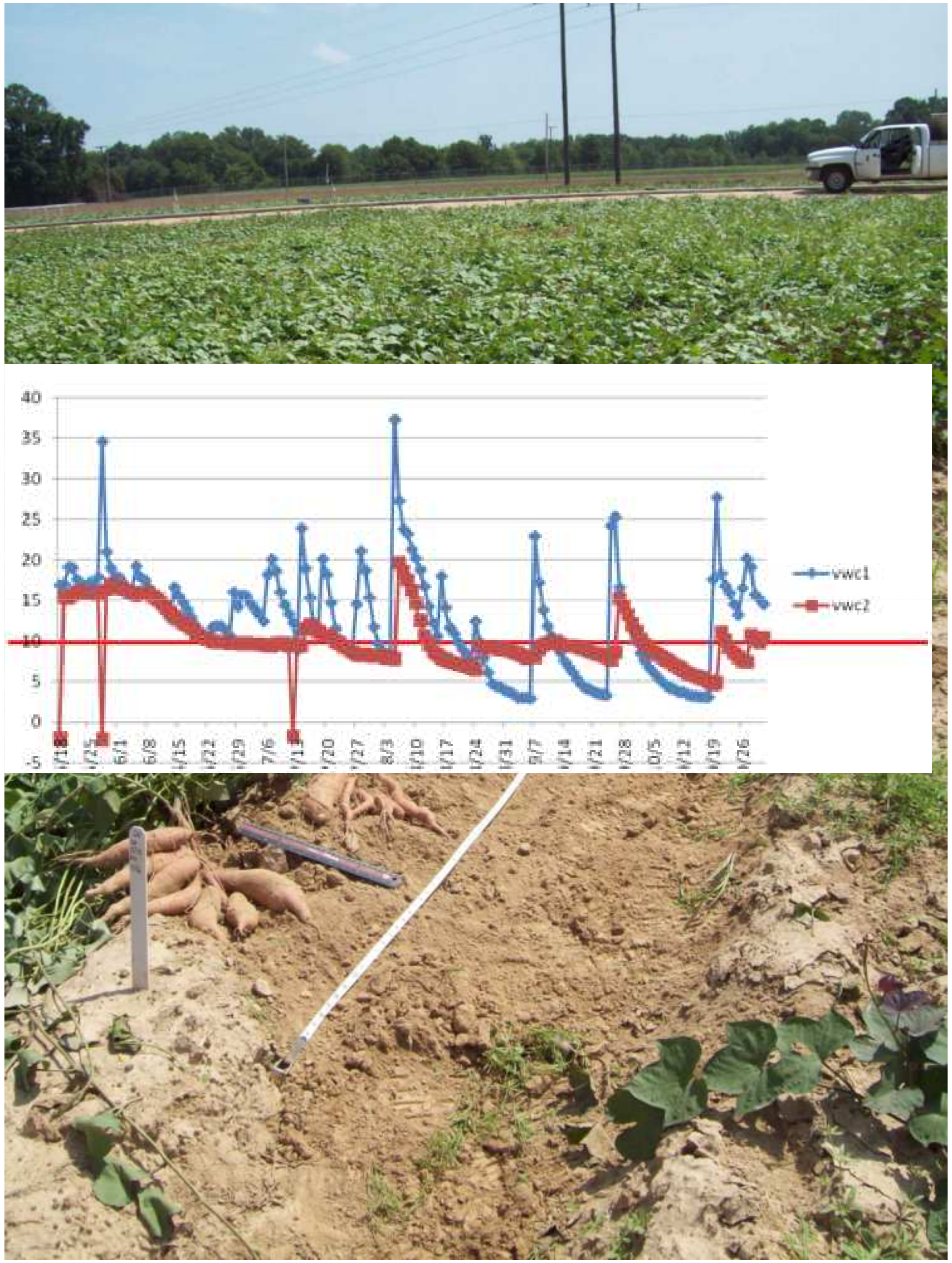


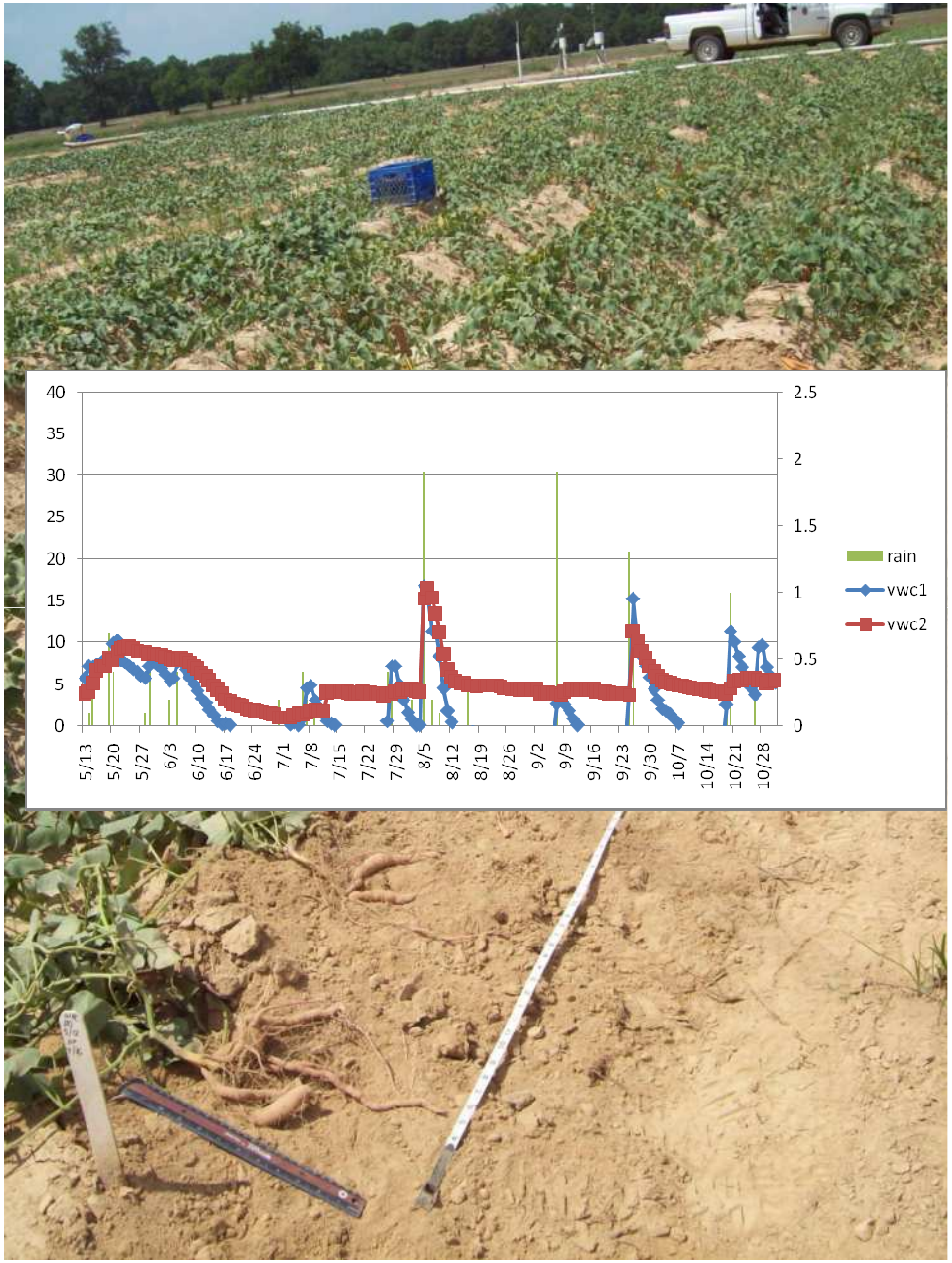
Drought,
16 to 40 days,
50 to 70 days



Drought towards the end of season?

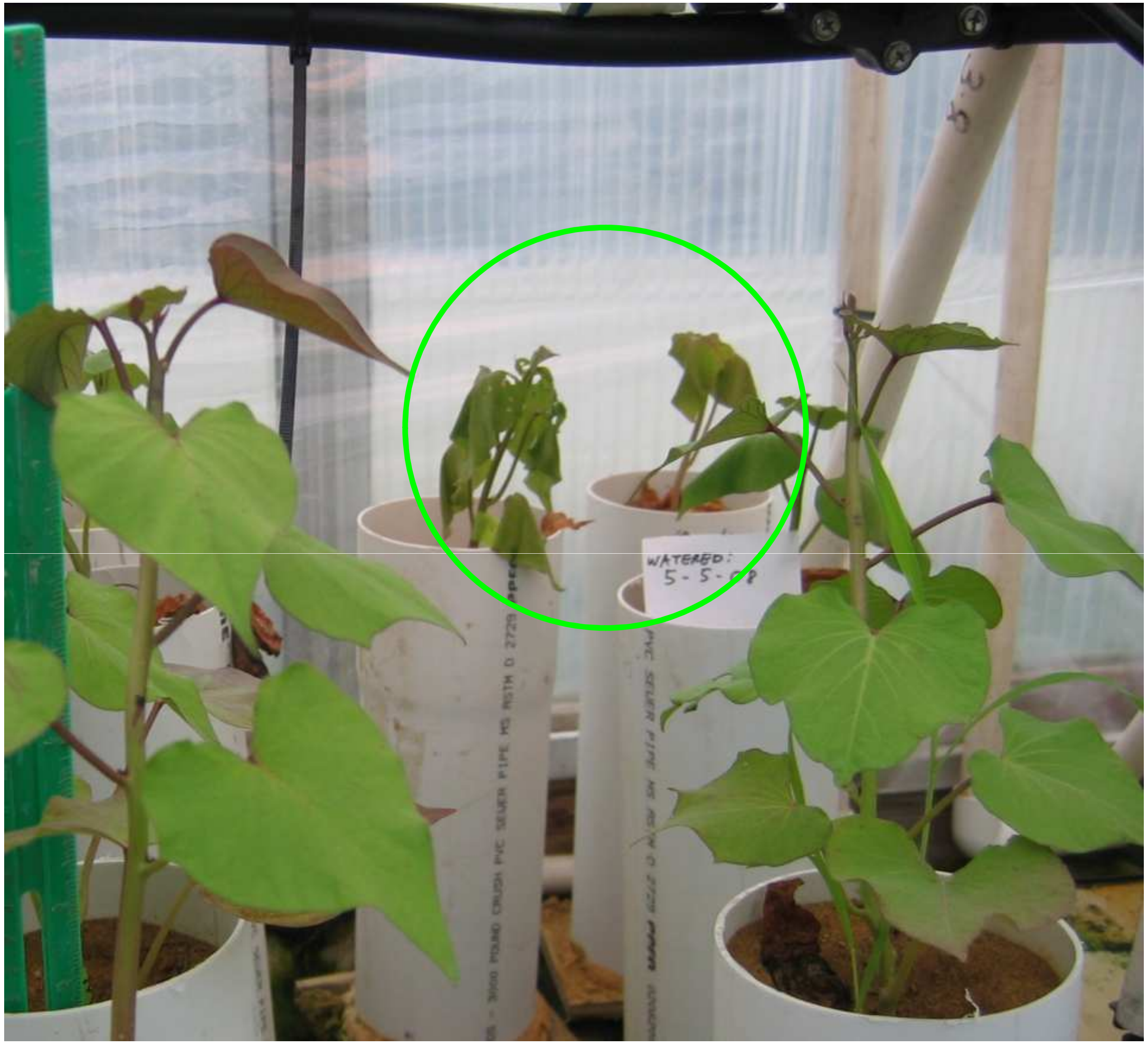
Planted: 5/12, Observed 7/1



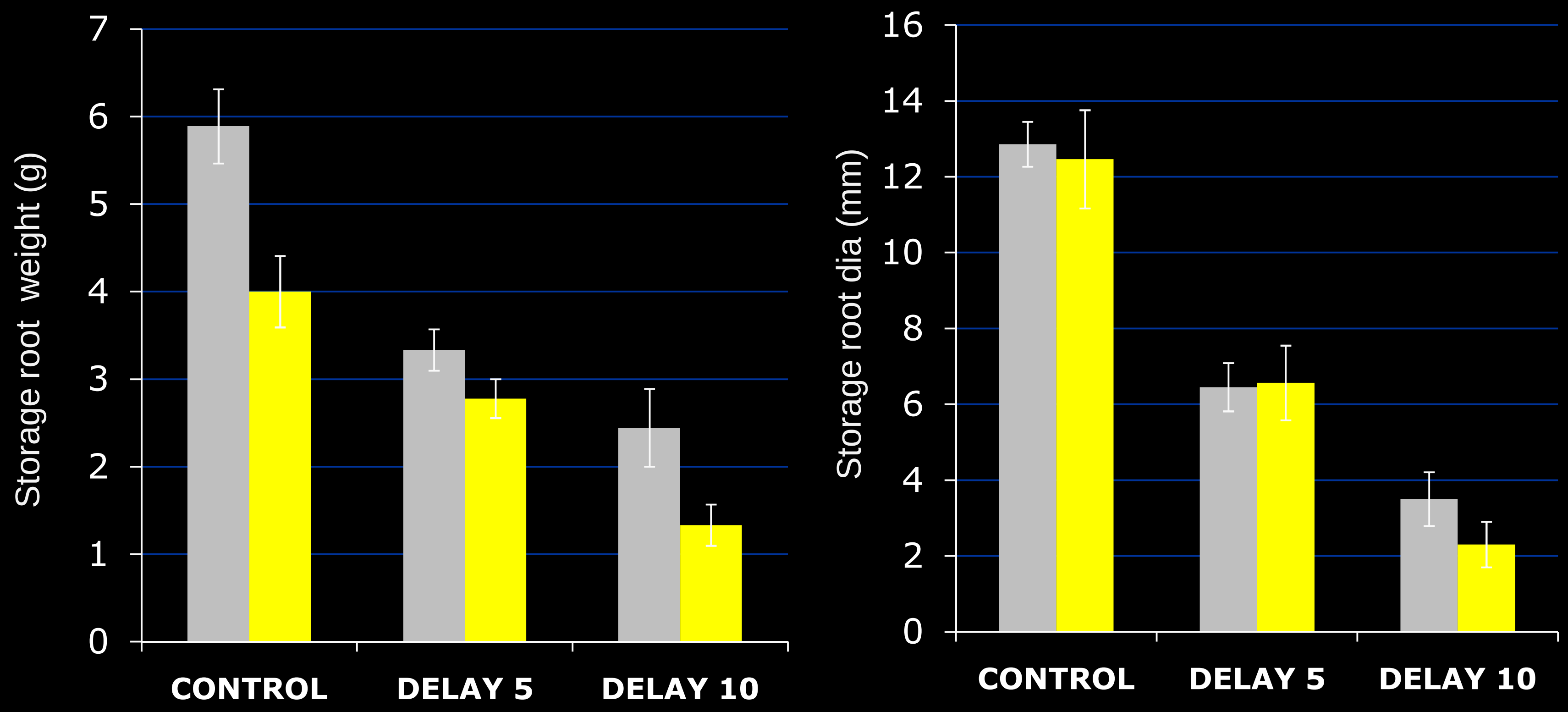


Planted: 5/12, Observed 7/1

Simulated drought studies



Delay in watering (days after transplanting) : greenhouse studies)



Simulated drought at planting, field studies

Specialty Crops
Research Initiative

Drought at planting….

Sampling at
36 days:

Influenced by initial
soil moisture in the soil

Specialty Crops
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Drought at planting….

Comparison between sheltered and non-sheltered plants

Can this knowledge be used in plant breeding?

