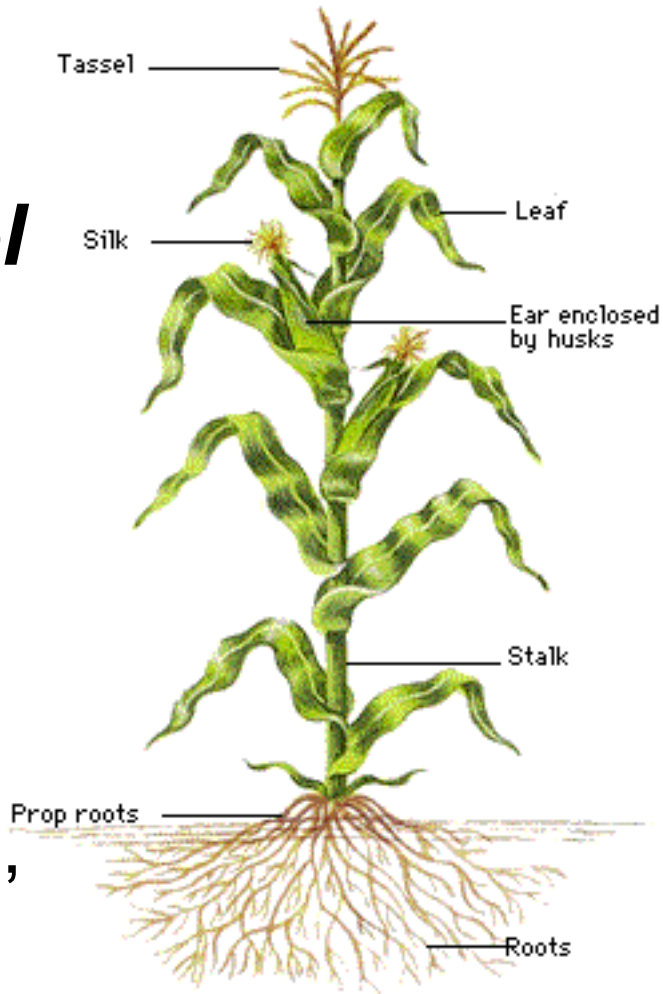
National Renewable Energy Laboratory

# *Near-Infrared Spectroscopy as a Genetic Screening Tool for Corn Stover Cell Wall Chemistry*




May 7, 2003

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A.D. Sluiter, D.W. Templeton,
K.W. Evans, B.R. Hames

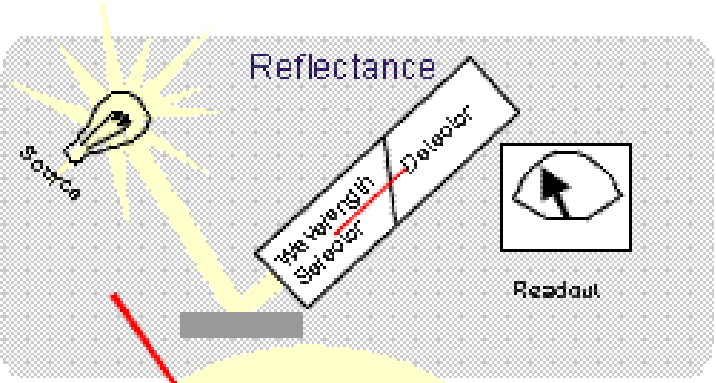
# Project Goals

- Develop a reliable, high throughput method for screening corn plants for differences in cell wall composition.
- Acquire positive control lines and incorporate into screen.
- Identify candidate mutant lines for further investigation.

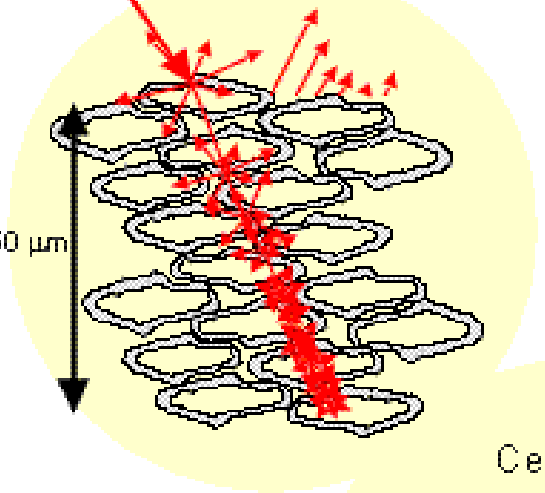
- Future: Isolate and characterize cell wall-related genes.

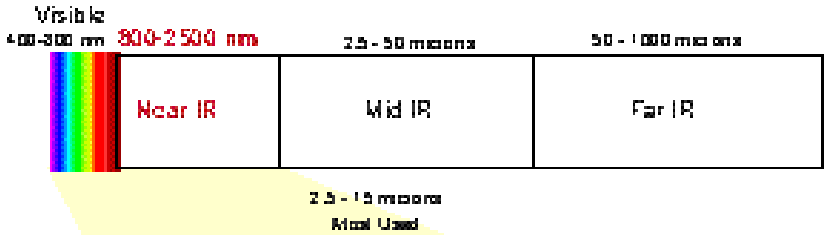
# Advantages of NIR Spectroscopy

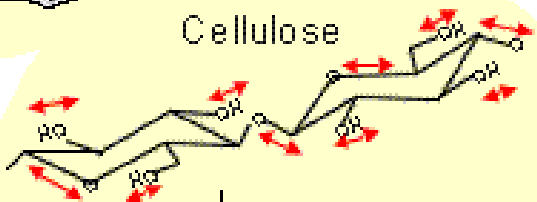
| | Wet Chemistry | Near-infrared spectroscopy |
|---|---|---|
| Analysis time/sample | 2 weeks | minutes |
| Throughput | 6/week | 500 -1000/day |
| Cost/sample | $1000 - 2000 | $10 - 20 |
| Technician | highly trained | novice |

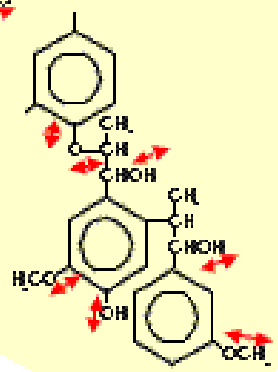


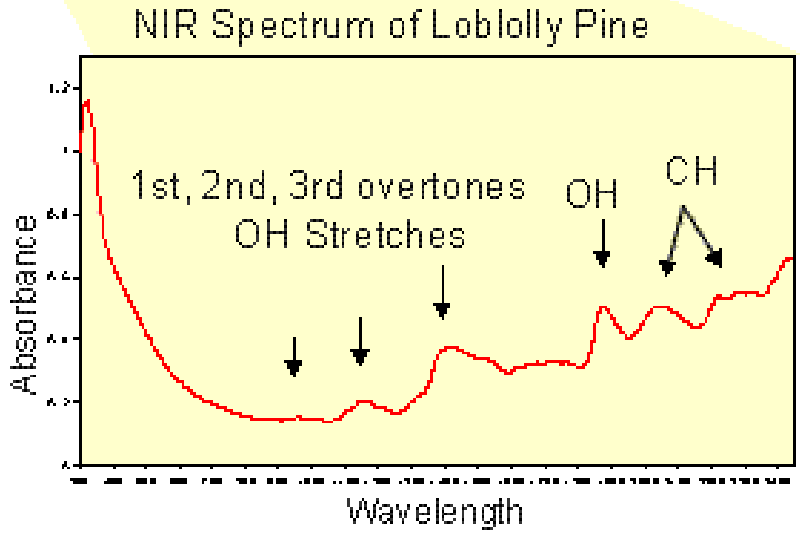

NIR is typically used for measurement of organic functional groups, especially C-H, O-H, N-H, and C=O



