

# A “nomics” Approach to Studying Abiotic Stress and Seed Vigour in *Brassica*

Dr. L.V. Gusta

Dept. Plant Sciences

University of Saskatchewan



# Research Interests

- ABIOTIC STRESS
  - Low Temperature/Freezing
  - Drought
  - Heat
- SEED VIGOUR
  - Low Temperature
  - Salinity
  - Aging

# Research Methodologies

- Genomics
  - Global Gene Expression Analysis/Microarray
- Proteomics
  - 2D-Gel Electrophoresis
  - 2D-Differential Gel Expression (2D-DIGE)
  - Multi Dimensional Protein Identification Technology (MudPIT)
- Metabolomics
- Hormonics
- Bioinformatics

# Genomics

- Currently have several subsets of slides available for analysis
  - *Brassica* regulatory genes
    - 7.4K cDNAs
  - *Brassica* seed specific genes
    - 9K cDNAs
  - *Brassica* stress-related
    - 9K cDNAs

# Genomics

## *Heat and Drought Studies*

- Transgenics :
  - Rob5, CBF, Dhn, PPA, SOD
- 5 Field locations across western Canada
  - AB, SK, MB
- Two years (2002 and 2003)
- Analyzed for physiological responses due to heat and drought

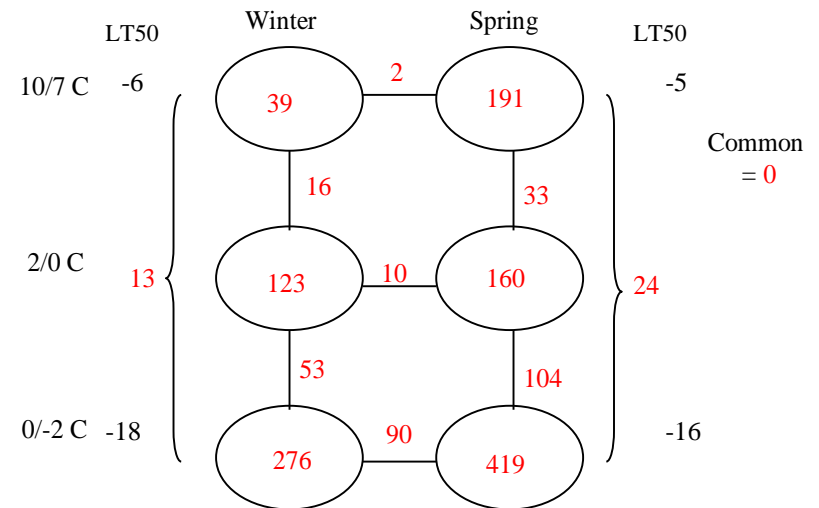
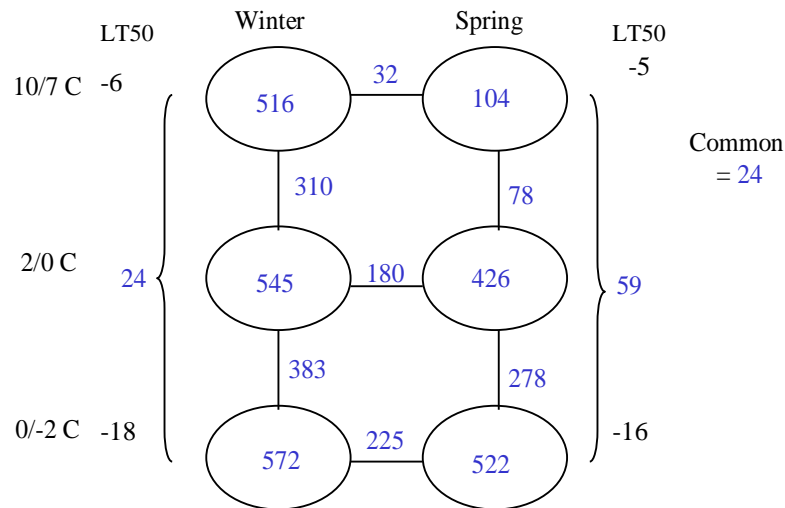
# Genomics

- Rob5 and CBF Transgenics
- Agronomic Analysis
  - 35% yield advantage
  - Earlier flowering (2 wks)
  - Earlier maturity (10 – 14d)
- Genomics
  - Constitutive stress tolerance
  - Fewer genes up-regulated in response to stress