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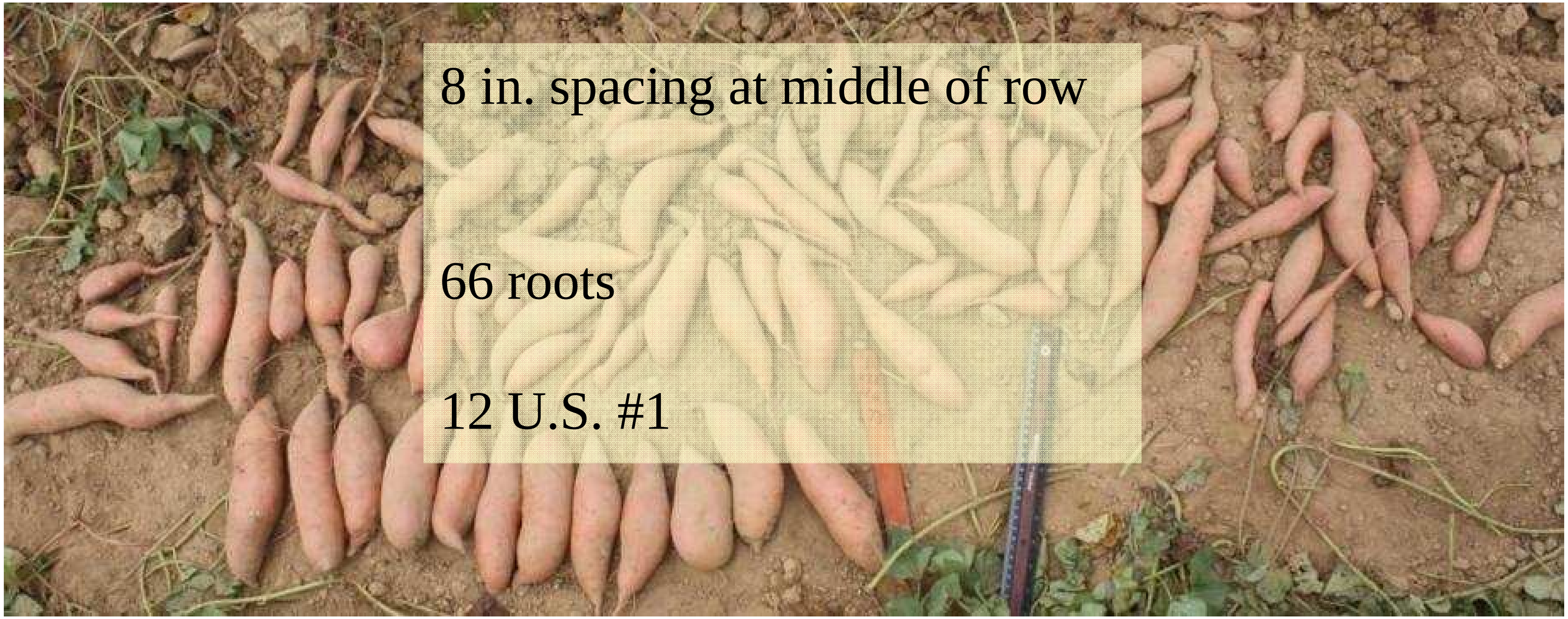
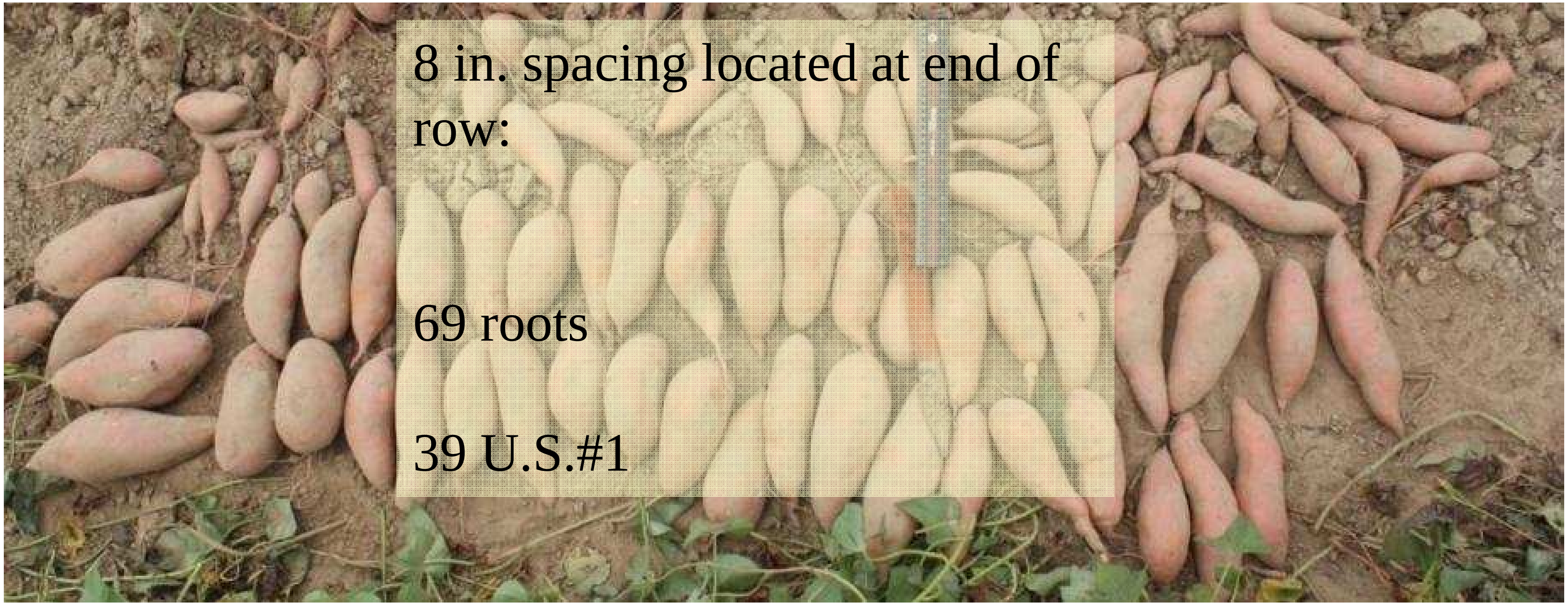
8 in. spacing located at middle tier of row:


8 in. spacing located at end of row:


8 in. spacing at middle of row
66 roots
12 U.S. #1


8 in. spacing located at end of row:
69 roots
39 U.S.#1

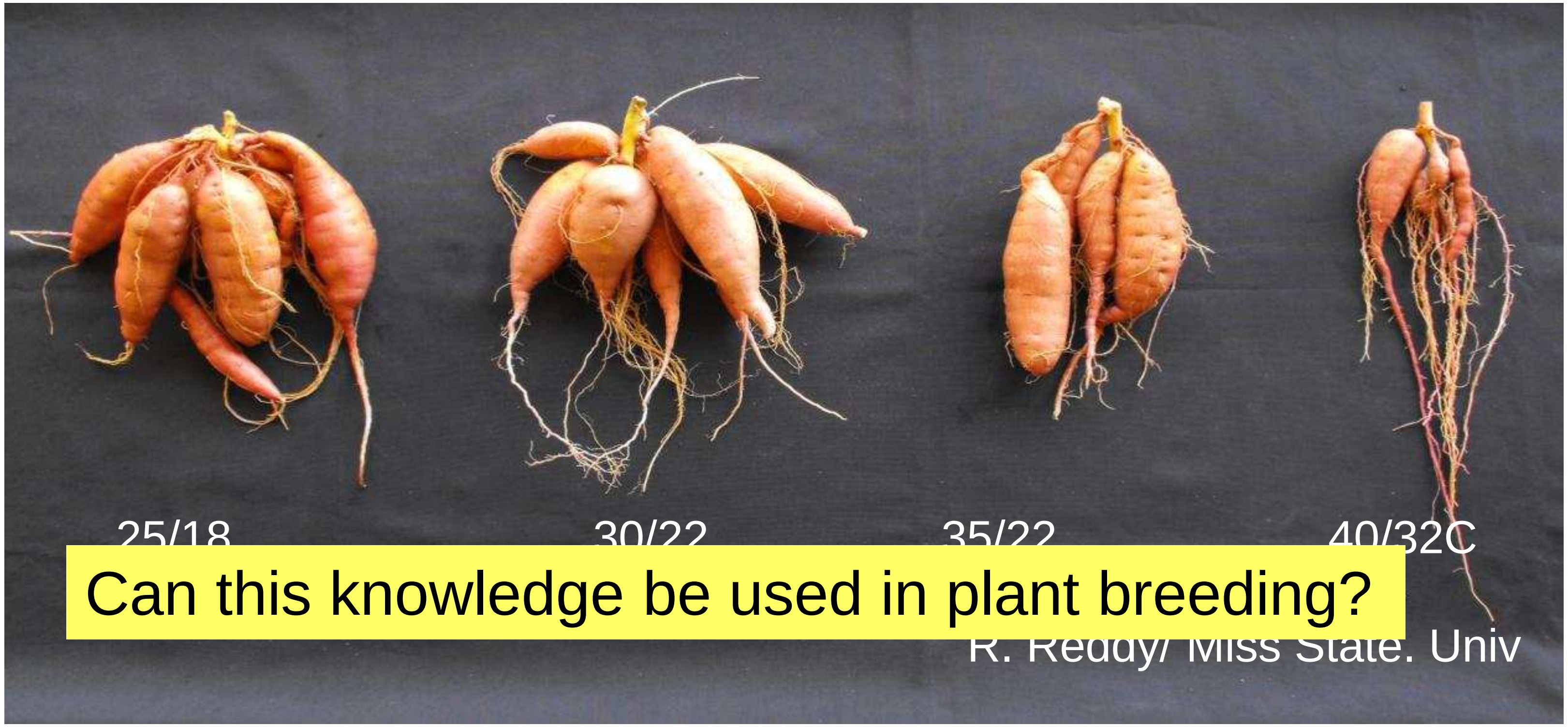
Impact of temperature: naturally lit growth chambers, Mississippi State U (R. Reddy)

Specialty Crops
Research Initiative

Temperature affects sweetpotato storage root development
(after 55 days)

Specialty Crops
Research Initiative

Temperature affects sweetpotato storage root development: optimum during the first 15 days; variable to 85 days



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Temperature effects on adventitious root emergence:

29C day
13C night

29C
16C

29C
21C

Nitrogen fertilizer rate effects on adventitious root emergence

Can this knowledge be used in plant breeding?

Transplant attributes:

With leaves and terminals

With terminals, without leaves

Important variables….

- Water
- Initial temperature (above 18 C and below 35C)
- Season long growing temperature (25 to 30C)
- Fertility - *likely*
- Plant quality
  - leaves vs. no leaves
  - age of plants

Storage root development and root architecture (lateral root development)

Prior research, or how we stand on shoulders of giants:

Lateral roots “may be very important to supply the internal growth elements of the formation of root-tuber” (Koshimizu and Nishida, 1949)

小清水卓二，西田 緑：甘藷蔓苗の体内擴散型生長素の動靜と結藷との關係

Takuji KOSHIMIZU and Midori NISHIDA: On the relation between the distribution of free-auxin in the young sweet potato plant and its root-tuber formation.

緒　　言

甘藷蔓苗は，その榮養の大部分を直接その種藷から仰いでいたものを，採苗と共に急にその榮養關係をたち切られ，而も環境の著しく異つた圃場に挿植されるので，活着するまでの過程に於ける環境に對する苗の抵抗力や，發根とその生育に對する苗の体内生理的活動が結藷に大なる影響を及ぼす。

そのため甘藷栽培の技術者は，甘藷の結藷能力をあげるのに最も肝要なのは，良苗を得る事であるとしている[6, 8, 18, 19)]。又蔓苗の中特に結藷の著しい節位は苗の中央部とされている[12, 17, 34)]。

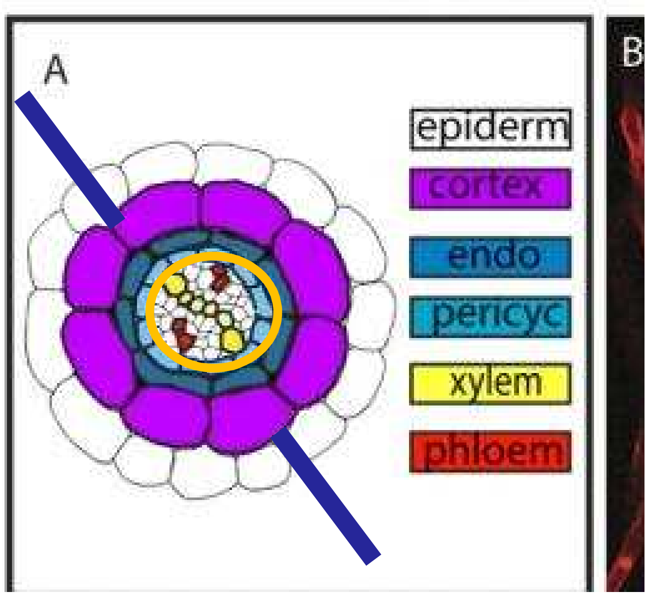
又蔓苗の親葉と側芽とが，結藷或は發根に對し極めて有効に作用するという者[5, 14, 19, 26)]と，親葉よりも側芽が有効であるとする者[12)]と，側芽は發根とその伸長とには有効であるが，塊根形成には却つて不利であるとする者[4)]等がある。然しこれ等の理由を主として外的要因に結びつける者が多く，内的要因の方面からは單に蔓苗の全糖量や，灰分の動靜と發根關係を調べた[20)]位に過ぎない。

玆に於て著者等は，甘藷蔓苗の内的要因として重要視すべき体内擴散型生長素の動靜が，發根，側芽發生，結藷等と如何なる關係を有するか，又側芽，親葉等が如何に結藷に影響するか等に就き，'43年から京大榎本教授の主催する學術振興會の甘藷班に屬して5カ年間研究を續行し，良甘藷苗の具備すべき必要

What is known about lateral roots

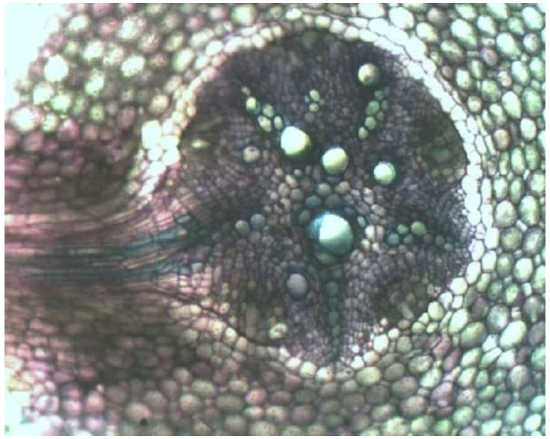
- Detect and find soil moisture
- Detect and find nutrients
- Conduct water, “act” as plumbing system
- Determine root architecture
- “Die off” in response to drought
- Regrow with moisture?

Origin of lateral roots:

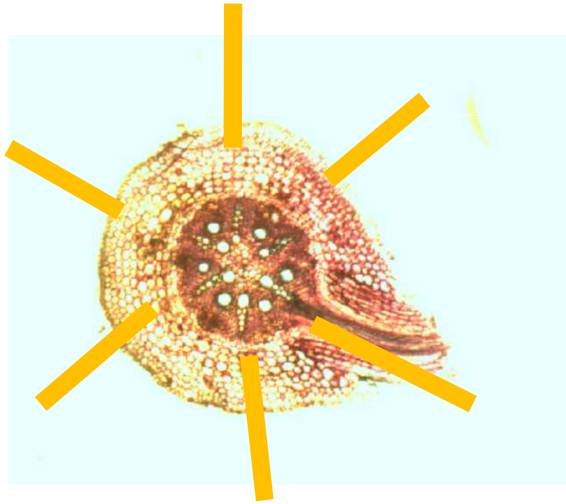


Arabidopsis: diarch protoxylem

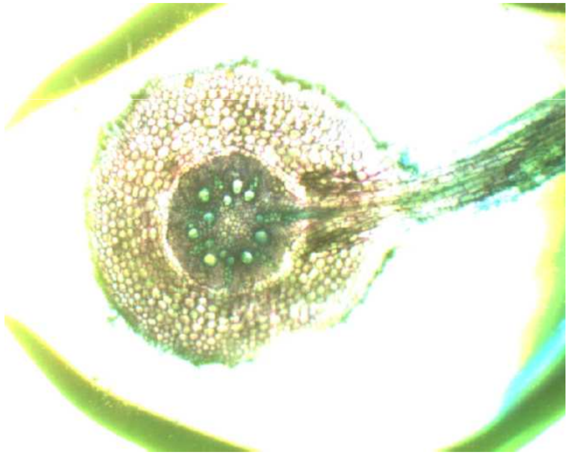
Sweetpotatoes: polyarch protoxylem



pentarch

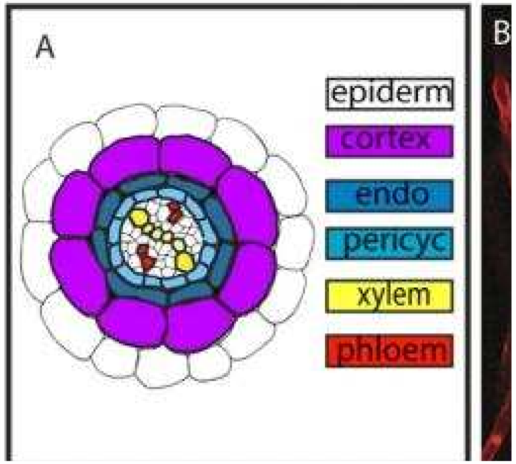


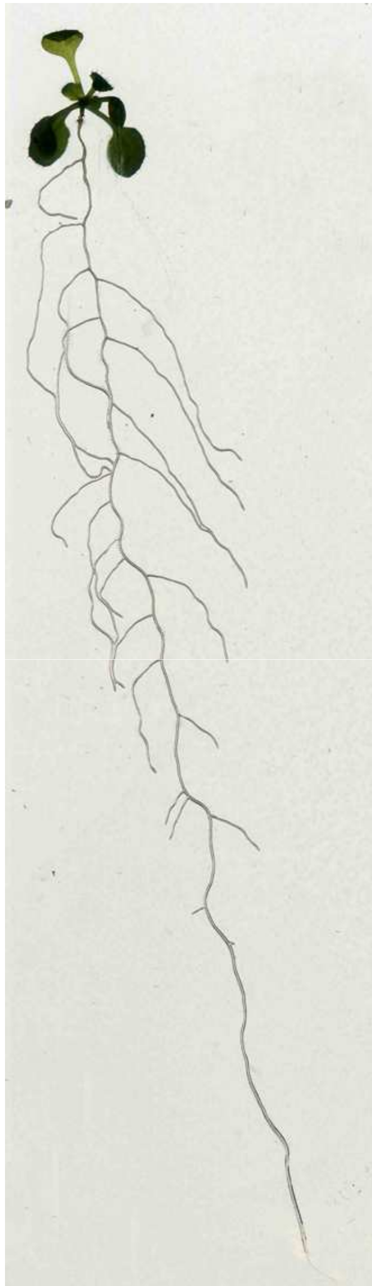
hexarch

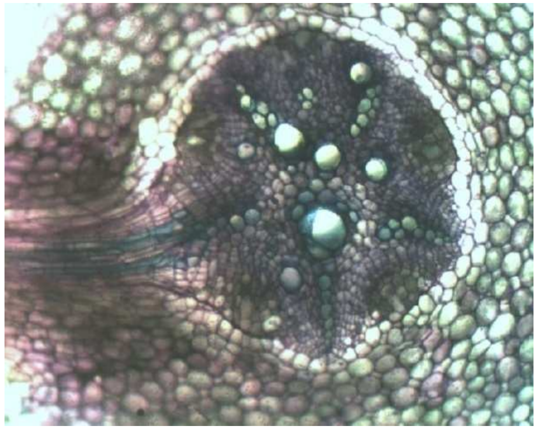


septarch…

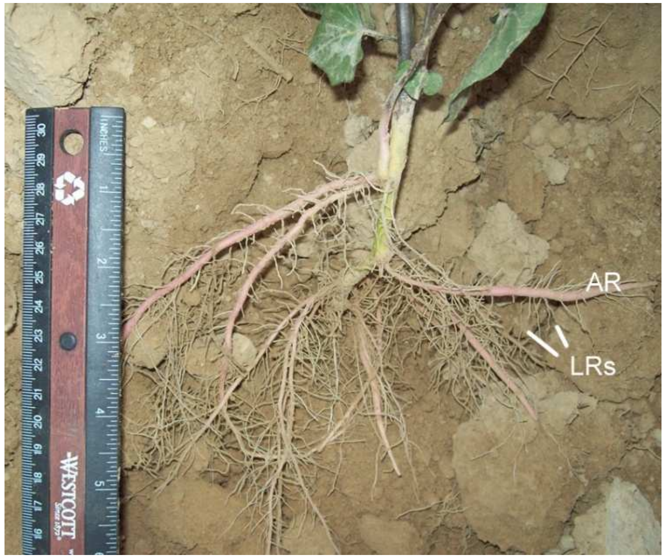
Diarch Arabidopsis: Two rows of lateral roots