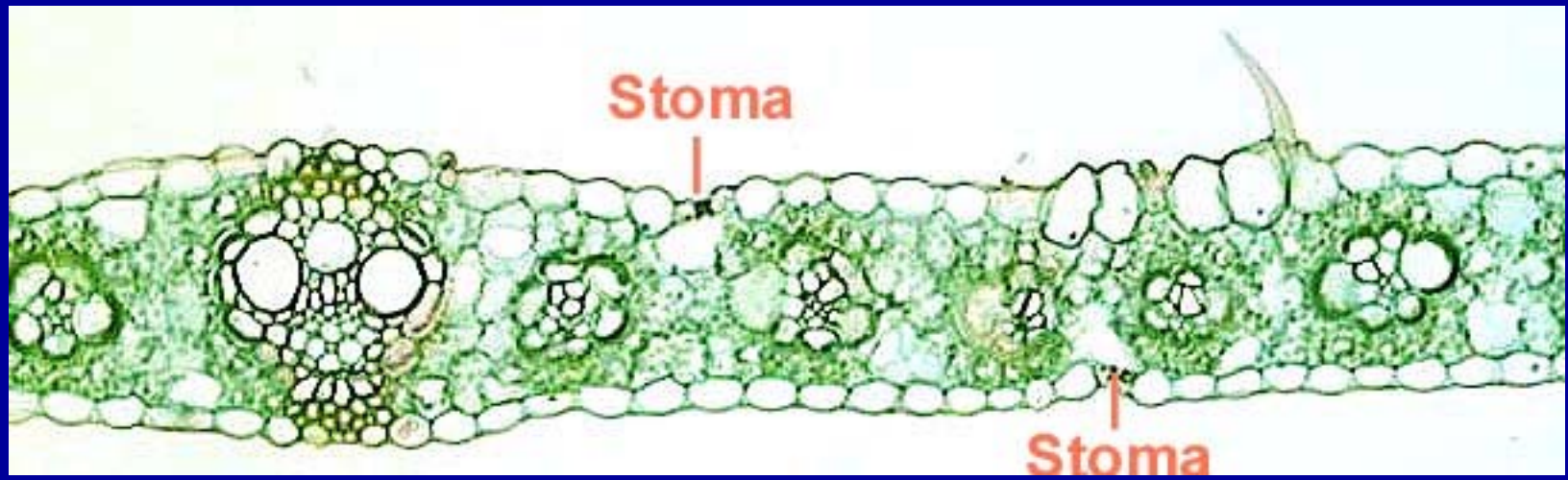



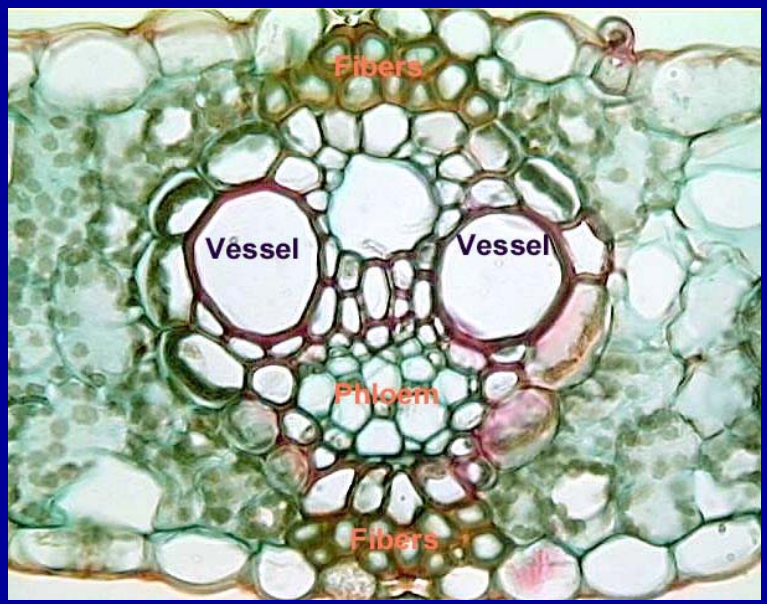



# Corn Leaf Anatomy




http://www.lima.ohio-state.edu/biology/leaves.html

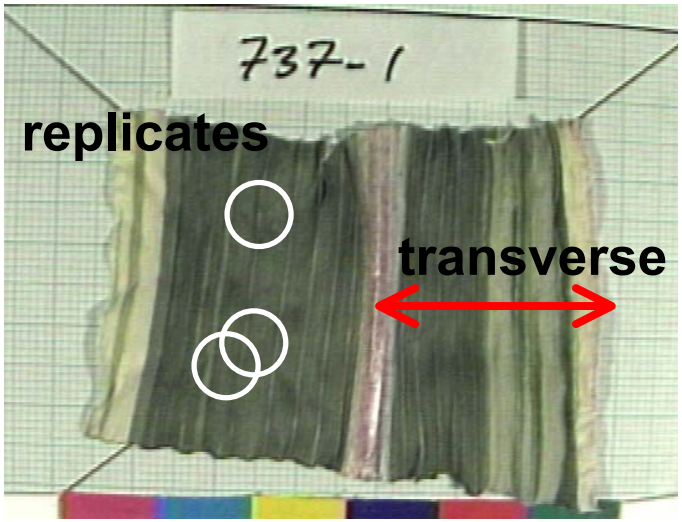
# Principal Component Analysis

- Computerized data reduction technique that facilitates identification of unusual samples in complex data sets.
- Analysis assumes a normal distribution for all PCs.
- Correlates each data point with every other in data set.
- Spectral correlations are grouped into orthogonal (independent) principal components (PC).
- Each PC can be inspected separately for features of interest (PC loading).
- PC1 explains highest proportion of variance among samples (PC1 > PC2 > PC3…).
- PC score is expressed as variance from a mean.

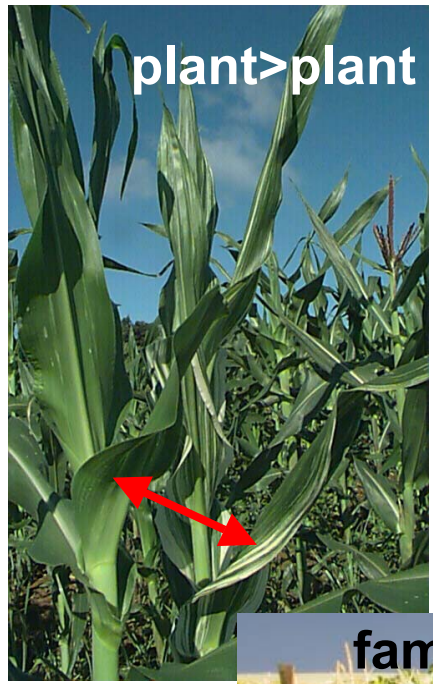
# Resources and Tools

- Corn seed from segregating F2 mutant families from a Mu transposon insertion library generously provided by Erik Vollbrecht and Rob Martienssen (Cold Spring Harbor Laboratory, NY).
- NIR Spectrometer: ASD FieldSpec Pro FR (Applied Spectral Devices, Boulder, CO).
- Multivariate statistics software: The Unscrambler (CAMO).