# Genomics

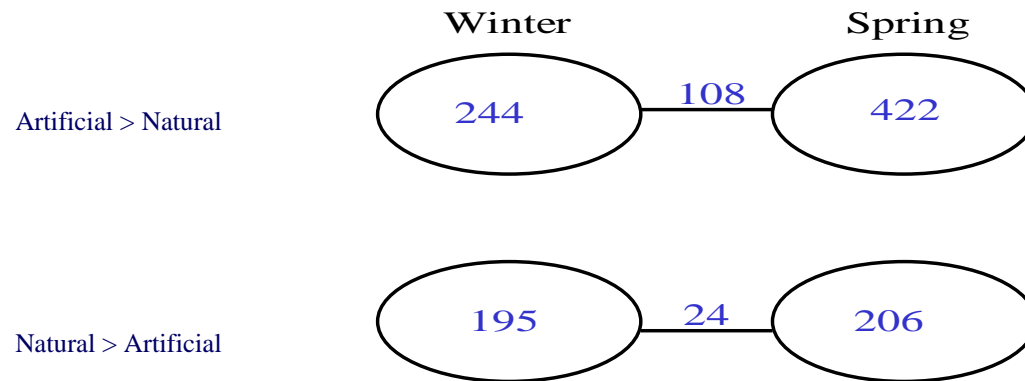
- *Brassica napus* during cold acclimation
  - Different stages of acclimation
  - Spring (cv. Quest) vs. winter (cv. Express)
  - Field (natural) vs. Growth Chamber (artificial) acclimation
  - Correlated to physiological data for candidate gene identification

# Genomics



The number of genes **up-regulated** (blue) and **down-regulated** (red) ( $> \log_{10} = 0.5$ ) in leaf tissue of acclimating winter and spring canola (*Brassica napus*) compared to non-acclimated leaf tissue. Numbers outside each oval represent similar genes between each change in temperature and / or canola type. The number beneath “common” represents the same genes down regulated in both canola types during cold acclimation.

# Genomics



- different genes up regulated in the growth chamber vs. the field

The number of genes up-regulated ( $> \log_{10} = 0.5$ ) in leaf tissue of acclimated winter and spring canola (*Brassica napus*) from controlled environment chambers (Indoor) compared to field acclimated plants (Field).

# Genomics

- *Brassica napus* seed vigour
  - 24°C vs. 8 °C germination
  - Saline germination
  - ABA priming
  - Different aged seed (natural and artificial)
  - Extensive physiological testing for correlation to microarray data
- Currently being analyzed

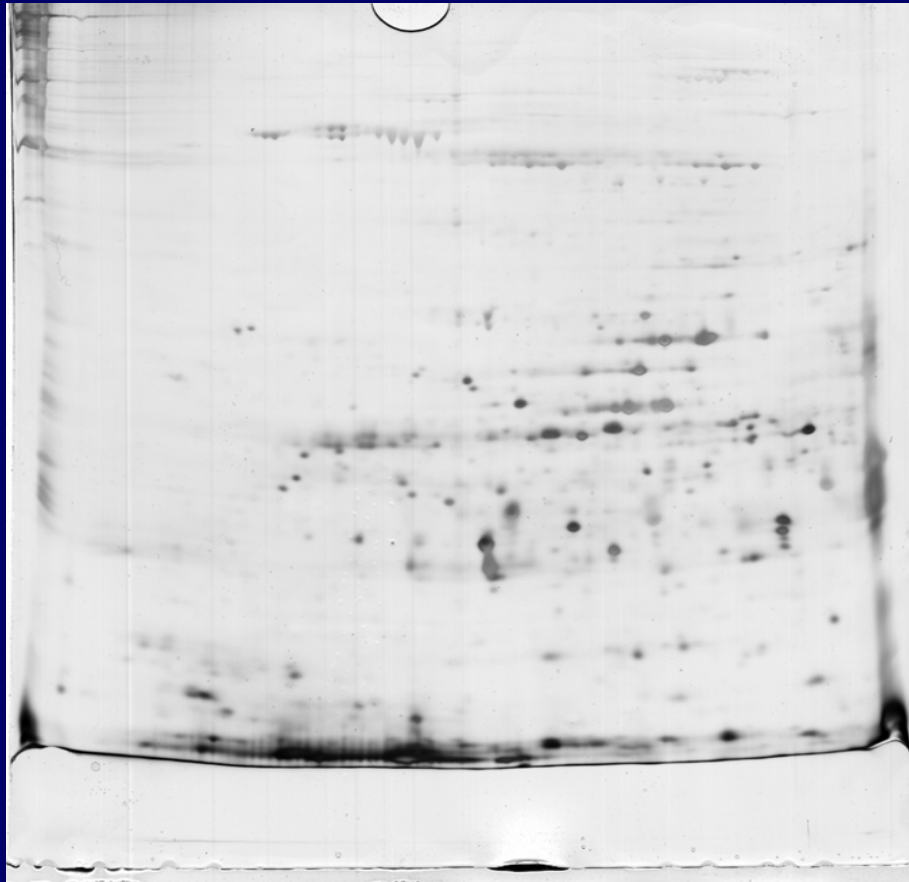
# Proteomics

- Currently utilizing multiple techniques
  - 2 Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE)
    - Protein identification/Post translational modification
  - 2 Dimensional Differential Gel Expression (2D-DIGE)
    - Protein accumulation quantification
  - Multi-dimensional Protein Identification Technology (MudPIT)
    - Protein identification/quantification (low abundant proteins)