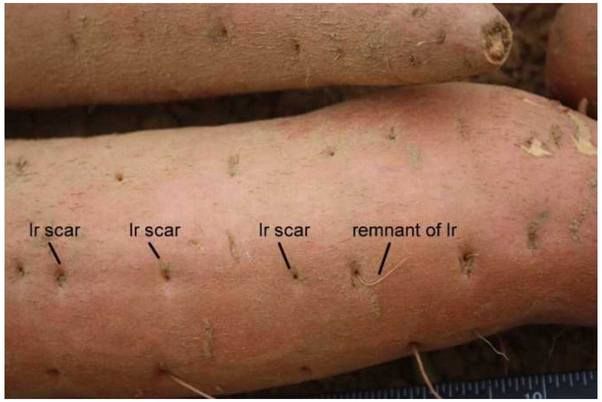


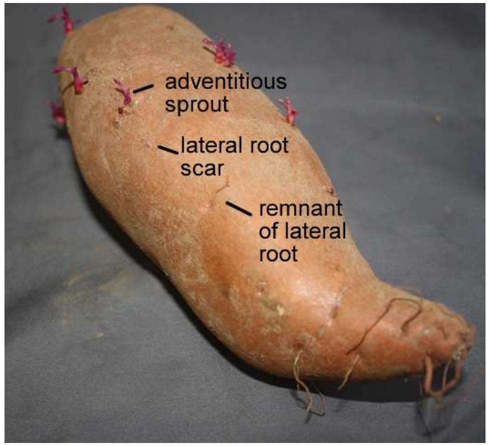


Pentarch sweetpotato



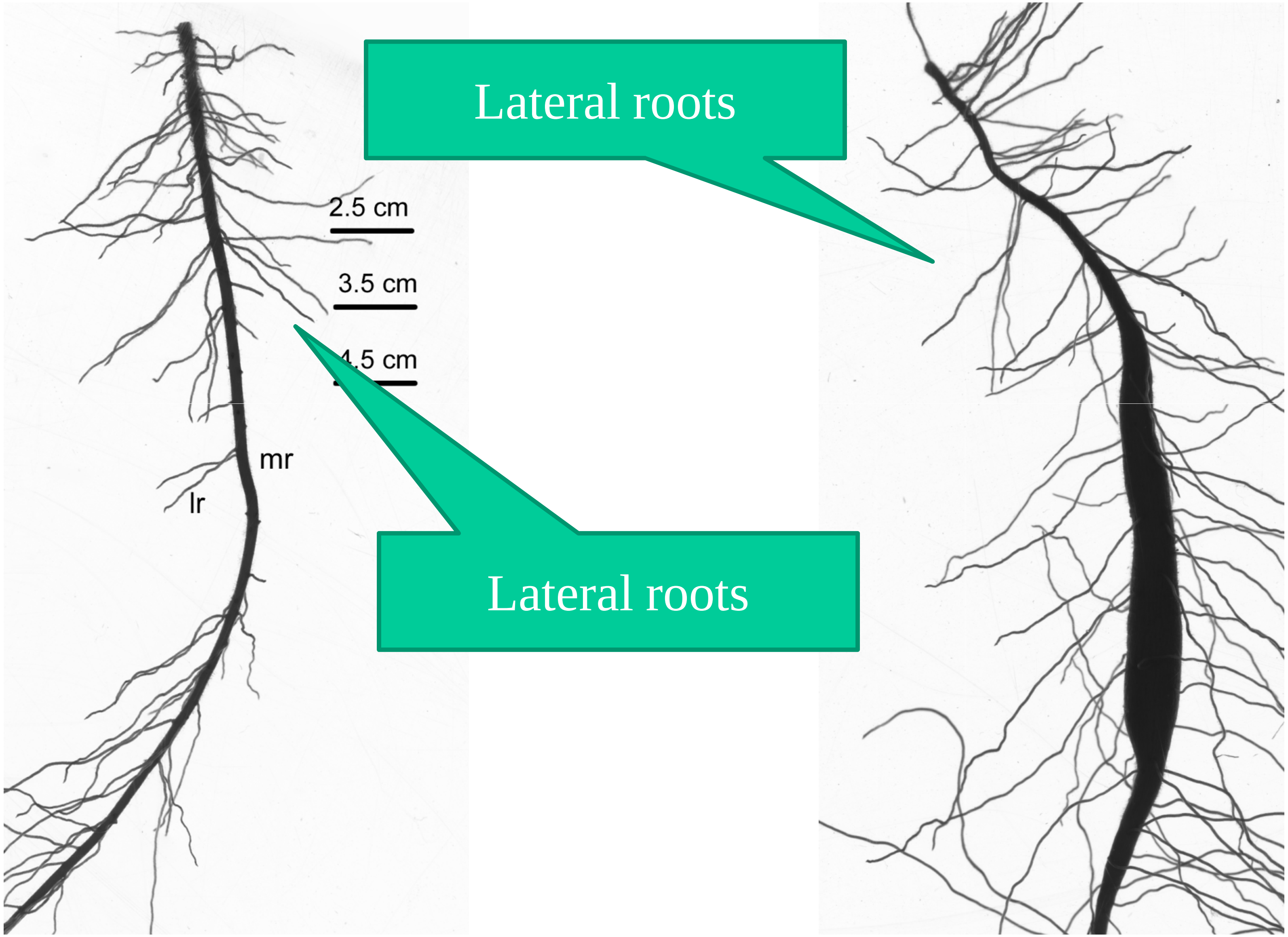
Five rows of lateral roots





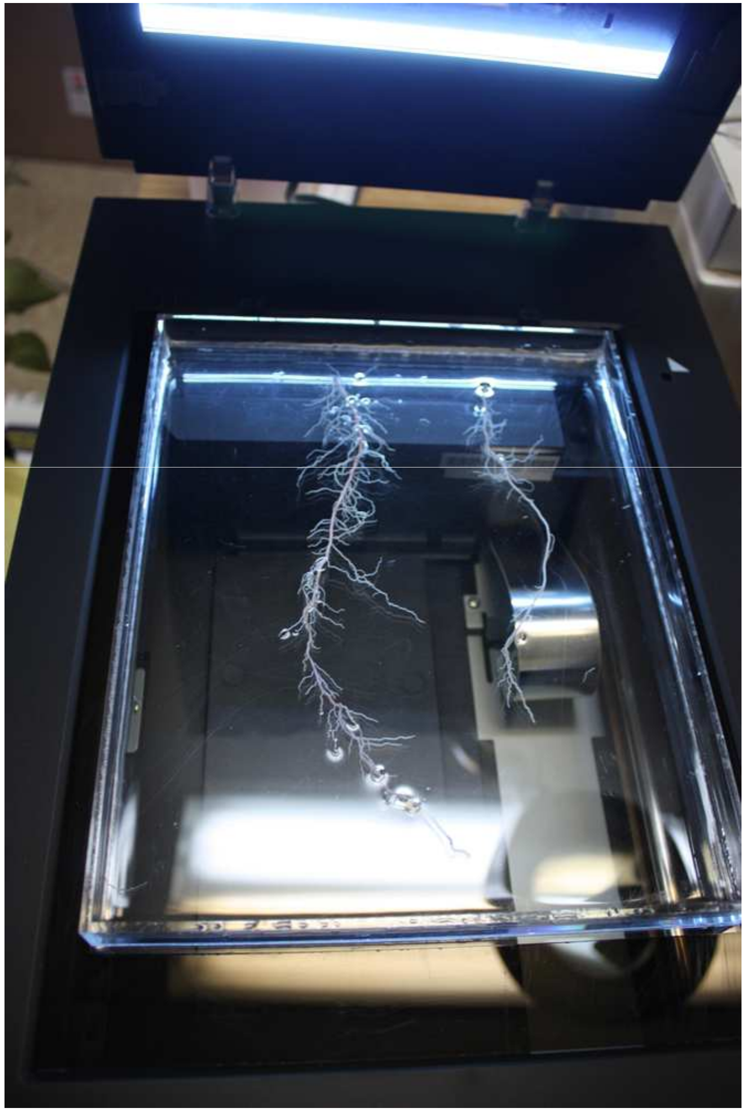
Five rows of lateral root scars

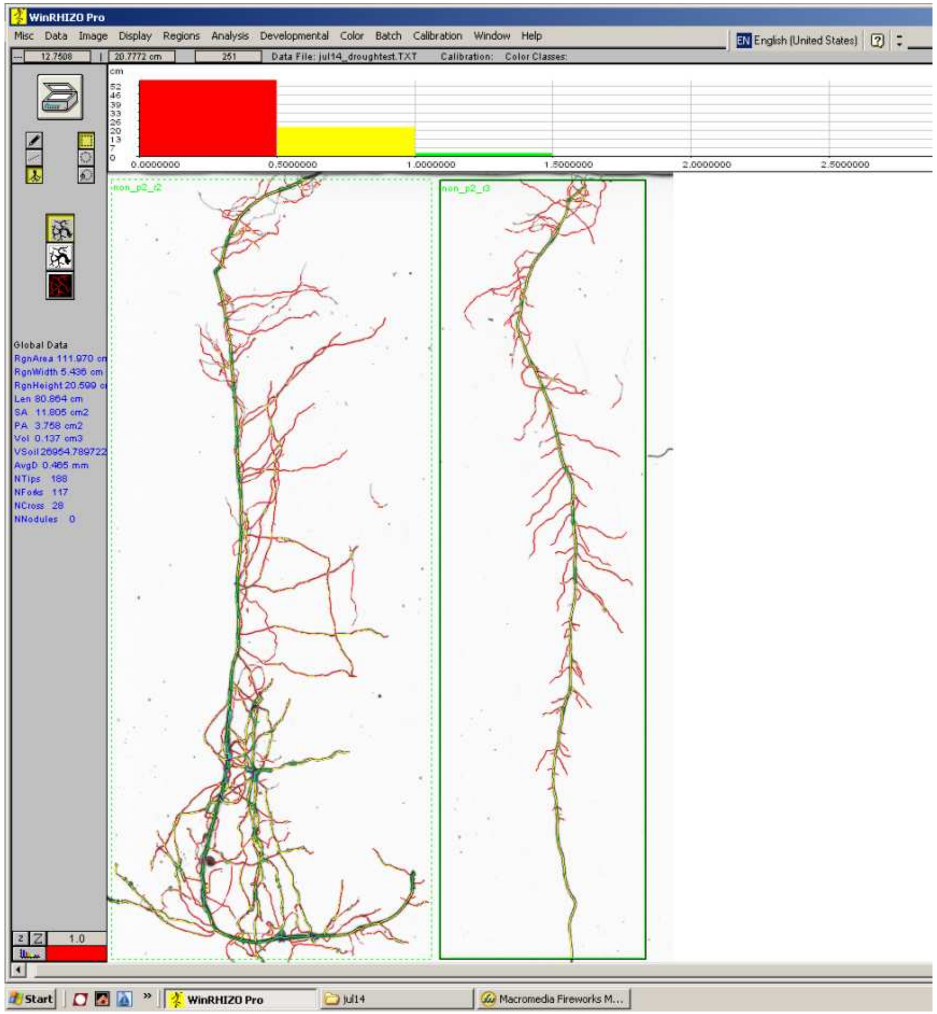
Is lateral root developmnet related to storage root formation?



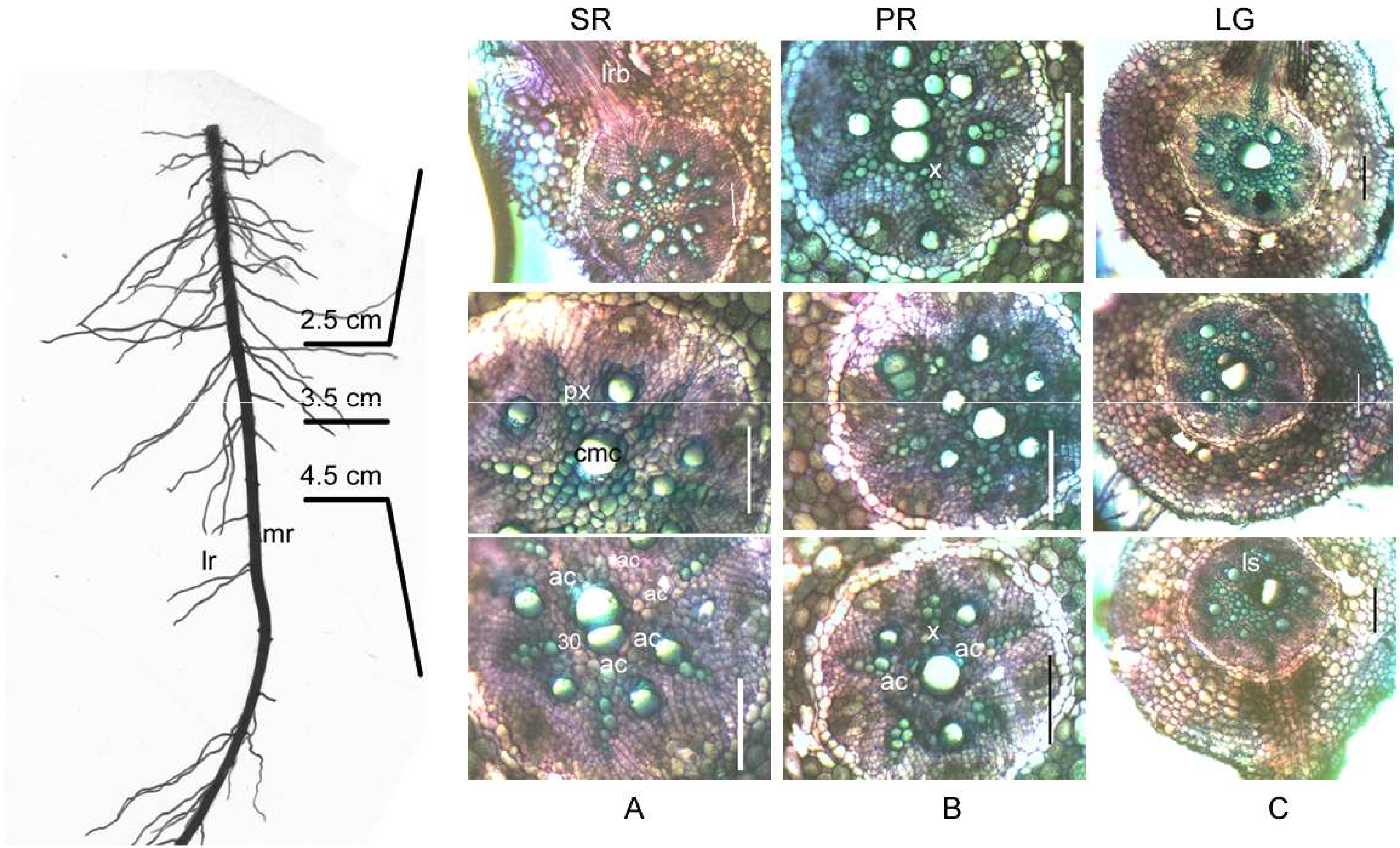
Recovery of near-intact root systems was important:

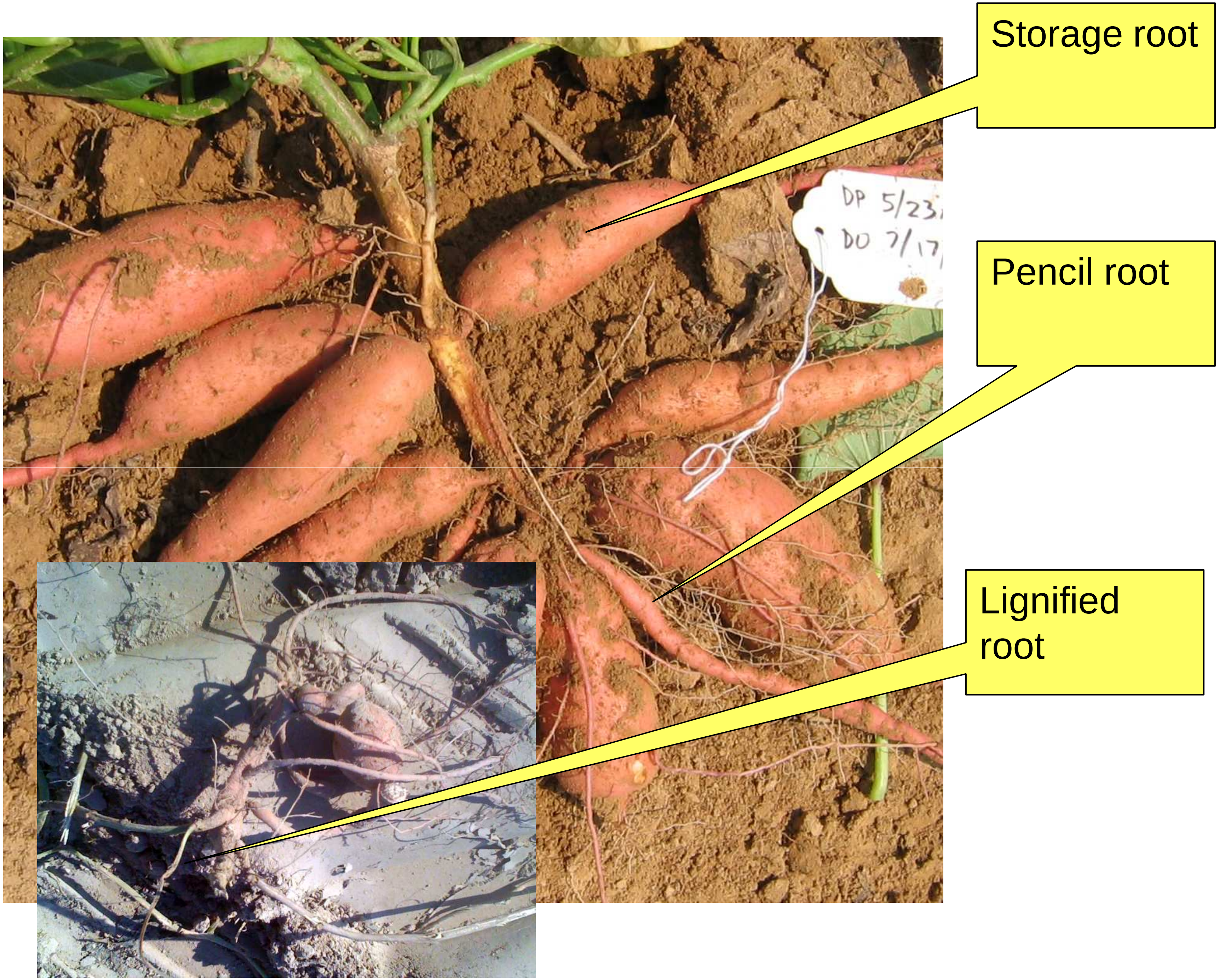
scanner-based root images





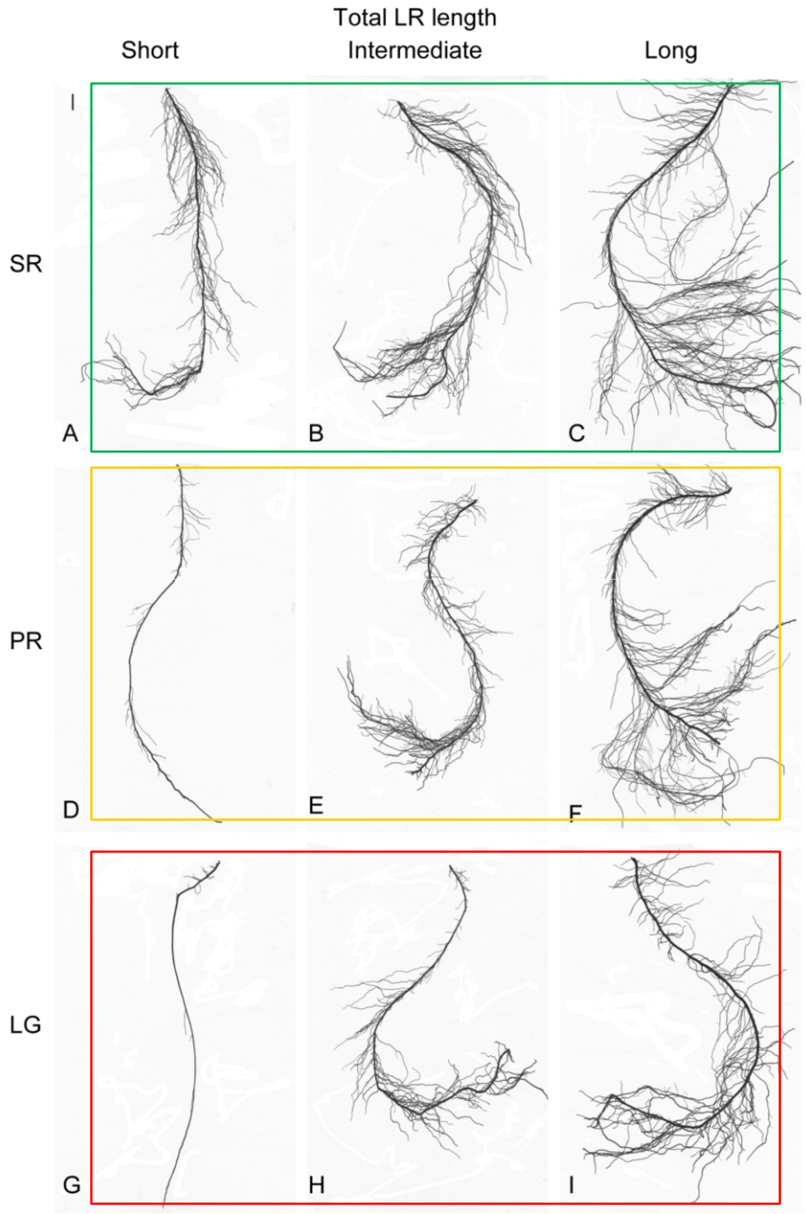
Each adventitious root was sectioned as indicated, and classified as storage root, pencil root, or lignified root at 20 days:



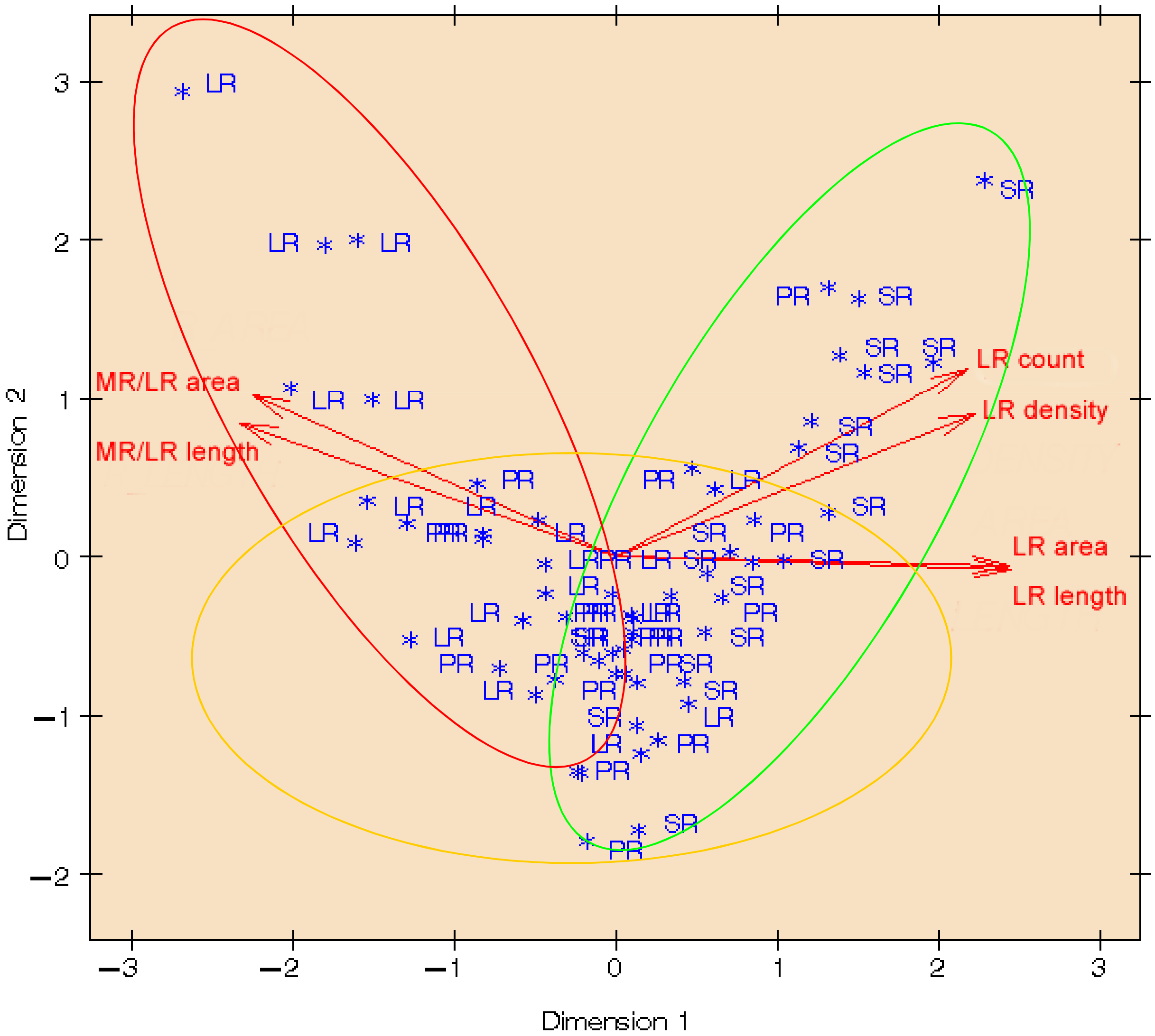

Storage root
Pencil root
Lignified root
DP 5/23
DO 7/17

Experimental results:

Representative roots
for each developmental stage
representing shortest, intermediate, and longest total LR length.

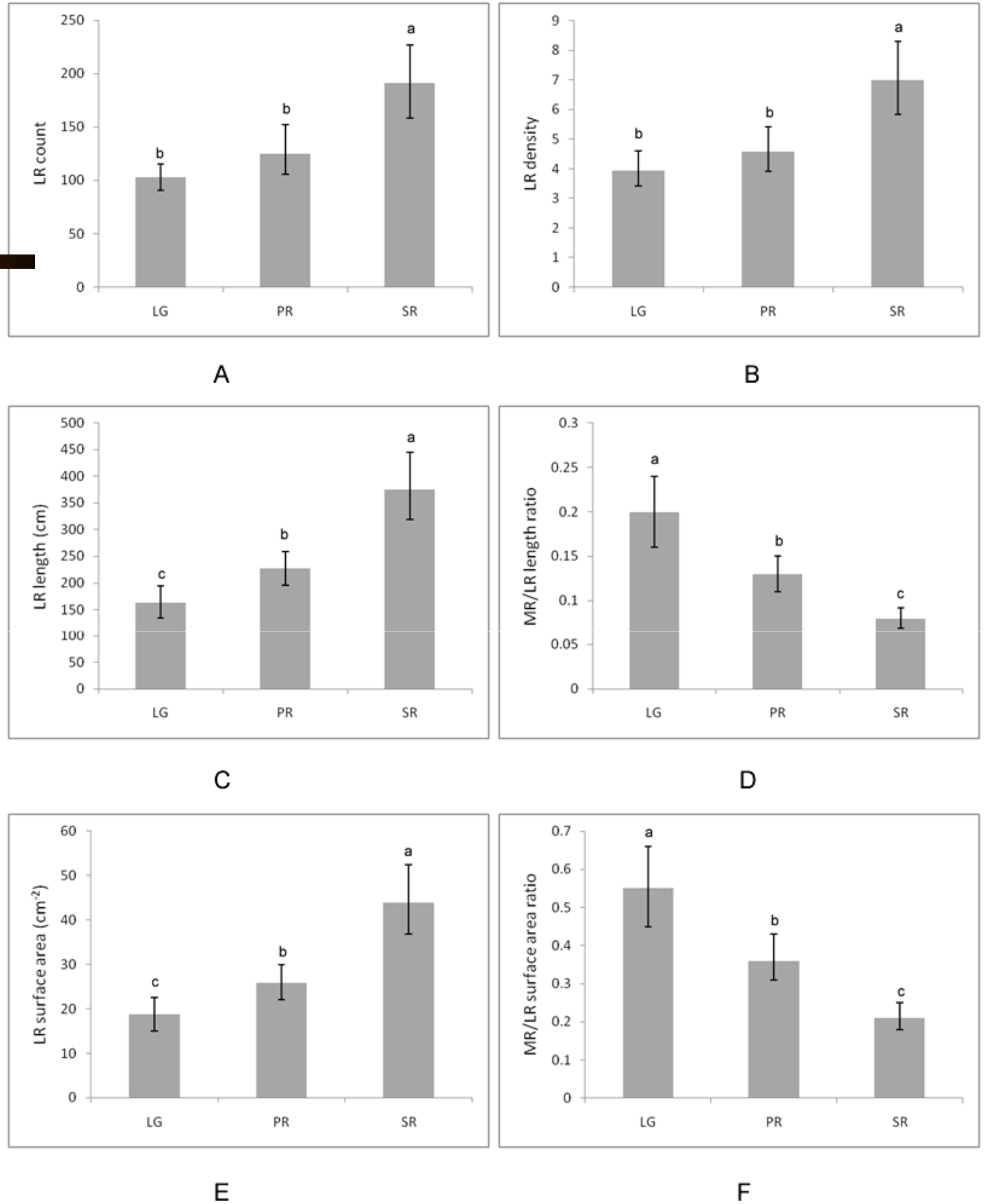


Principal components analysis of lateral root attribute data: developmental trajectories and adventitious root fates



Experimental results:

Descriptive statistics of lateral root attributes of lignified roots, pencil roots, and storage roots.



Storage root Lignified root

Experiment #2:

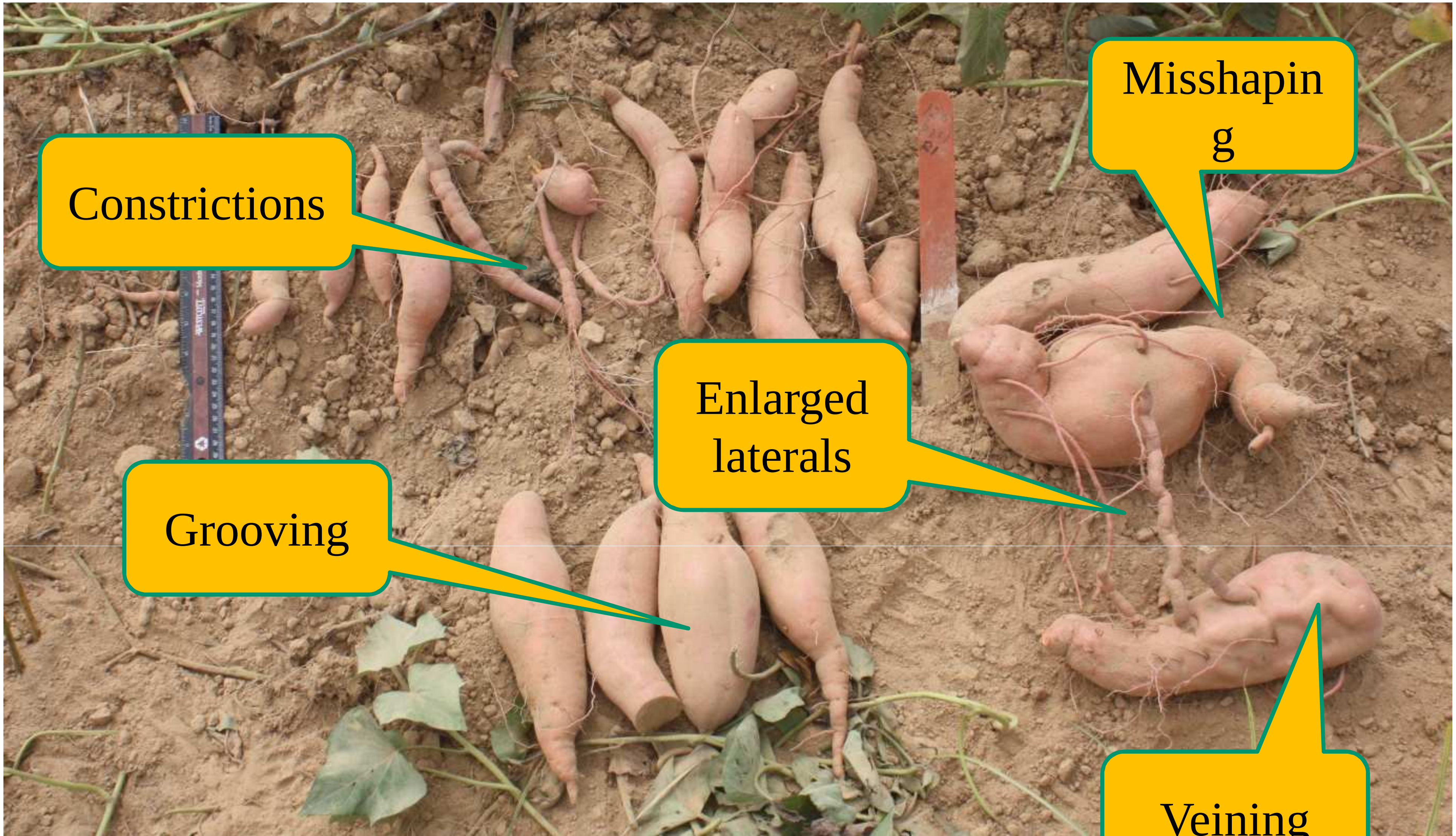
Root plasticity in response to soil water temporal variability (20 days)

Can this knowledge be used in plant breeding?