# Sample Collection

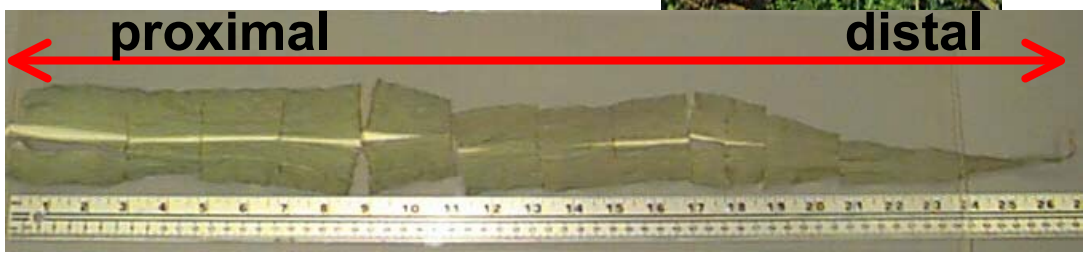



Abaxial vs. adaxial

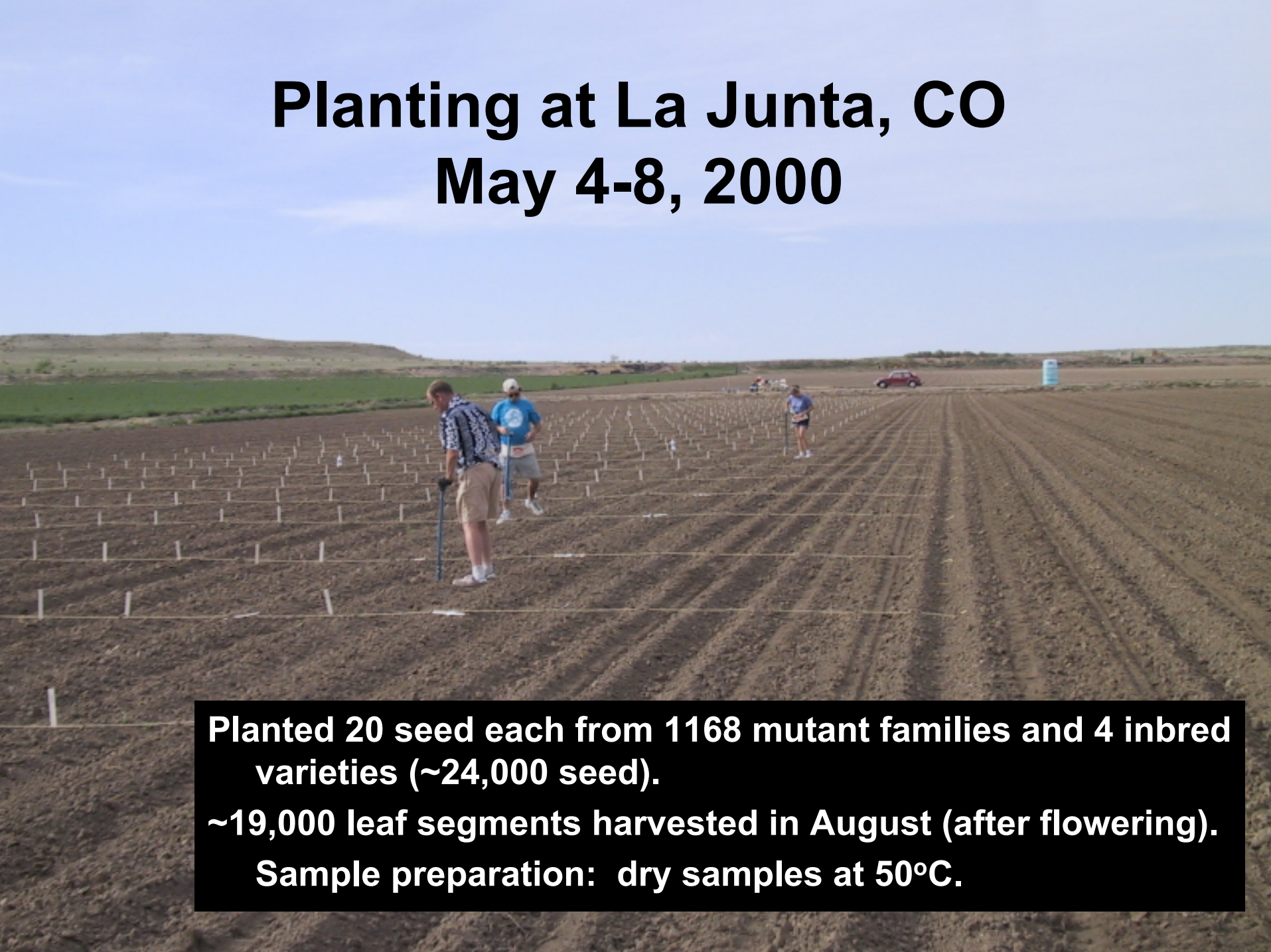





**Harvest 2-inch segment from central third of 5th mature phase leaf blade.**



# Planting at La Junta, CO
# May 4-8, 2000

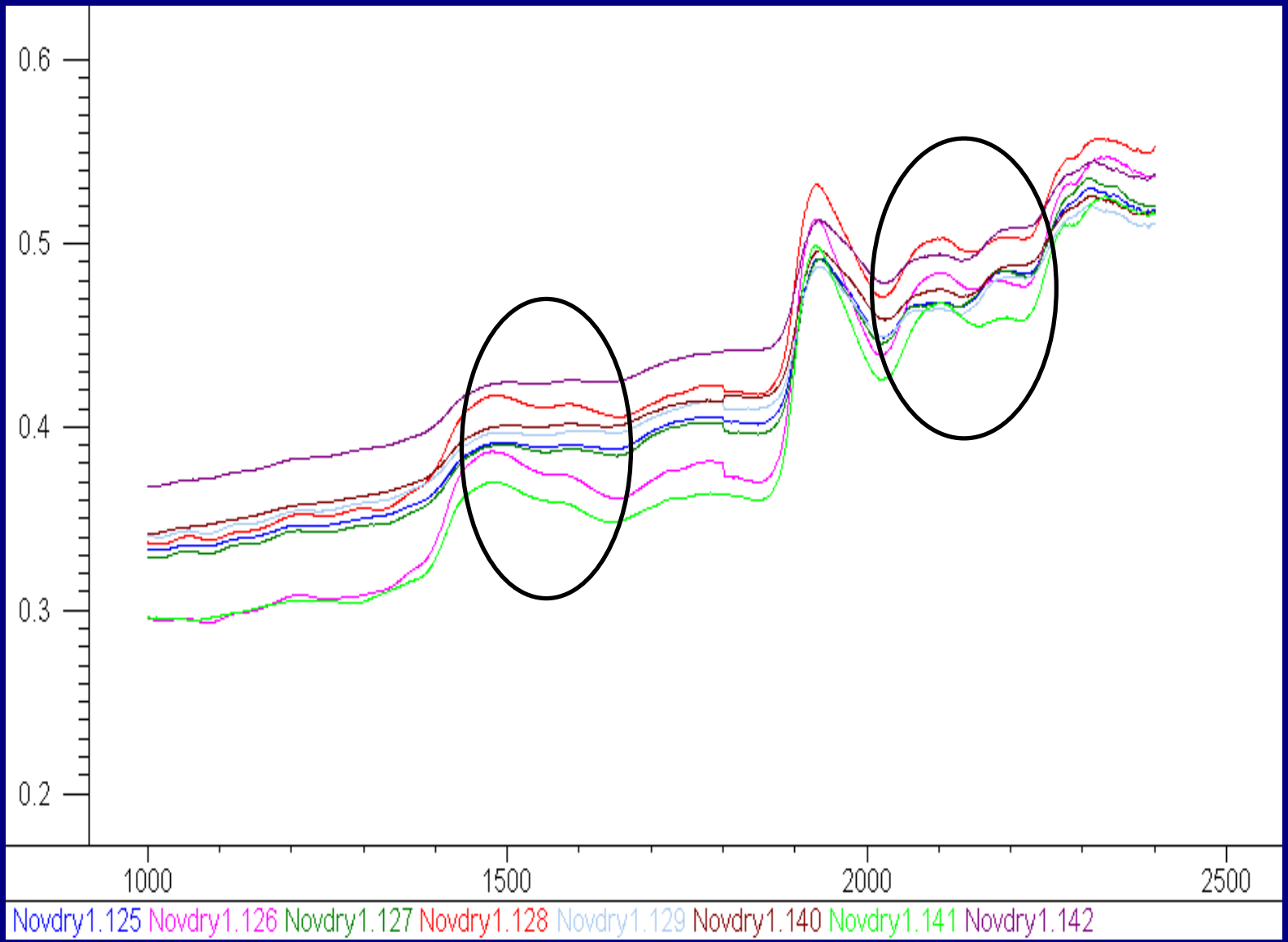


Planted 20 seed each from 1168 mutant families and 4 inbred varieties (~24,000 seed).

