Seed Biology, Production and Quality 2015

Imaging Seed Internal Morphology Ronald Michaels, PhD Technical Director Phenotype Screening Corporation

Seed Quality Assessment Commercial Applications

- X-ray primed seed (Incotec, tomato)
- IR-spectrum (Syngenta, beet)
- In both cases, goal is uniform germination

X-ray Image of Tomato Seed





Germination = normal seedling

Germination = abnormal seedling

X-ray images of primed tomato seeds by Incotec

http://www.seedquest.com/~seedqu5/technology/from/Incotec/upgrading/default.htm

IR Spectrum of Beet Seed

- Developed and patented by Syngenta
- Classify seeds based on IR Spectrum
- Determines "field emergence characteristic, a chemical quality, a structural quality, a sensoric quality or a functional quality and/or a combination of one or more thereof"

Syngenta Patent: http://www.google.com/patents/EP2627458A1?cl=en

PSC's Morphology Based Approach

- Image and Analyze Individual Corn Seeds
- Germinate and Analyze Seedlings
- Grow and Analyze Plants
- Identify seed properties having predictive power
- Develop automated imaging and analysis methods for sorting seeds
- Develop Seed Multiplication for High Vigor Seeds (based on Genetic and Epigenetic Inheritance?)

Why Corn Seed Vigor?

- Seed Vigor is a "good thing"
- PSC has seen Lab results suggesting that higher vigor seeds are more responsive to a biostimulant.



Selected High Vigor Seeds - 2013

No Seed Selection - 2012

Total Root Length

X-ray Imaging of Corn Seed



X-ray image of corn seed 01_010

X-ray Imaging

- Image capture rate of 4 seeds per minute
- Optical Resolution of 20 microns/pixel
- Greater Res \rightarrow a LOT more time and money
- Imaging based on x-ray attenuation of seed constituents
- Phenotype Screening Corporation has imaged about 1500 corn seeds, of which about 400 have been germinated.

X-ray Image Acquisition

- 32 Corn Seeds in fixture
- Computer controlled vertical and horizontal positioning.
- Automated Image Capture



OCT Imaging of Corn Seed



OCT image of corn seed 01_010

Optical Coherence Tomography

- Southwest Sciences Inc. has imaged four seeds for proof of concept.
- Imaging based on variations in index of refraction – shows surfaces and interfaces
- A wide range of NIR wavelengths can be used, possibly delineating variations in constituency or chemistry
- Resolution 20 microns per pixel, greater resolution is possible

OCT Image Acquisition

Proof of Concept Lab Setup



Seed Analysis



Example: Scutellum Area done manually using ImageJ

Seedling Image Acquisition

- Seeds placed in germination pouches
- Pouches on hangers
- Each pouch photographed 4 days after germination



Seedling Analysis



Example: Primary Root Length done manually using ImageJ

Plant Growth



Seed vs Seedling

	Seed Internal Structure					
	ScutellumAreaPx	InternodalArea	SeedMass/RadicleArea	ScuA/seedA	EmbryoAxisA/SeedA	
NumberOfSeminalRoots4DAI	0.305348372	0.390666137	-0.134750918	0.216031893	0.156178176	
PrimaryRootLength4DAI	0.412293364	0.170812209	0.167621338	0.253794135	0.274469306	
ShootLength4DAI	0.604765501	-0.004477519	-0.299471811	0.642179711	0.14983289	
AxisSum4DAI	0.526426356	0.128586543	-0.043142298	0.457195579	0.243288934	
SumLengthOfSeminalRoots	0.917195554	0.350312993	-0.339688862	0.864025158	0.51521736	
VigorRating	0.923471607	0.331052187	-0.296644496	0.858368748	0.503077961	

We found high correlation coefficients, highlighted in rectangles and bold font, between

the internal seed structure of the embryo's scutellum area

and

the seedlings' sum of the root lengths of the seminal roots four days after the start of imbibition.

Seed vs Plant

	Seed Internal Structure				
	RadicleArea	ScuA/seedA	RadicleA/SeedA	PluA/InterA	
HeightatV6(mm)	-0.402993629	-0.529233373	-0.393430539	0.217730979	
V6Date	0.125953443	0.10573364	0.144666434	0.728915869	
DaysToV6	0.125953443	0.10573364	0.144666434	0.728915869	
HeightAtV5(mm)	-0.697007613	-0.667458299	-0.708944656	0.196562404	
V5Date	0.087595686	0.037891182	0.090944806	0.726376639	
DaysToV5	0.087595686	0.037891182	0.090944806	0.726376639	
HeightV4(mm)	-0.794815444	-0.849886148	-0.799598381	-0.094424125	
V4Date	0.249859467	0.081561666	0.255539703	0.822131391	
DaysToV4	0.249859467	0.081561666	0.255539703	0.822131391	

We found relatively high correlation coefficients between

the internal seed structure of the embryo's radicle area

and

the height of the growing plant at vegetative development stage V4.

Preliminary Conclusions

- Seed germination and vigor are correlated with nonlinear combinations of seed feature measurements.
- Other internal seed properties not yet measured are important in determining seed germination and vigor.
- It is possible to raise the percentage of high vigor seeds in a seed lot by nondestructive analysis.
- Trait propagation through seed multiplication may solve numbers problem.

Further Work

- OCT Imaging
- Higher Resolution
- Larger sample sets
- Automation
- Integration with other Testing Methods