Uniform watering During first 10 days;

Drought: withhold next 10 days

Saturated: 2x watering next10 days



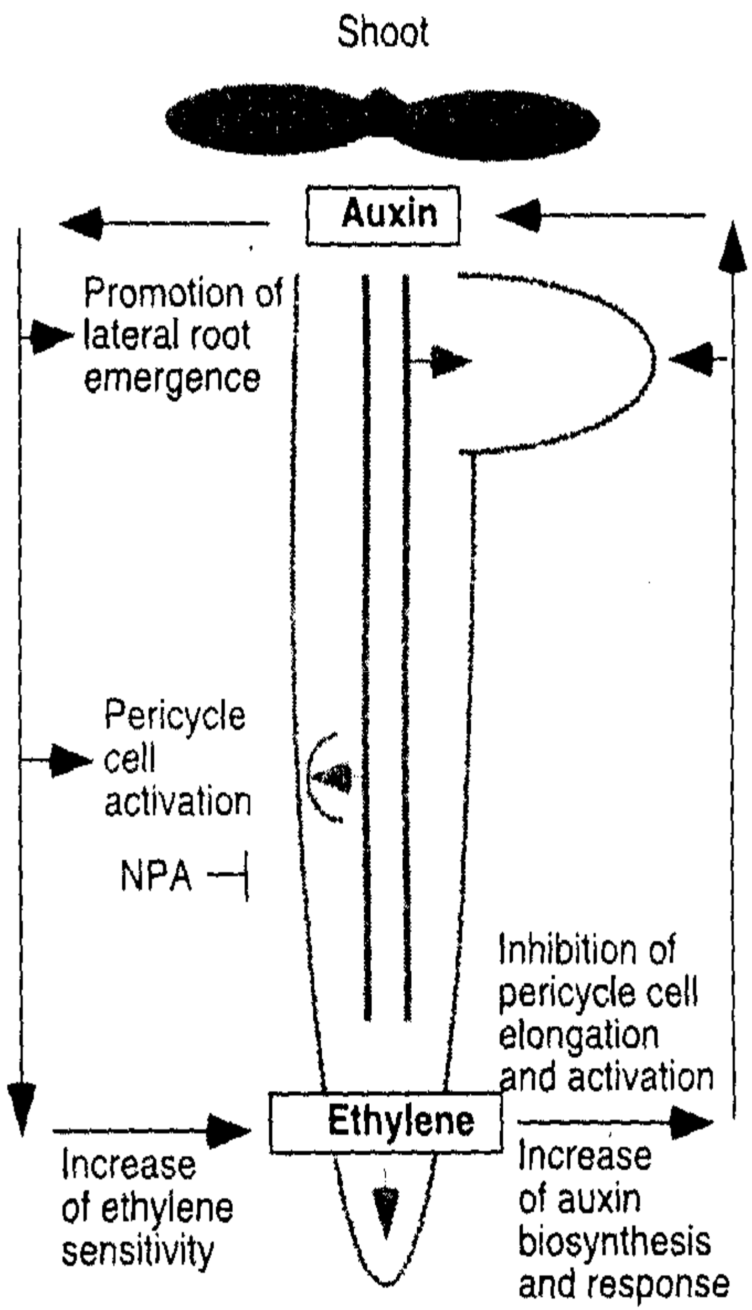
w/o supplemental irrigation

Planted 5/12; Harvested 9/7

Linking basic knowledge with observations

- ✓ Hormones and plant growth
- ✓ Importance of lateral roots

Simple model for ethylene-auxin crosstalk in determining lateral root development



Ivanchenko, et al, 2008 Plant Journal 55:335-347

Adopting a model:

ethylene ↓ auxin ↑ :
Lateral root primordia are activated

ethylene ↑ auxin ↓ :
-root initials remain arrested
- stops elongation at tip

it is all about *stress and opportunity!*

Hormones.....

√ Ethylene

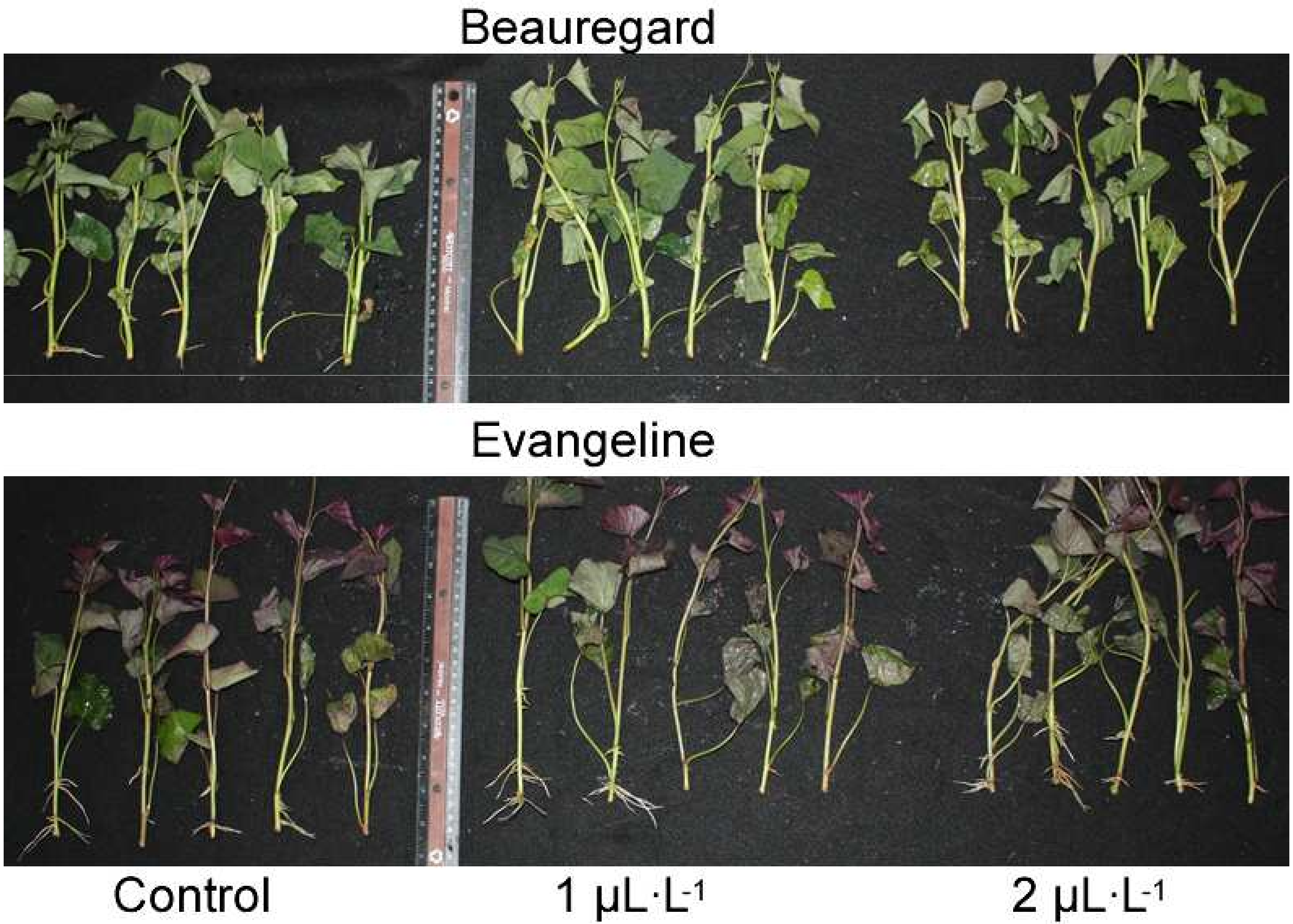
- produced in all parts of the plant
- associated with stress
- Induces lateral roots
- induces root hair growth
- Interferes with auxin transport (when auxin is high)

Hormones.....

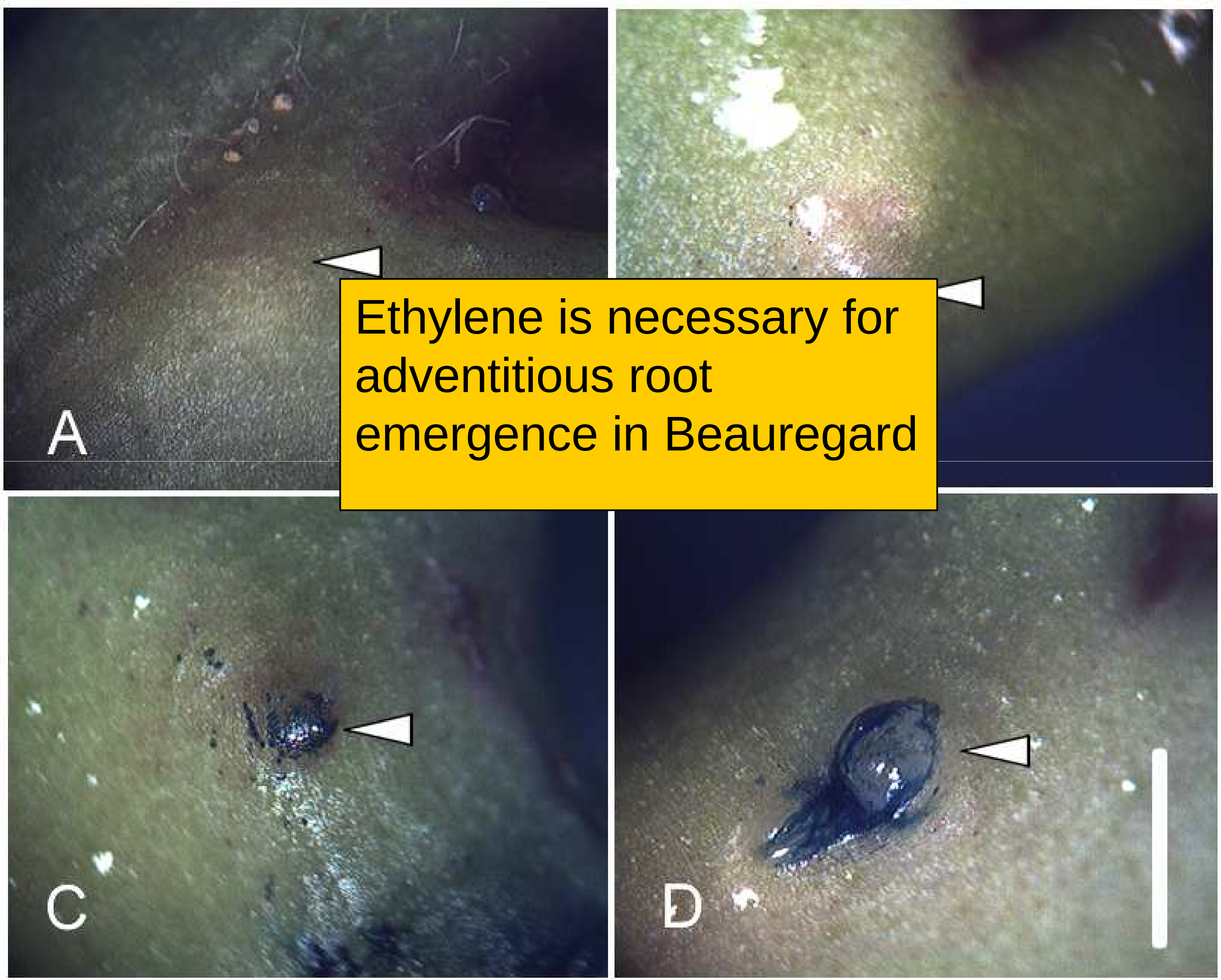
√ Auxin

- essential for cell growth – stimulate cell division, cell differentiation, elongation
- promotes adventitious root formation, induces growth of existing roots, and organ shape
- formed in apical meristems – transported through phloem

Genotypic variability in ethylene response – variable adventitious root development in response to 1-MCP pretreatment of cuttings:

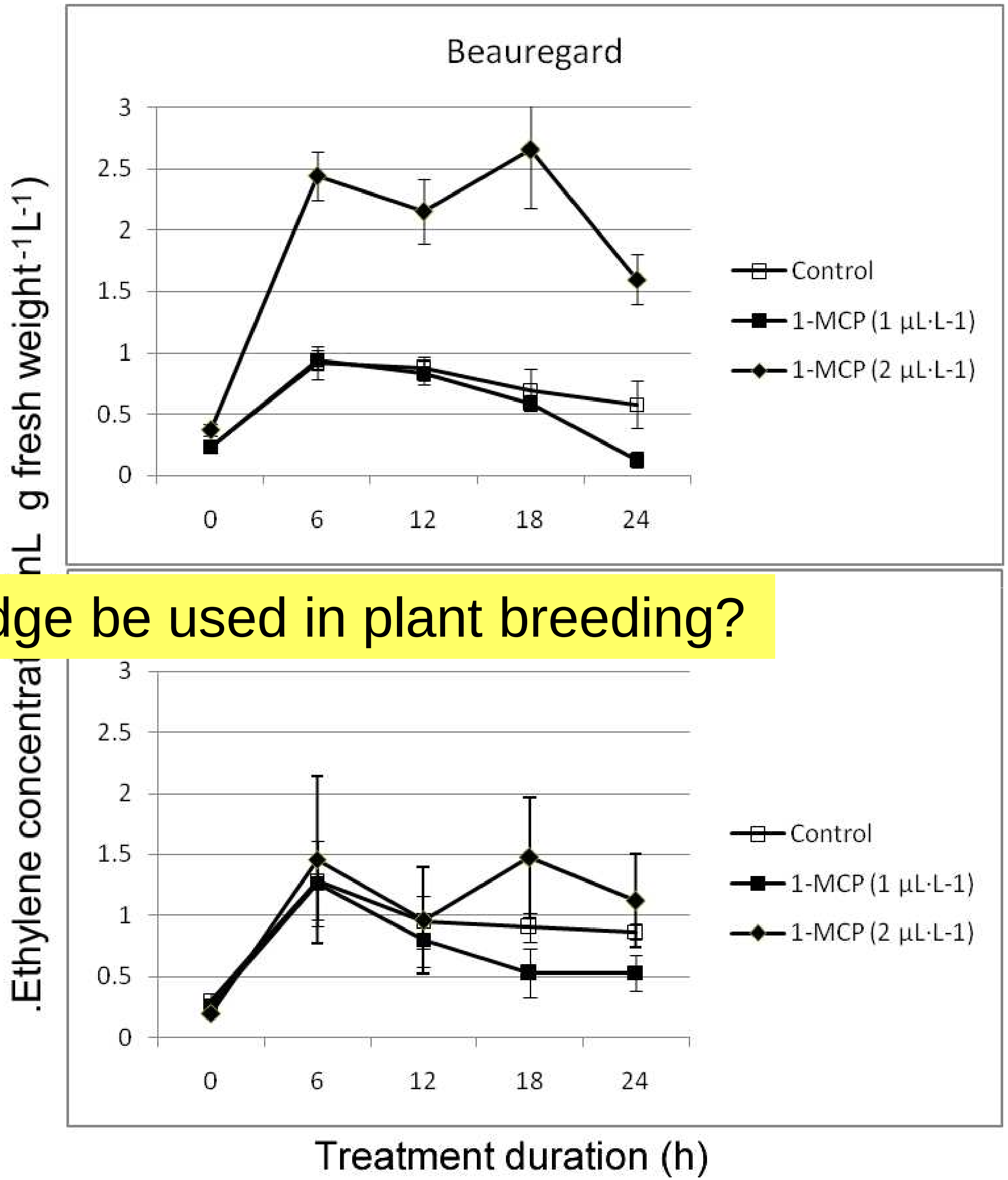


1-MCP delayed cell death at the site of adventitious root emergence



Ethylene is necessary for adventitious root emergence in Beauregard

Ethylene is naturally produced during cutting:



Can this knowledge be used in plant breeding?

Prior research:

Distribution of free auxin (dotted) on sweetpotato cutting (Koshimizu and Nishida, 1949)

験 方 法

各節位の節間及び葉身附葉柄から擴散 生長素を分離するのに，始 ーテル・アルコホル・水等で抽出する方法をとつたが，これ等の抽出法 は複雑な他の生長素が同時 抽出されて思はしくないので，寒天

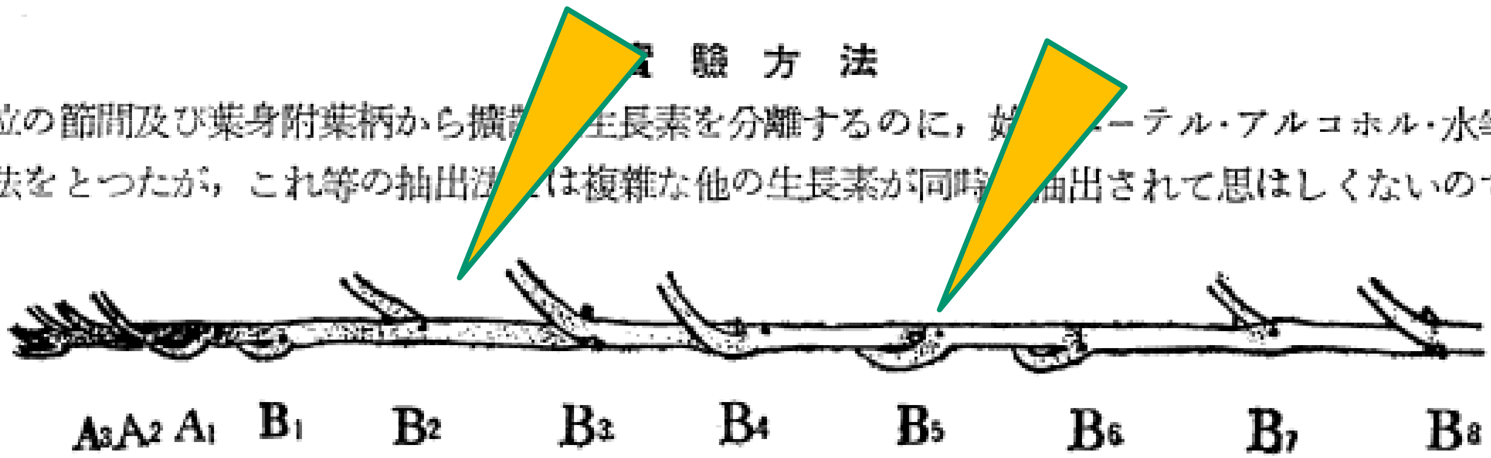


Fig. 1. Diagram of the distribution of free auxin (dotted) in the body of the young sweet potato plant. A_1, A_2.... orders of node bearing the close leaf ; B_1, B_2.... orders of node bearing the open leaf.