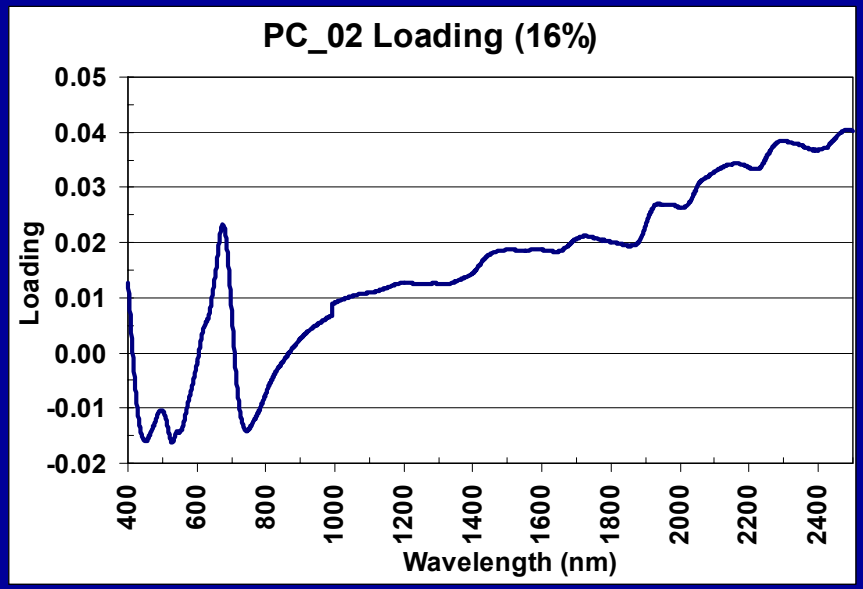
~19,000 leaf segments harvested in August (after flowering).

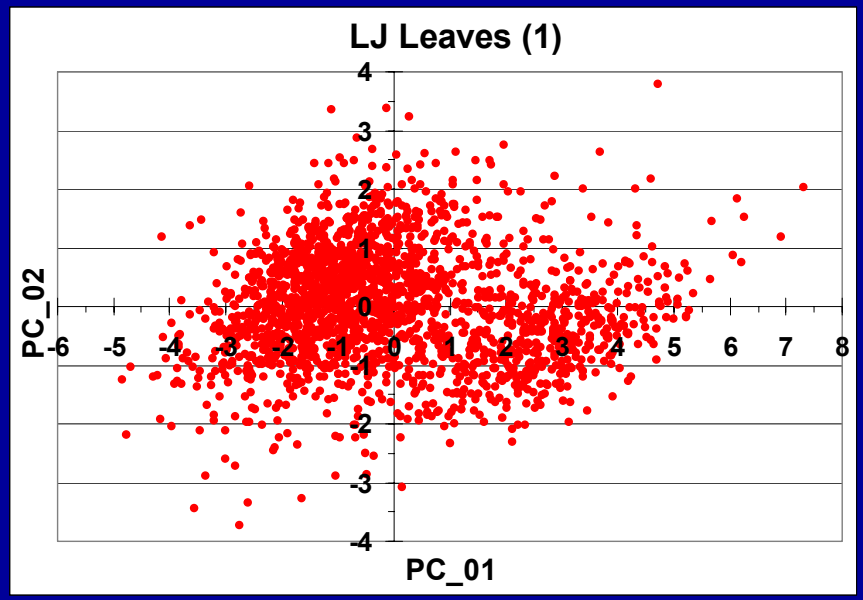
Sample preparation: dry samples at 50°C.

# Spectral Differences Among Samples



# Principal Component Map of *Zea mays* Mutants



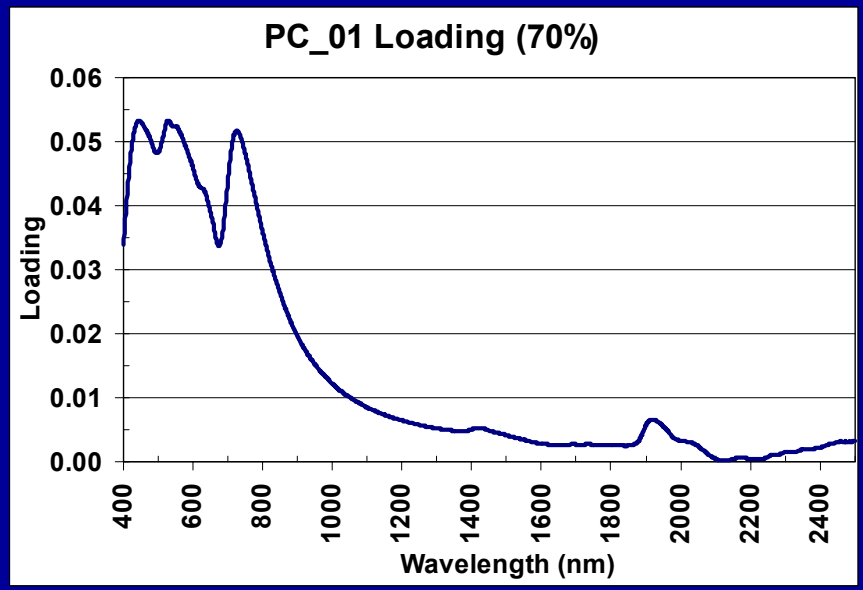

Model 1

Samples: 15,712

2000 individuals shown

Variables: 400-2500 nm

Weights: 1.0



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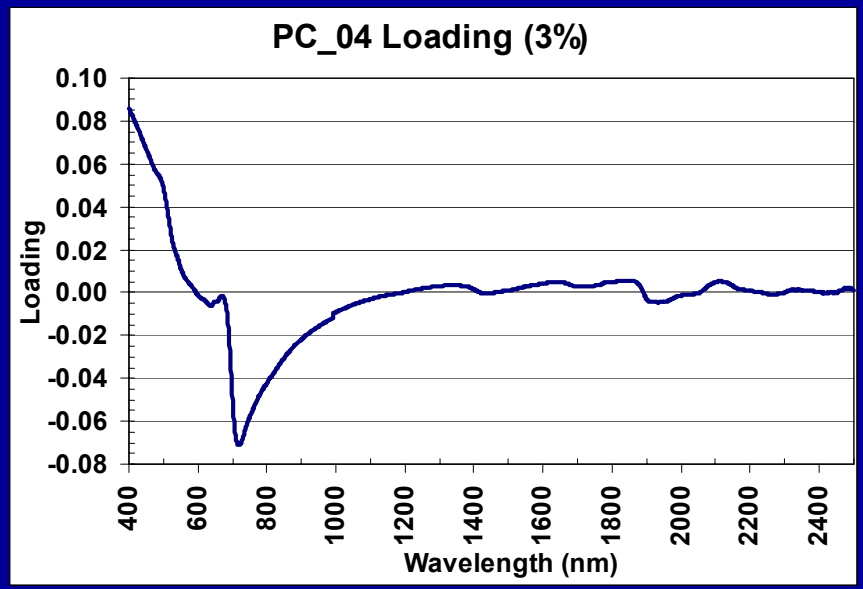


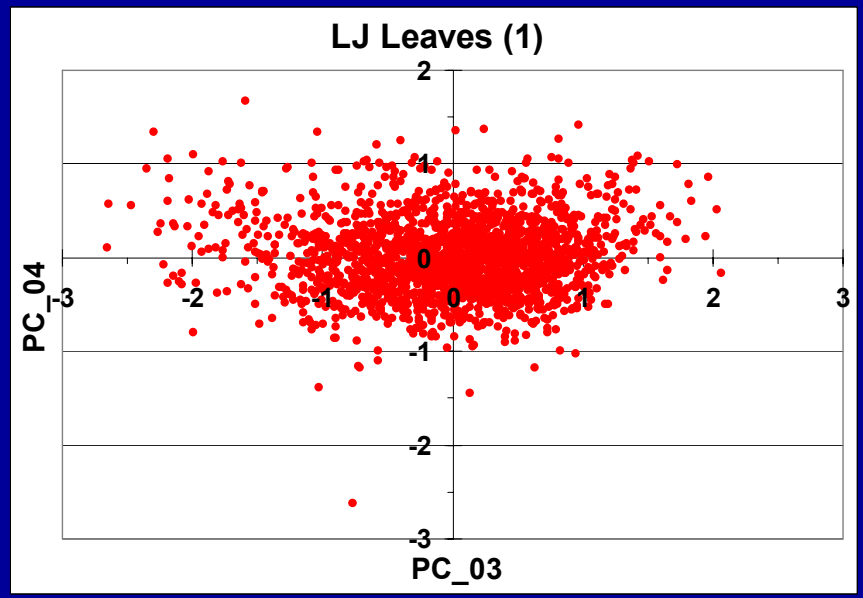


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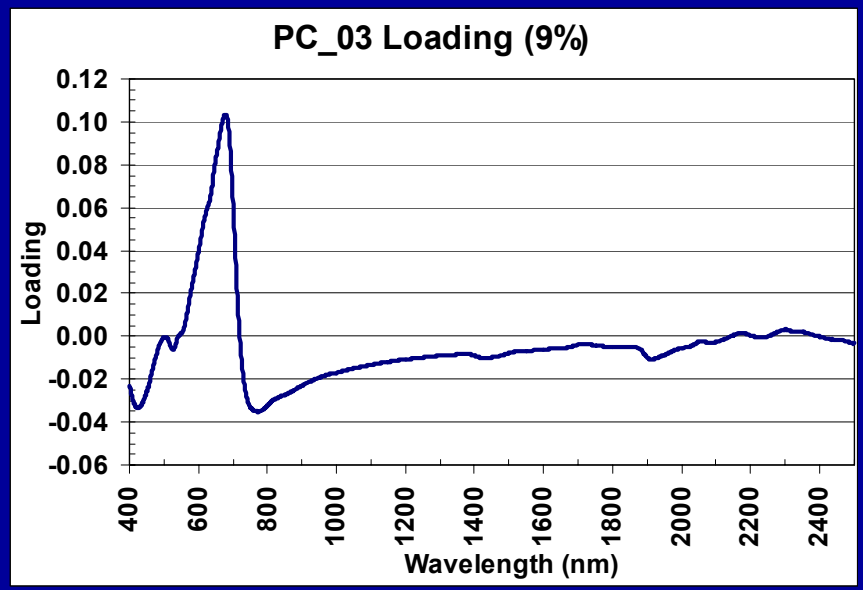

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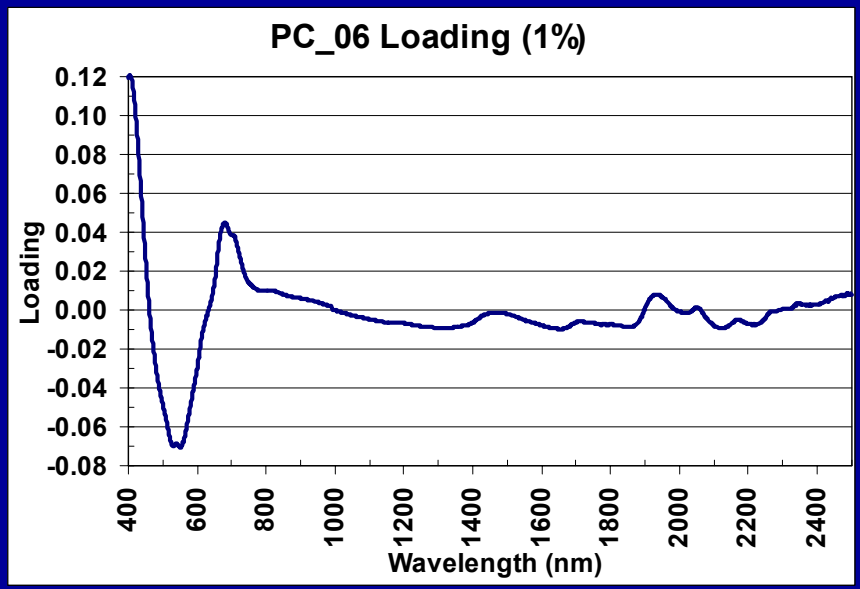


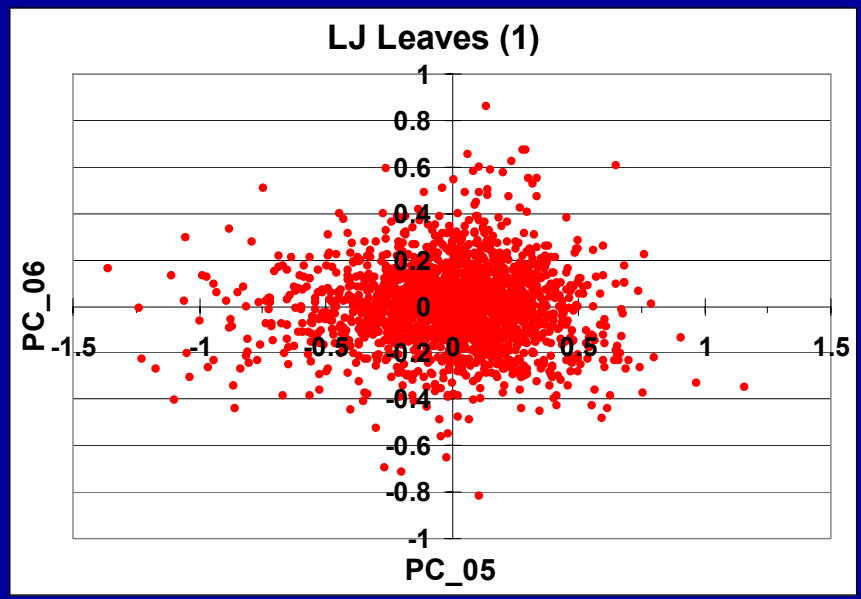


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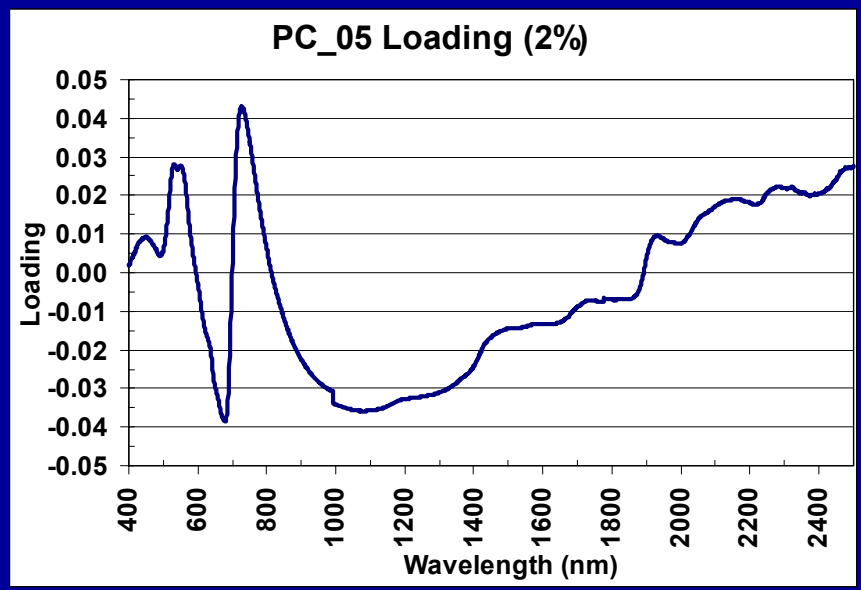


# Summary of La Junta Leaf Results

- ~19,000 leaf specimens were collected from ~1168 mutant families and inbreds.
- 15,712 spectra were collected from 776 mutant families and analyzed by PCA (~12,100 individuals).
- Five PCs explain >99% of the variance in the system.
- Loadings for PCs 2 & 5 contain information about cell wall polymers.
- 484 individuals lie outside the 99.5% confidence interval for at least one PC.
- 26 families contain 3 or more individuals scoring very high/low on PC2 &/or PC5 (3.5% of families screened).
- These families became the focus of subsequent work.