Genomics and Biotechnology for Sweetpotato

What can we do?

Complicated…

Hexaploid

a.) allopolyploid and autopolyploid nature

b.) altered segregation ratios:

- chromosomal segregation
- random chromosomal segregation
- maximum equational segregation

c.) 6 loci and multiple alleles

Quantitative inheritance

a.) bad - no simple inheritance

b.) good – traits are stable

Complicated…

Incompatibility

a.) ensures outcrossing and allelic diversity
b.) limits backcrosses
c.) favorable cross combinations

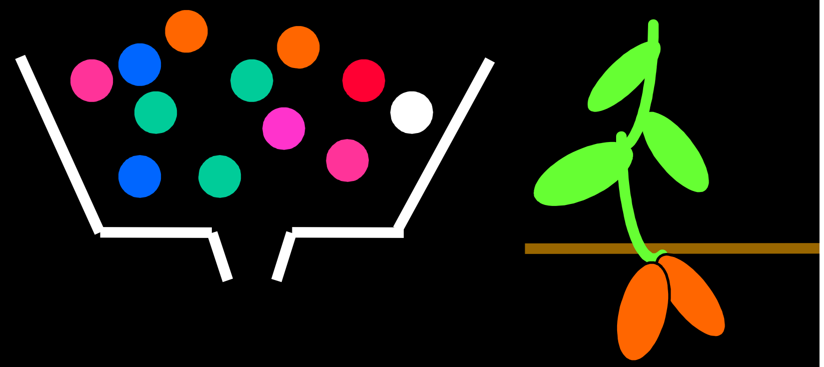
Basically a mess!

Valuable alleles are out there

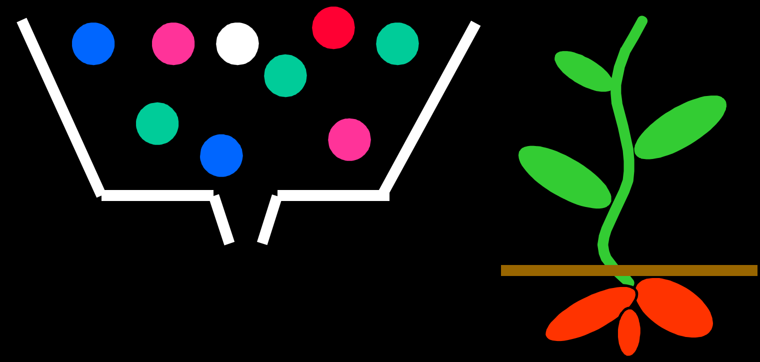
Value

Identify new genetic sources

Early domestication

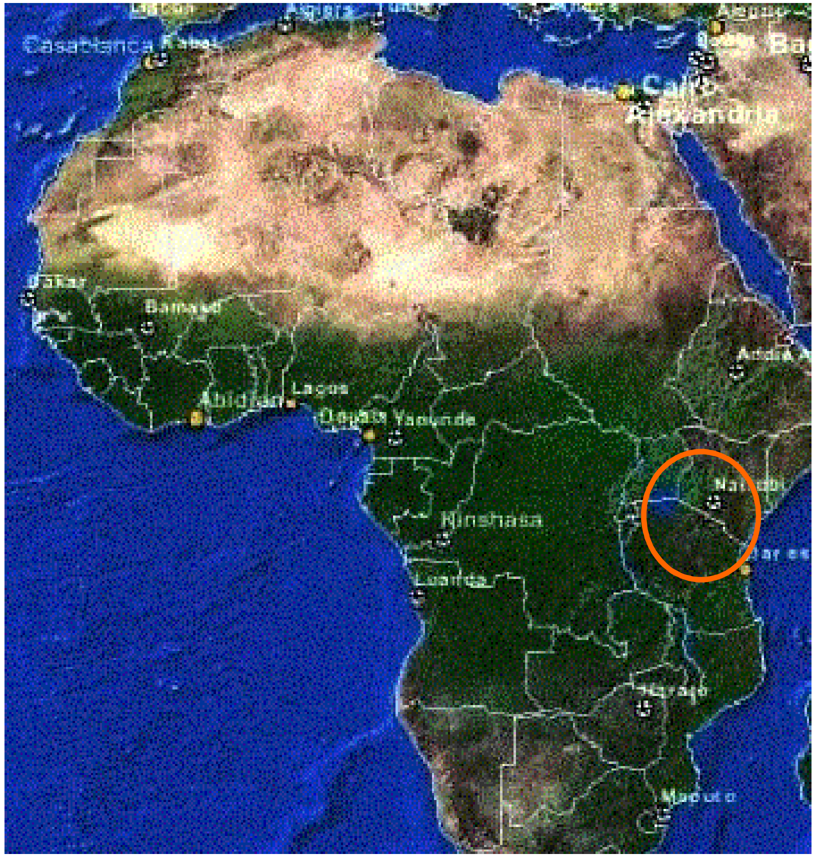


Domesticated



Transferring valuable alleles

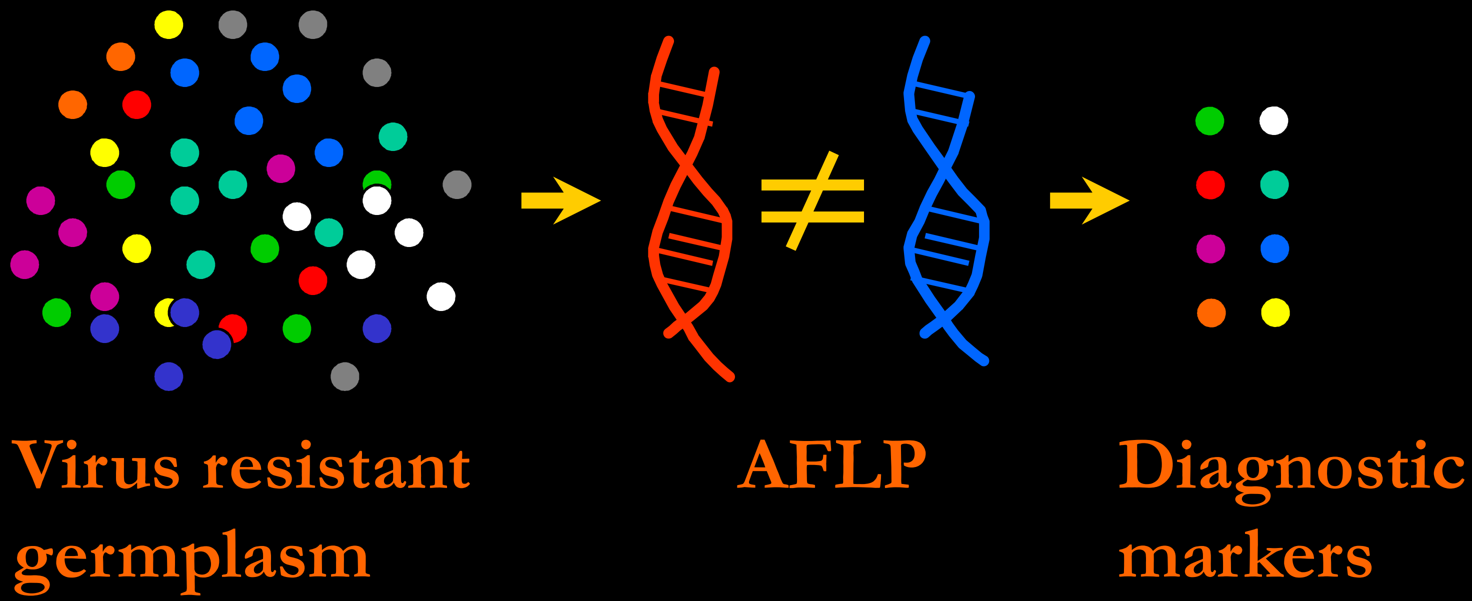
- ***Key regional populations: virus resistance***



Finding new alleles

✓ *Wild species*

Identify important markers



What can we do…

Marker selection protocols

traditional mapping is doable

1:1 ratio-simplex (Aaaaaa) x recessive (aaaaaa)

4:1 ratio-duplex (AAaaaa) x recessive (aaaaaa)

drawbacks

problems

-most genes don't fit ideal segregation patterns

-limits to progeny population size

-progeny are skewed, e.g., ↑ susceptible: resist ↓

-resistance is muted

What can we do…

Discriminant analysis **- *s*tudy differences between two groups with respect to several variables simultaneously.**

Characteristics

- ☑ **multivariate technique**
- ☑ **predictive, allocate genotypes**
- ☑ **flexible population structure**

Marker selection protocols

Example **- identifying origin and type of tea based on metal content (Fernandez-Caceres, 2001).**

- ***no differences in metal contents (Al, Cu, Fe, K, Mg + 7 more)***

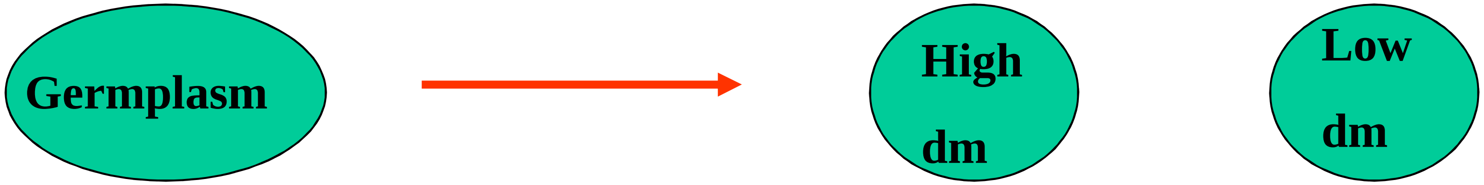
- **DF1= -1.16Zn - 0.88Mn + 1.72Fe - 2.18Mg etc. (Function that best separates the 2 classes of tea)**

predictive

Statistical analysis

A) Cluster analysis

Phenotypic group verification

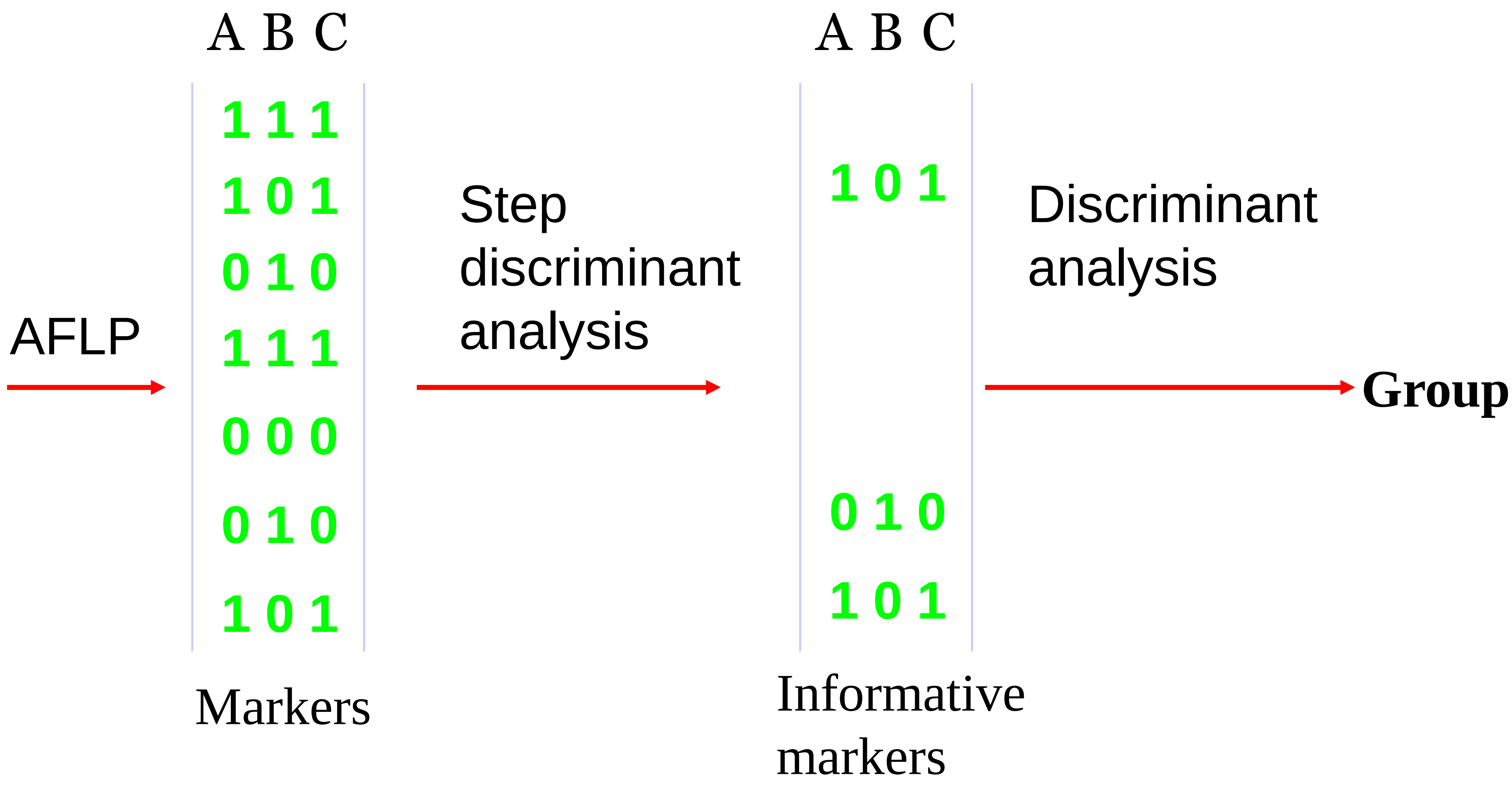


B) AMOVA

Genotypic group significance

Discriminant analysis - the process

. Stepwise discriminant analysis



Objective

- ☑ ***Identify molecular markers linked to dry matter content***
- ☑ **Phenotypic vs. genotypic clustering**
- ☑ ***Predictive power of discriminant analysis***
  - ***-cross validation***

Materials

- ☑ **68 clones - 34 high dry matter (36-43%)**
  - 34 low dry matter (12-22%)
- ☑ **Training sample – 58 clones**
- ☑ **Test sample – 10 clones**

AMOVA for 68 genotypes using AFLP markers

Source	df	Variance	Prob
Group	1	0.49	<0.001
Genotypes	66	28.15	

6 Predictor markers as selected by the STEPDISC procedure

Marker	Step	Wilk's λ	Pr < λ
cta084	1	0.84	0.019
cag185	2	0.69	<0.001
cag235	3	0.51	<0.001
cag148	4	0.42	<0.001
cta212	5	0.36	<0.001
ctt183	6	0.28	<0.001

Rate of correct classification of 10 test clones

Number of predictor markers	Clones misclassified	% correct classification .
6	3	70.0
10	2	80.0
12	0	100.0
14	0	100.0

Caveats

- ☑ **Issues (Discriminant analysis)**

	Yes	No
False positives	☒	☐
Clearly defined classes	☒	☐
Precedence	☒	☐

Advantages…

False positives are good – identify new sources of allelic variation

Assemble diverse populations to find markers

Good linkage with markers on traditional maps

Bias nurseries with marker bearing parents

Finding genes of interest……..