# La Junta Stover Screen

- 1879 whole plants were collected at the end of the growing season (no cobs or grain included).
  - 119 mutant families + 4 inbred lines.
- Plants dried and milled individually.
- NIR spectra collected and chemical composition determined using NREL's calibrated NIR method.
- Two standard deviation range about the mean was calculated for each constituent (95% confidence interval).
- Families containing approximately 25% of siblings outside the 95% confidence limits for the same constituent(s) are candidates for further investigation.
- 25 families selected.

# LJ Stover: Low Xylan Family

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| < M-2stdev | 0.2 | 38.1 | 15.3 | 11.3 | 1.6 | 1.8 | 2.6 |
| > M+2stdev | 2.6 | 45.5 | 22.0 | 18.2 | 6.5 | 9.5 | 4.3 |
| | | | | | | | |
| Sample | GH | glucan | xylan | lignin | protein | st_inorg | acetyl |
| 3165-1 | 3.3 | 40.0 | 15.2 | 14.8 | 4.5 | 9.0 | 3.3 |
| 3165-2 | 1.4 | 42.9 | 16.2 | 16.9 | 2.8 | 6.8 | 3.0 |
| 3165-3 | 1.4 | 41.8 | 16.1 | 15.6 | 3.4 | 7.8 | 3.1 |
| 3165-4 | 2.0 | 44.0 | 17.3 | 16.9 | 3.0 | 5.3 | 3.5 |
| 3165-5 | 1.1 | 42.4 | 16.4 | 15.6 | 2.7 | 8.0 | 2.8 |
| 3165-6 | 2.1 | 43.7 | 15.7 | 16.9 | 2.4 | 7.6 | 2.4 |
| 3165-7 | 0.7 | 42.6 | 18.5 | 16.7 | 3.0 | 5.6 | 3.6 |
| 3165-8 | 1.6 | 44.5 | 14.6 | 15.8 | 2.3 | 7.1 | 3.1 |
| 3165-9 | 1.4 | 43.6 | 15.8 | 16.3 | 2.9 | 5.6 | 3.3 |
| 3165-10 | 2.8 | 43.6 | 15.0 | 15.3 | 3.5 | 7.7 | 3.2 |
| 3165-11 | 2.0 | 44.4 | 14.1 | 13.4 | 3.8 | 8.1 | 3.2 |
| 3165-12 | 2.3 | 43.9 | 15.6 | 17.1 | 2.6 | 7.0 | 2.9 |
| 3165-13 | 2.0 | 44.1 | 15.9 | 14.2 | 3.2 | 7.2 | 3.4 |
| 3165-14 | 1.2 | 41.2 | 17.3 | 16.1 | 4.7 | 6.1 | 3.3 |
| 3165-15 | 2.3 | 43.1 | 16.0 | 16.2 | 2.9 | 7.7 | 2.7 |
| 3165-16 | 1.4 | 41.6 | 18.0 | 17.6 | 3.6 | 5.7 | 3.0 |
| 3165-17 | 1.6 | 43.2 | 18.1 | 13.5 | 4.4 | 6.6 | 3.0 |
| 3165-18 | 2.1 | 43.8 | 15.9 | 15.9 | 2.3 | 6.9 | 3.0 |
| 3165-19 | 1.9 | 40.4 | 15.5 | 14.3 | 5.1 | 9.0 | 3.2 |

# Unusual Stover Composition Patterns Observed

| Pattern # | Glucan | Xylan | Lignin | Acetyl | Protein | Ash |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |

**Low content**
**High content**
**@95% confidence**

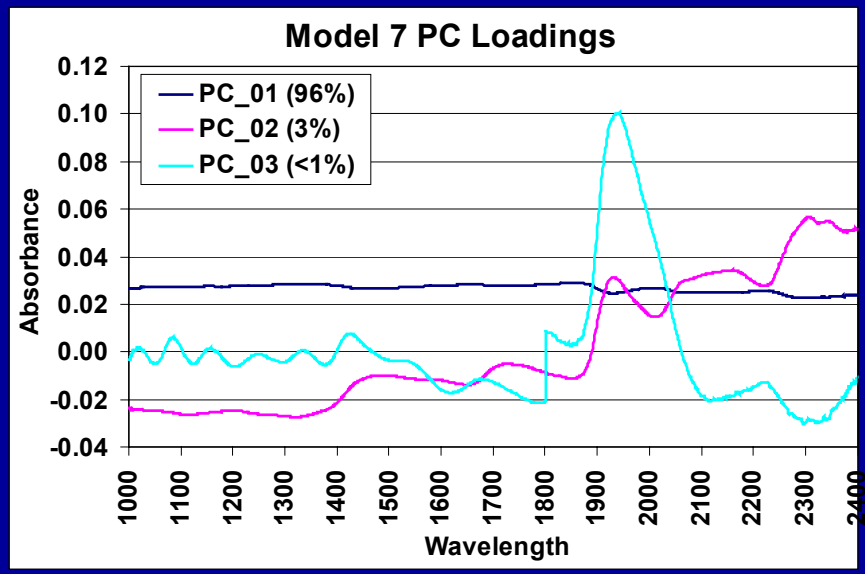
NREL

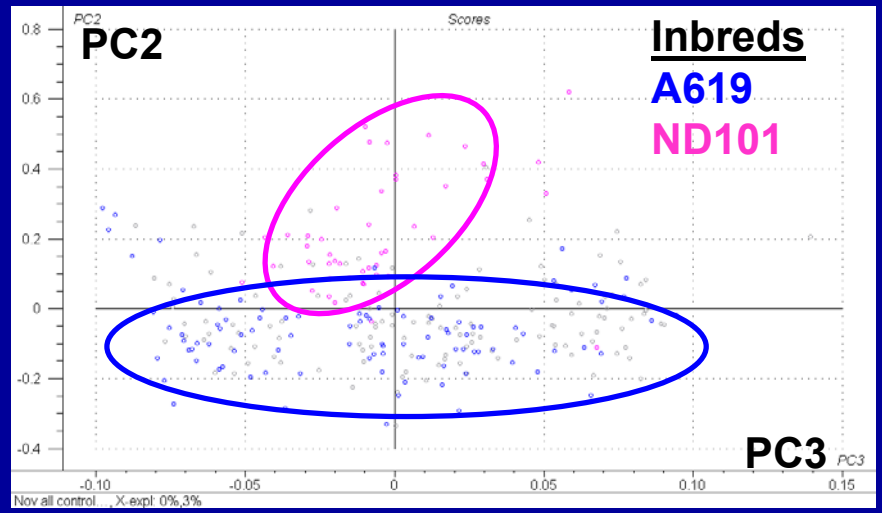
# 2002 Re-Screen

# Corn Lines Planted in 2002

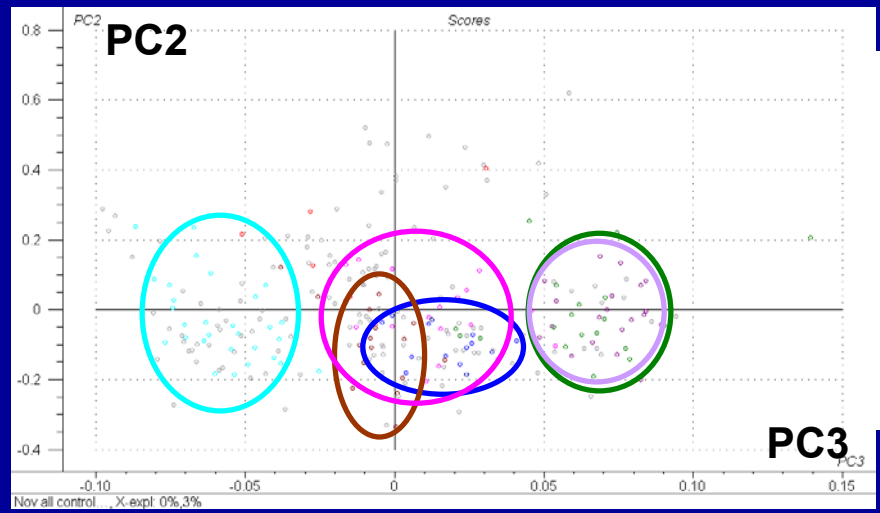
- CSHL MTM lines
  - 26 from La Junta leaf screen
  - 25 from La Junta stover screen
  - 24 lines related to stover lines
- 7 positive controls
  - Purdue *bm1*, *bm2*, *bm3*, *bm4* (in A619 background)
  - Mycogen F407 (*bm3*)
  - MGSC 515D (*bm1*)
  - MGSC 916C (*bk2*; brittle stalk)
- 6 inbred lines (negative controls)
  - A619, ND101, B73, Mo17, W22, Purdue A619
- 8 backcrossed MTM lines (from 2001)

# PCA Model - 2002 All Controls




Model 7
Samples: All controls
Variables: 1000-2400 nm
Weights: 1.0




Purdue bm1
Purdue bm2
Purdue bm3
Purdue bm4
Mycogen F407
MGSC 515D
MGSC 916C

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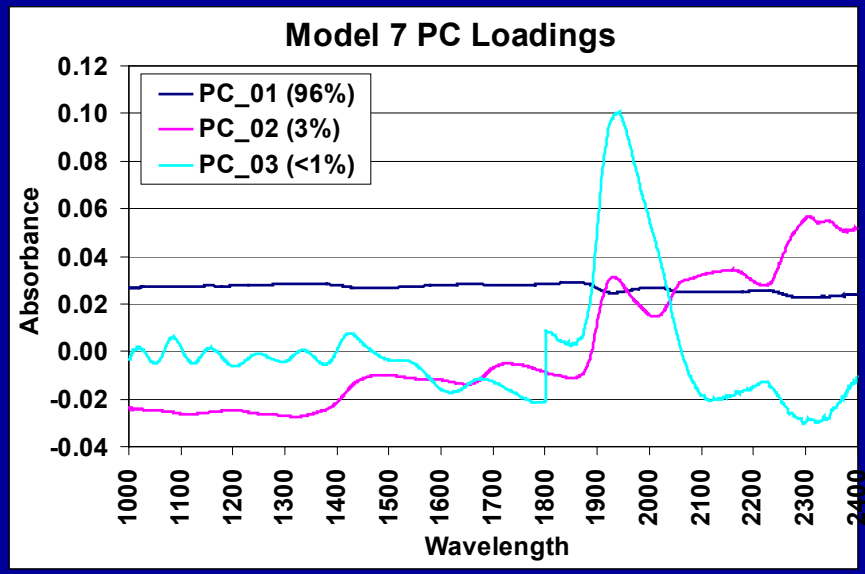
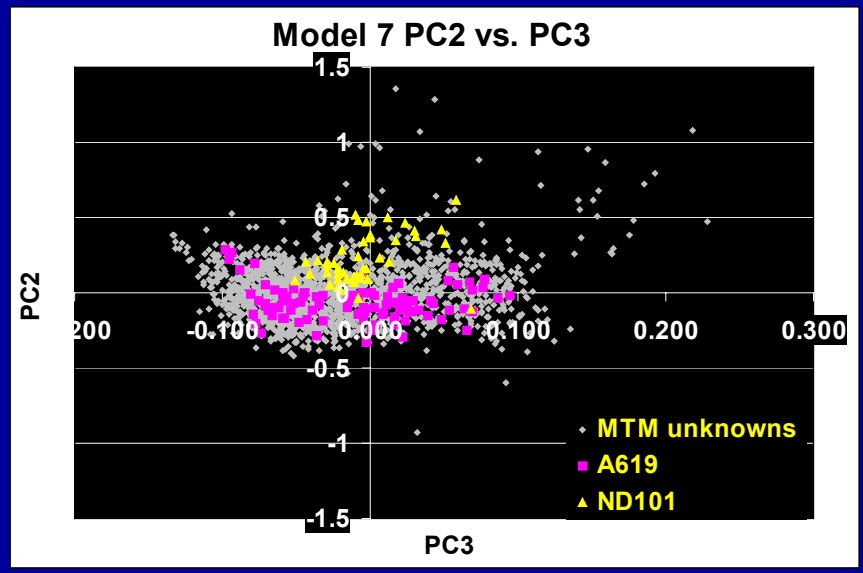
# Classify MTMs into PCA Model

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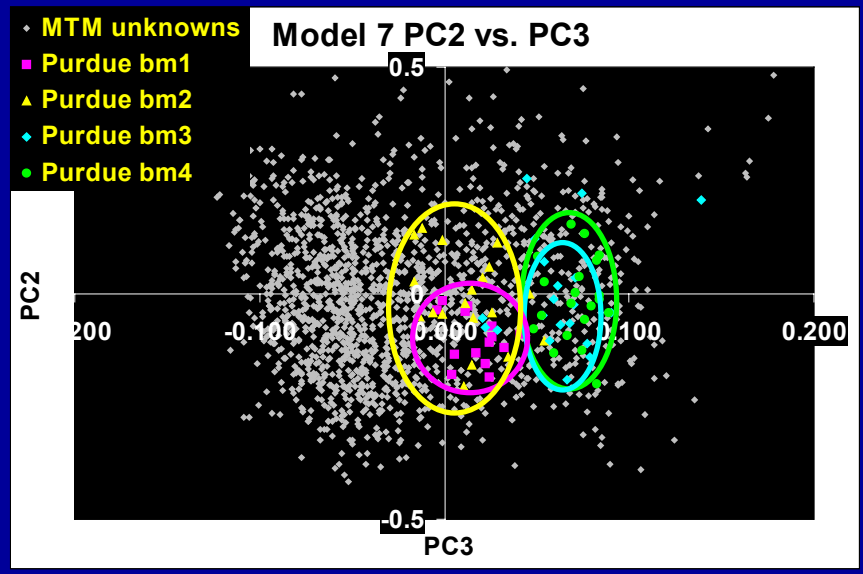
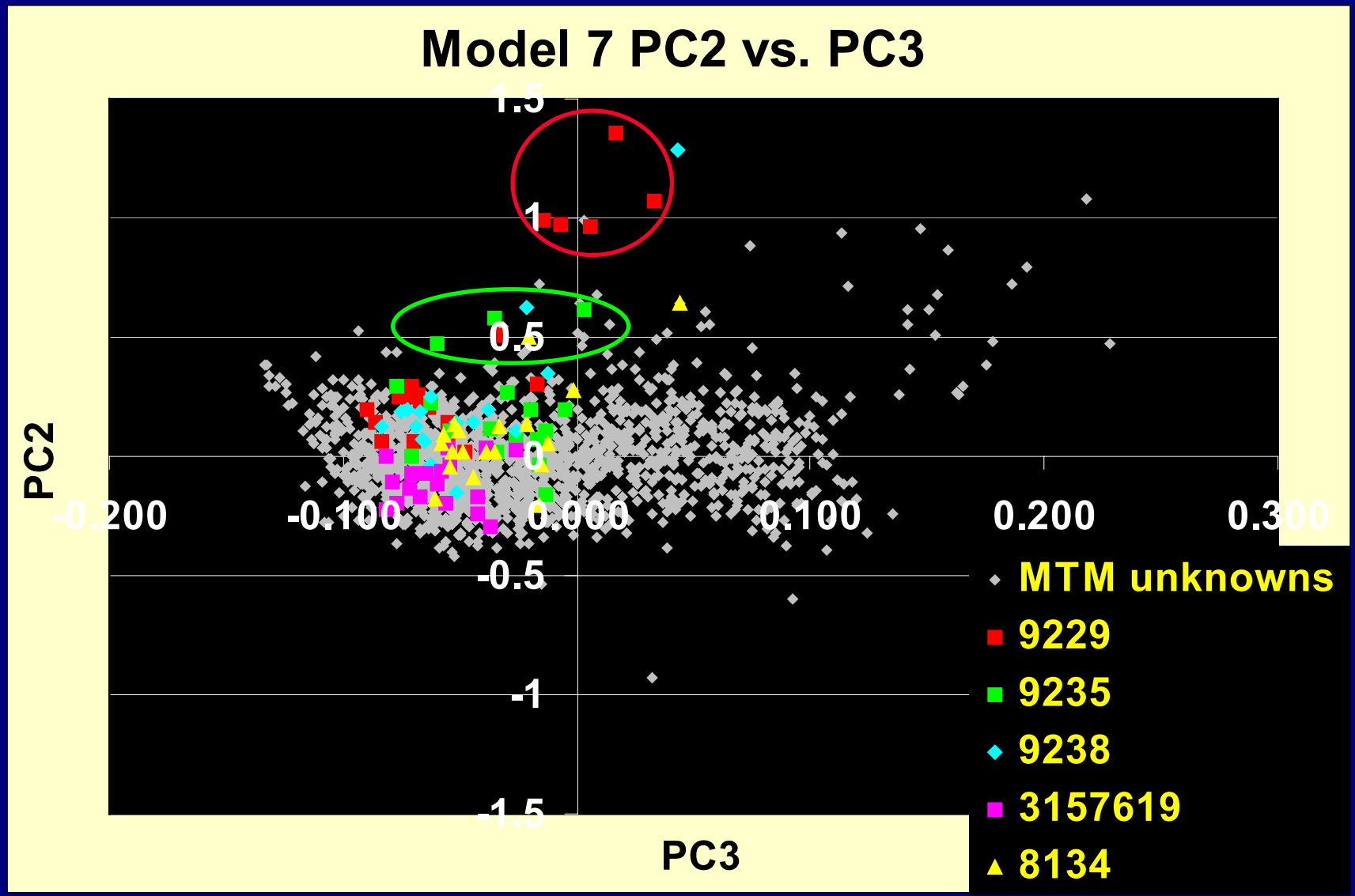