Differential gene expression

Tissue collection (~1 mm):

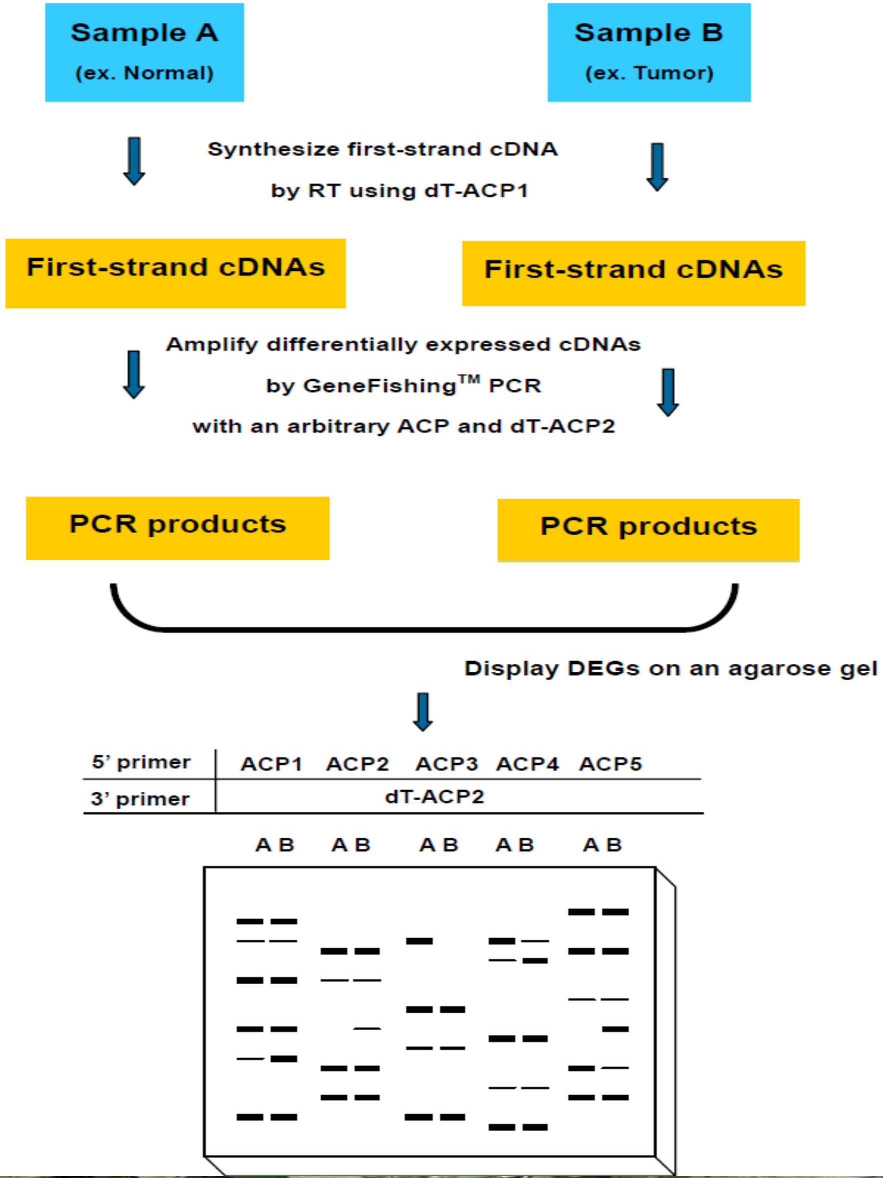
0 h, 2 h, 4 h, 8 h, 12 h

RNA extraction and cDNA preparation

ACP-primer based Gene Fishing

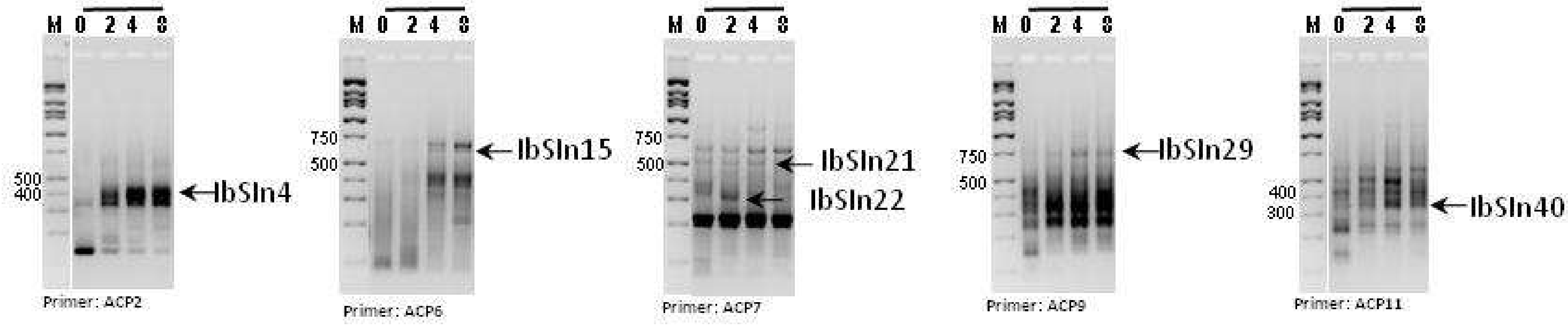
Cloning and Sequencing of DEGs

(Semi) quantitative Reverse Transcription Polymerase Chain Reaction Analysis of DEGs



Results

Isolation of DEGs under skinning injury



Isolation of DEGs for skinning injury

A total of 70 DEGs (250 clones) were isolated and sequenced

65 DEGs match plant-specific cDNA sequences

Differentially expressed genes in root tissues

DEG	Similarity	Length (bp)
IbTH2	Thioredoxin H2 mRNA	266
IbCyc	Cyclophilin mRNA	524
IbSpor	Sporamin	414
IbTAL	Transaldolase	283
IbPPId	Peptidyl-prolyl *cis-trans* isomerase	343
IbTCTP	Translationally controlled tumor protein	608
IbTOM1	Target of Myb protein 1-like	451
IbRNP	Ribonucleoprotein	299
IbCCOMT	Caffeic acid 3-*O*-methyl transferase	
IbRPK	Receptor protein kinase	481
IbSRP	Serine rich protein	344
IbHLH	Helix loop helix	327
IbCyt450	Cytochrome P450	276
IbnifU	Nitrogen fixation Unit	171
IbELIP	Early light inducible /Dessication stress protein	344
IbGDC	Glutamate decarboxylase	357
IbPAL	Phenylalanine ammonia lyase	
IbEXT	Extensin	

Abiotic

Wounding

Lignin/Suberin

TF/Signaling

Advantages…

Good way to identify genes related to a trait

-survey wild species (salt vs no salt) & seek sequence homology with I. batatas

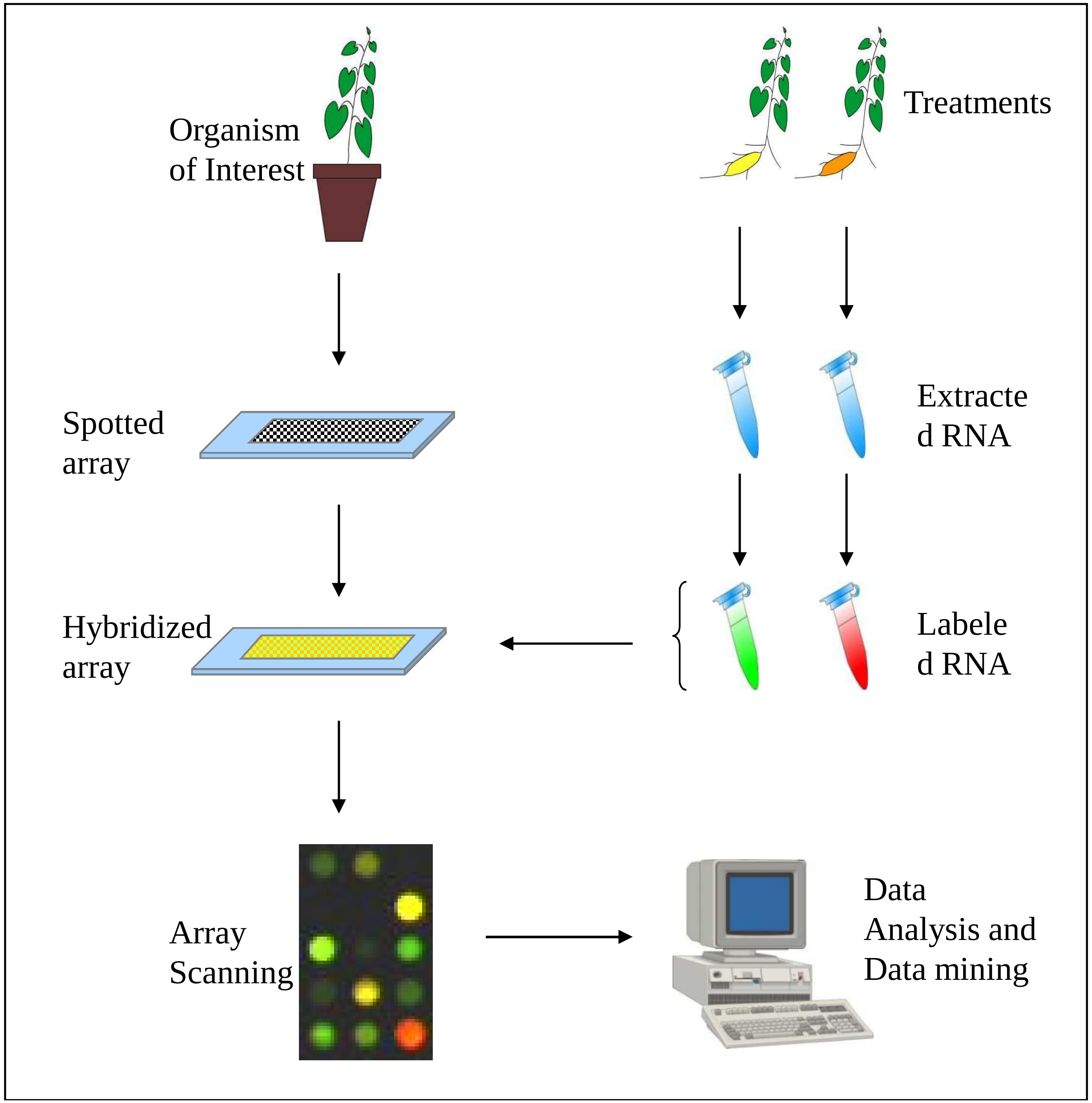
-bioinformatics straightforward

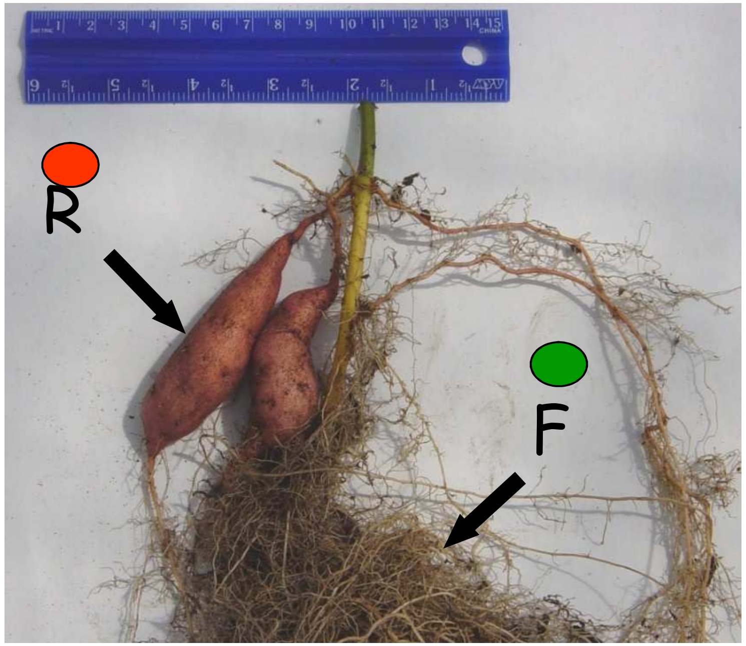
Can do time point studies

Disadvantages…

Some genes found, some lost….

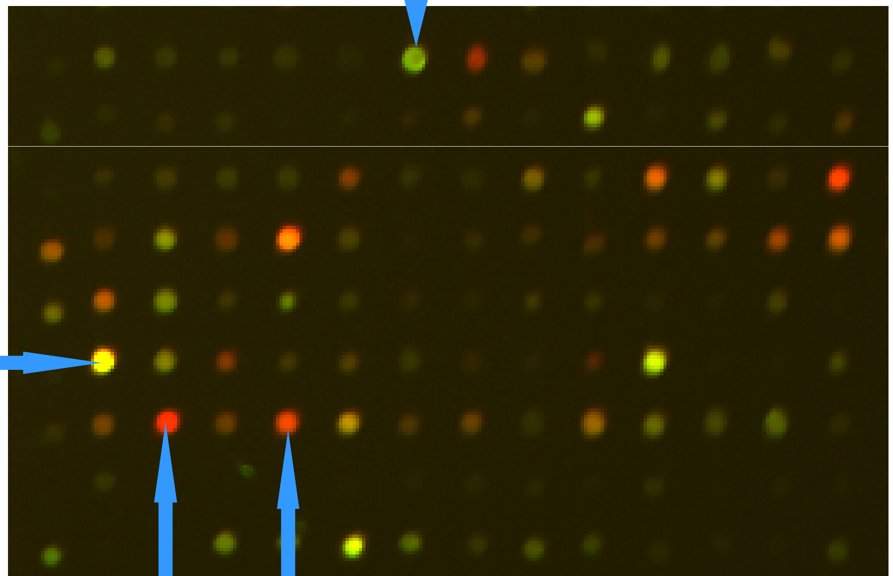
Microarrays





Expressed more in Fibrous Roots

Expressed equally in Fibrous and Storage Roots



Expressed more in Storage Roots

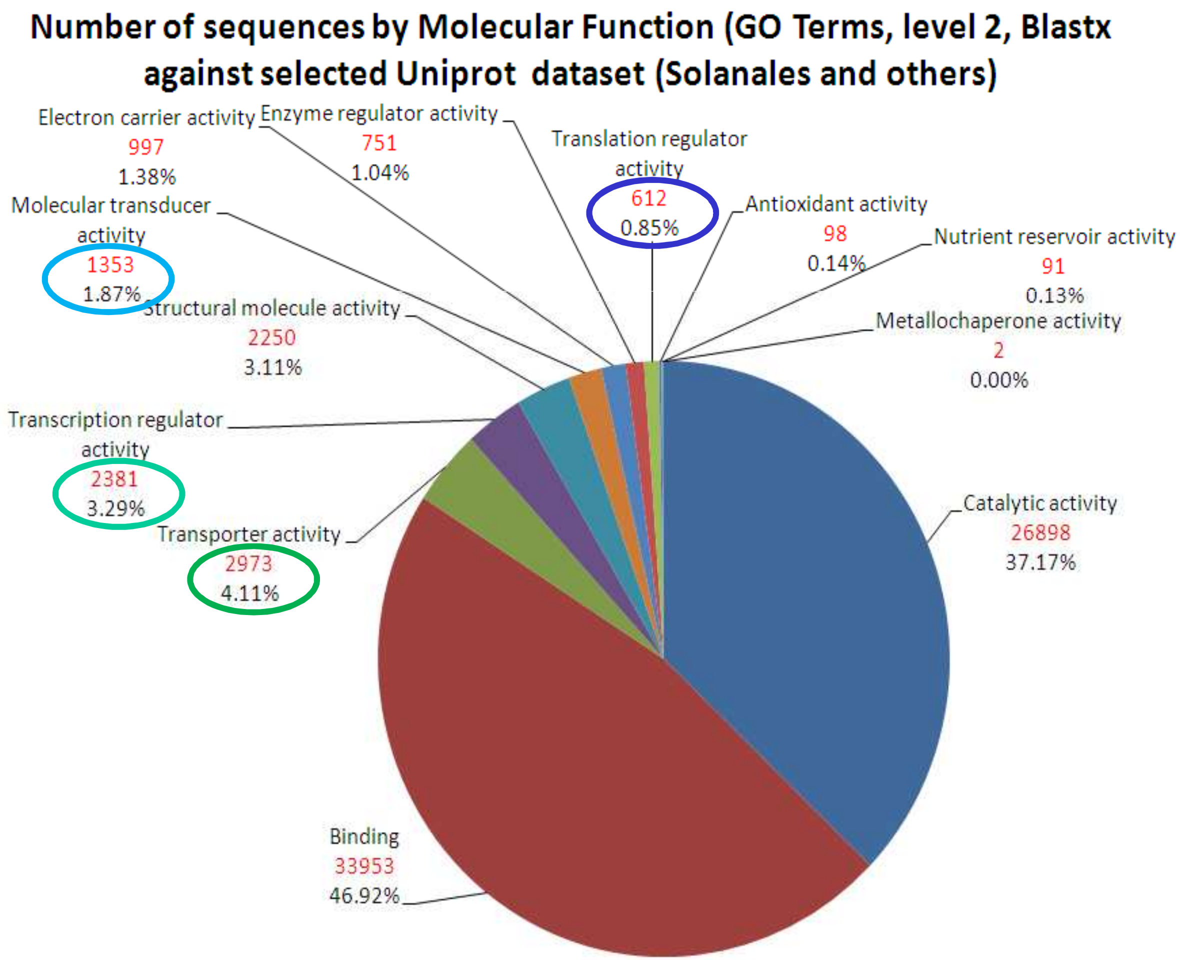
Result

Assembly	# Unigenes	Average Size (bp)	Total Annotated Unigenes
Cap3	56,270 (contigs) 18,961 (singlets)	659.8	**52322** (*49,147)
Newbler	35,069 (contigs) 42,594 (singlets)	629.1	46,967