# Segregating MTM Families in Model 7



# Results of Secondary NIR Screen

| PC2 | PC3 | PC2 & PC3 |
|---|---|---|
| ●3163 | ●3319 | ●6959 |
| ●3168 | ●3347 | ●8133 |
| ●3170 | ●4976 | ●8136 |
| ●3985 | ●5998 | |
| ●3992 | ●7249$1-2 | |
| ●7249$6-5 | ●7249$12-7 | |
| ●8137 | | |
| ●9229 | | |
| ●9235 | | |

# Summary and Conclusions

- Reliable and repeatable NIR spectra can be obtained from the surfaces of dried corn leaf segments.
- PCA identified 26 candidate mutant families that segregate (3:1) for differences in leaf cell wall chemistry.
- A significant fraction of unusual families from the initial leaf screen were confirmed in a secondary screen.
- Available positive controls were of limited usefulness.
- 25 candidate families were identified in a NIR-based compositional screen of whole stover.
- A calibrated PLS1 model that determines the chemistry of samples makes identification of unusual individuals (and *why* they are unusual) much more straightforward.

NREL

# Acknowledgments


Regulars
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Bonnie Hames
Tammy Hayward
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Bob Meglen
Amie Sluiter
David Templeton

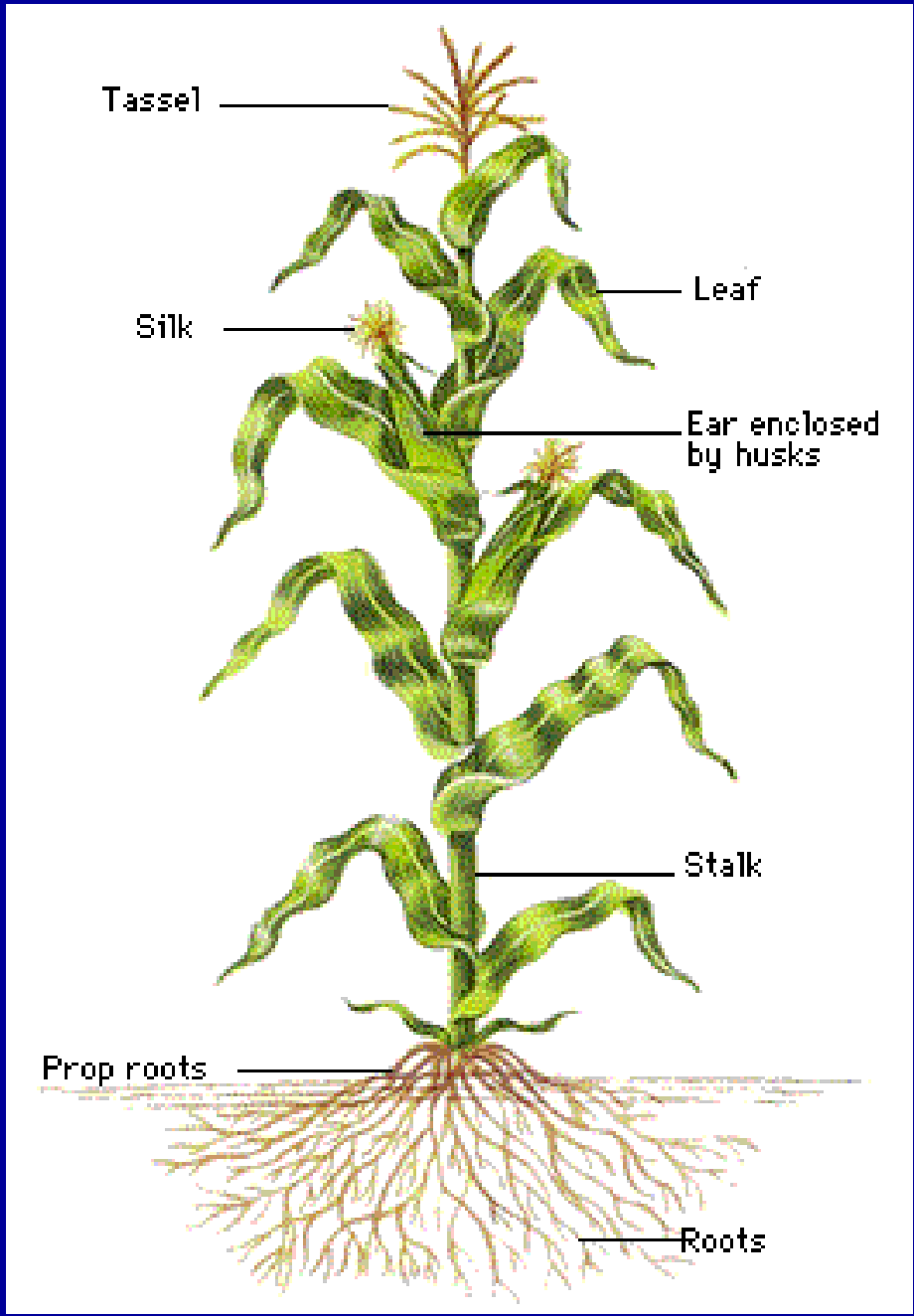
DDRD
Carol Riordan
Bobi Garrett

Recruits
Mark Davis
Greg Flack
Rick French
Jenny Hamilton
Eric Jarvis
Mike Looker
Rich Lozano
Gary McMillen
Millie Newman
Joe Patrick
Chris Roth
Chris Scarlata
Justin Sluiter
Liz Willson

Summer Interns
Martha Gitt (2000)
Nicole Buyck (2000)
Evan Thomas (2001)
Adam Zachary (2001)
Charnell Clark (2002)
Jonathan Meuser (2002)

Collaborators
Erik Vollbrecht (CSHL)
Rob Martienssen (CSHL)
Pat Bedinger (CSU)
Wilfred Vermerris (Purdue)


# end


Tassel
Leaf
Silk
Ear enclosed by husks
Stalk
Prop roots
Roots