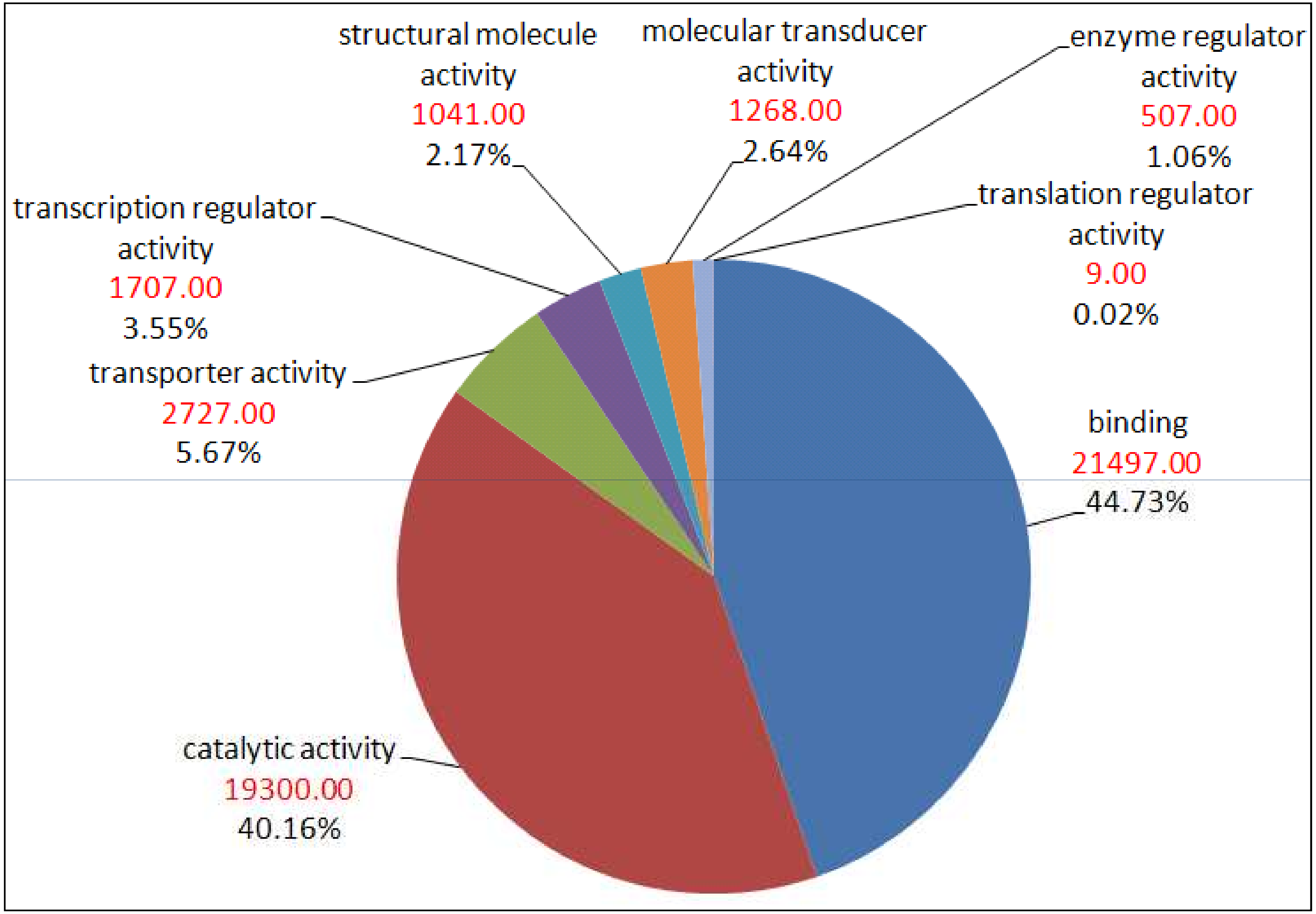
* Counts using a subset of Uniprot of protein sequences from Solanales and from some model plants(Arabidopsis, Vitis, Poplar, Rice, Coffea)

Microarray (454 sequences) 2 week initiating and lignified roots

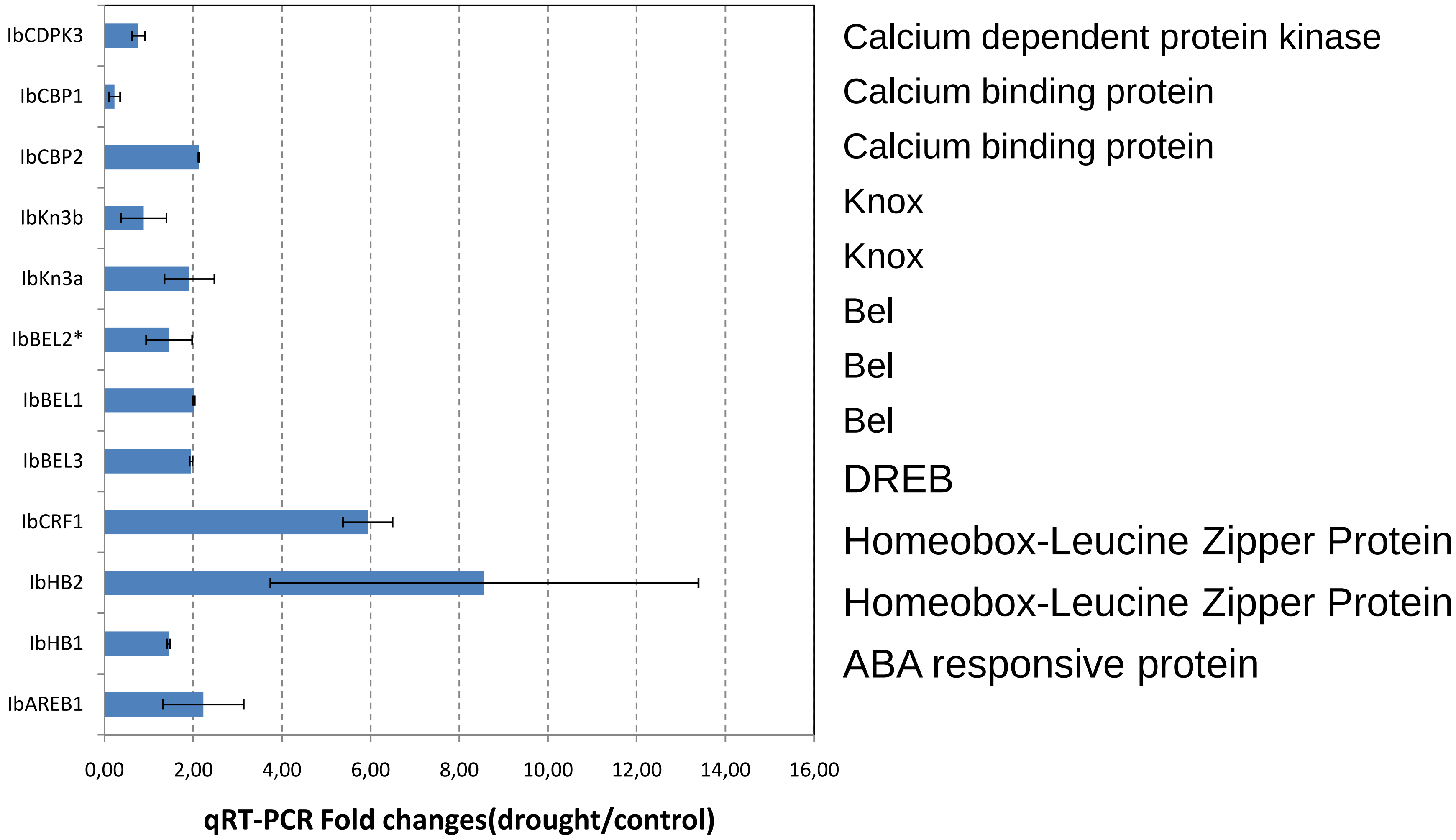
Start out with 500,000 sequences (read).
distilled to 55,000 contigs (overlapping sequences) + 45,000 singlets (single reads)
reduced further to 52,000 annotated (described genes)
finally an array built on 14,000 from 454 sequences and public databases.



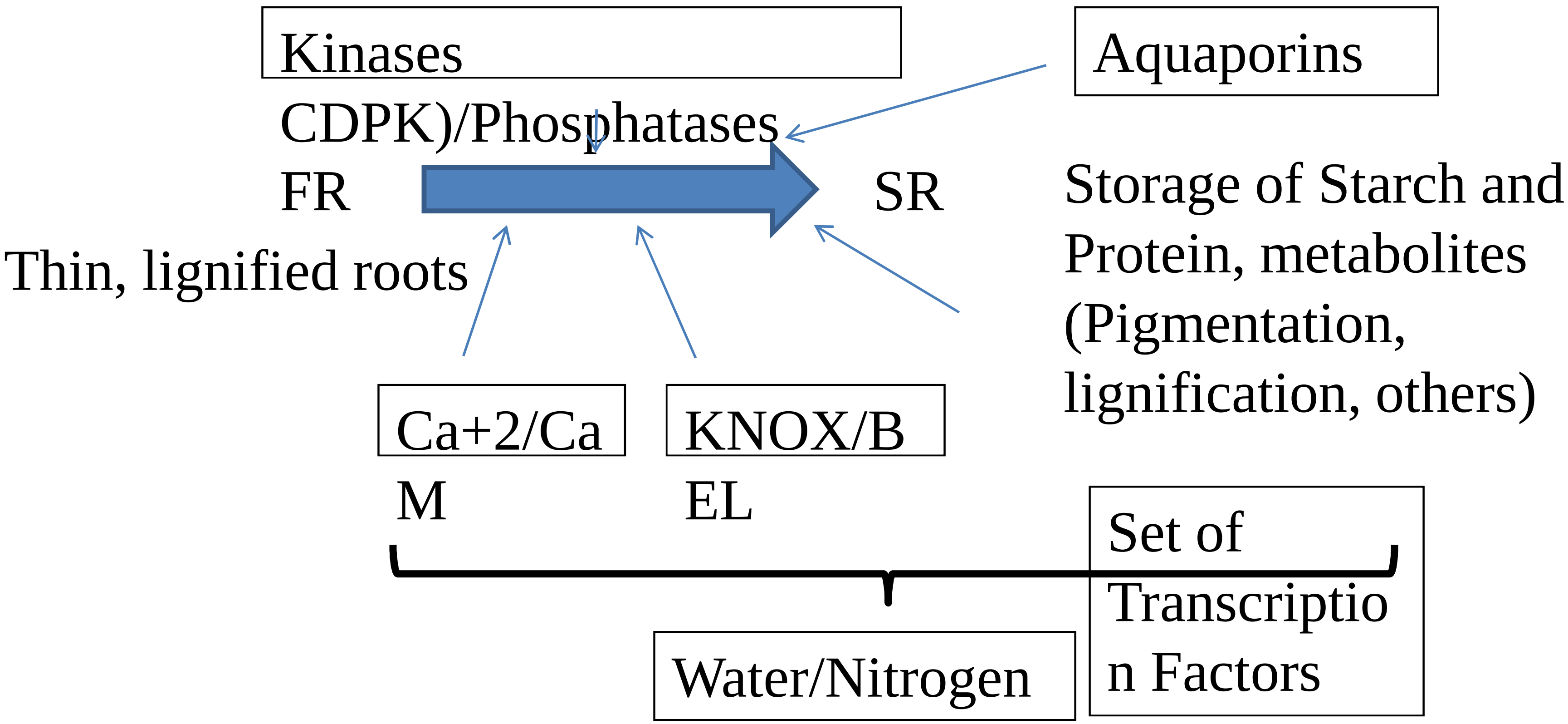
Classification of the transcriptome(cap3p80) by Molecular Function



Fold expression in drought stressed roots of sweetpotato



A HYPOTHESIS



- Calcium signaling is an important component in the onset of storage roots
- Regulatory transcriptions factor might act as a network that influence both metabolic pathways(enzymes) as well as transport and allocation of secondary metabolites
- Expression of Knox genes possibly suppressing the lignification in a similar way to Arabidopsis, Peach and Poplar.
- Post-transcriptional modifications involved in triggering the phase of FR to SR and in response to the environment

Microarrays

Advantages…

Good way to identify genes related to a trait

-global approach to understanding the genes underlying trait expression.

-can be combined with discriminant analysis

-can use an array on wild species to identify critical genes (not 100%).

-can build an array for wild species and then subject to abiotic stresses

-can do time point studies

Microarrays

Disadvantages…

-Requires specialized knowledge in bioinformatics to build the array

-Experimental designs are straightforward but analysis is not.

-Cost is an issue, but less so today

-confirmation studies with costly real time PCR

Genomics and sweetpotato - recap

√ Differential/discriminant analysis

-ease of comparisons
-chance to identify new alleles
-can build breeding population

√ Differentially expressed genes

-straightforward comparisons
resistant vs. susceptible
resistant/stressed vs. none
can identify genes

Mass selection

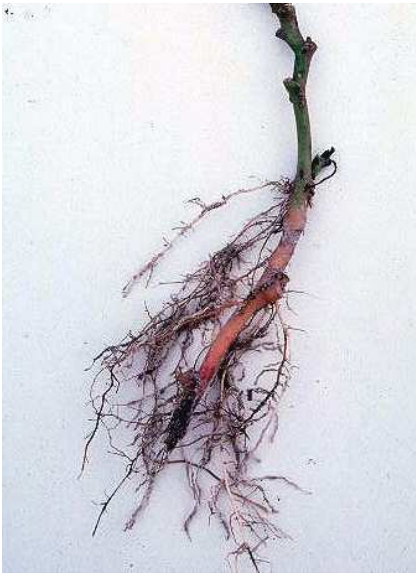
We can move a population towards a goal *if* we have solid selection criteria and we are patient

before

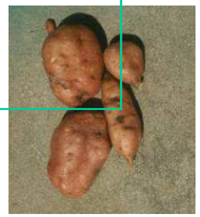
after

Streptomyces Soil Rot

(*Streptomyces ipomoeae*)



Feeder root necrosis reduces vine growth which in turn reduces yield.



Jewel = sus.

Resistant Susceptible

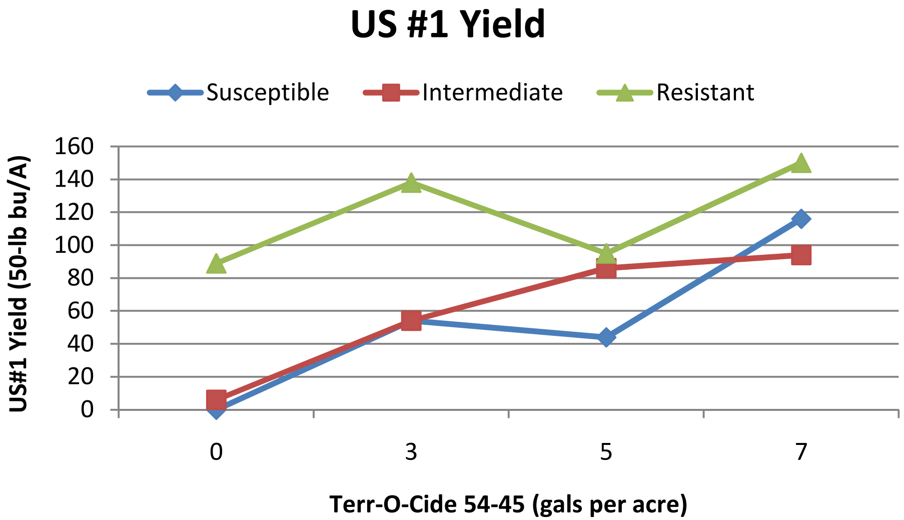
Beauregard = res.

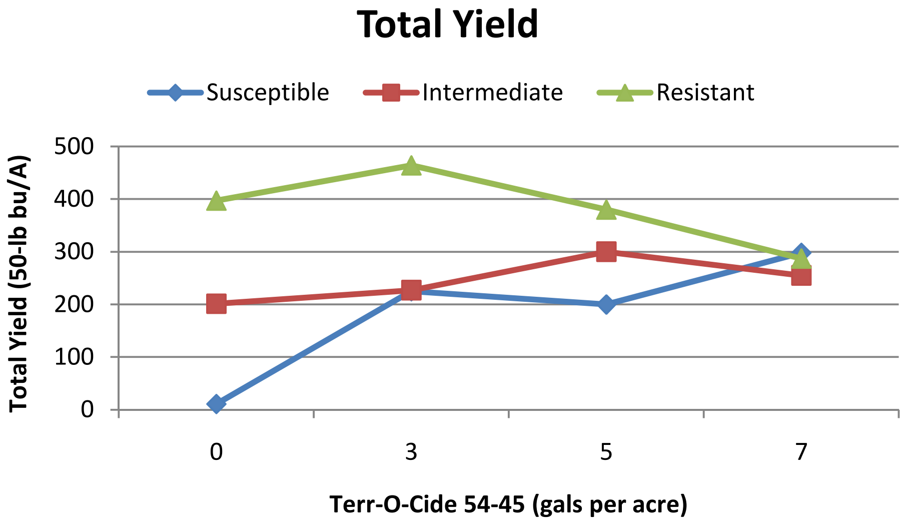
Streptomyces Soil Rot
(*Streptomyces ipomoeae*)

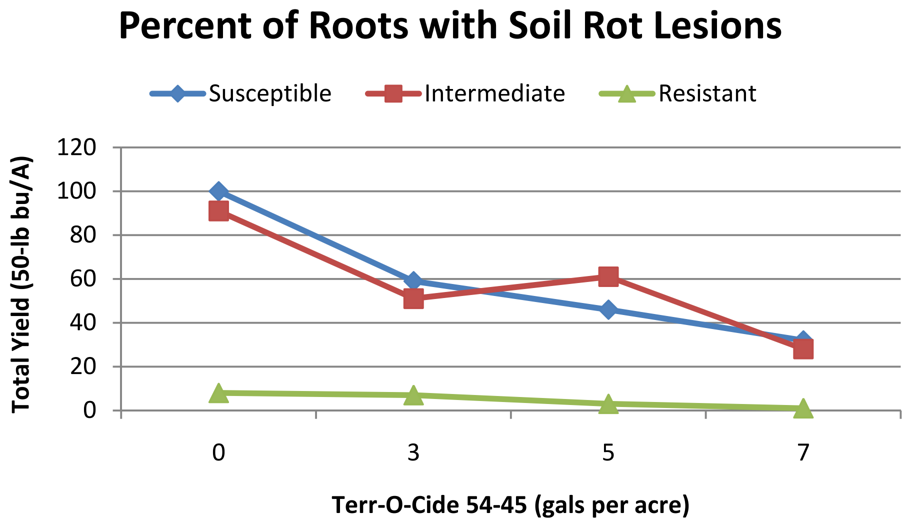
Necrotic lesions and constrictions reduce storage root quality and grade.

Streptomyces Soil Rot

(*Streptomyces ipomoeae*)







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