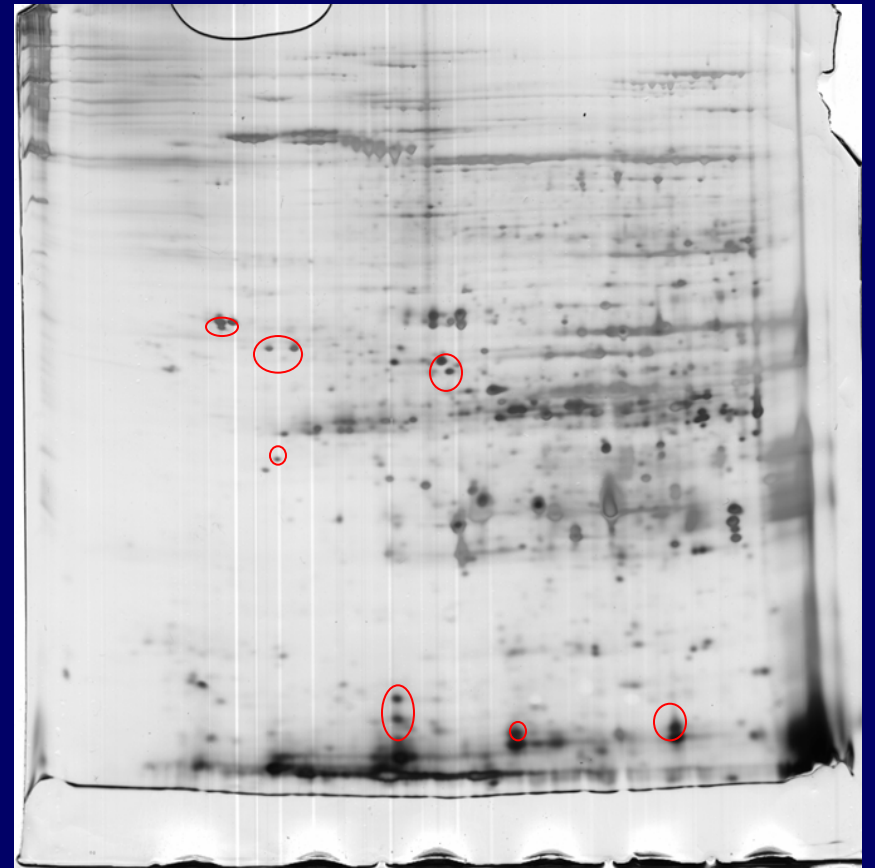
# Proteomics

- 2 Dimensional Polyacrylamide Gel Electrophoresis
  - Separates proteins based on pI and MW
  - Good resolution
  - Gel to gel variation
  - Slow turn around
  - Can determine post translational modifications
  - Spots picked and digested via robotics
  - Proteins identified via LC MS/MS

# Proteomics



48h at 8°C ddH<sub>2</sub>O



72h at 8°C KH<sub>2</sub>PO<sub>4</sub>

# Proteomics

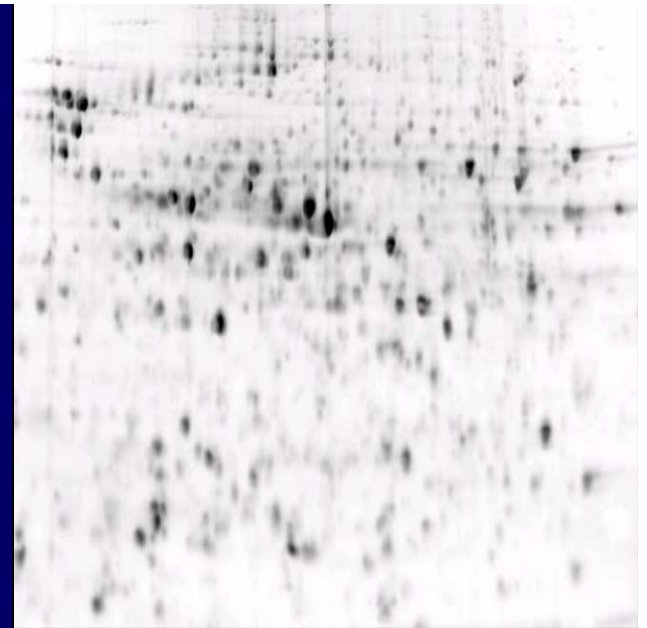
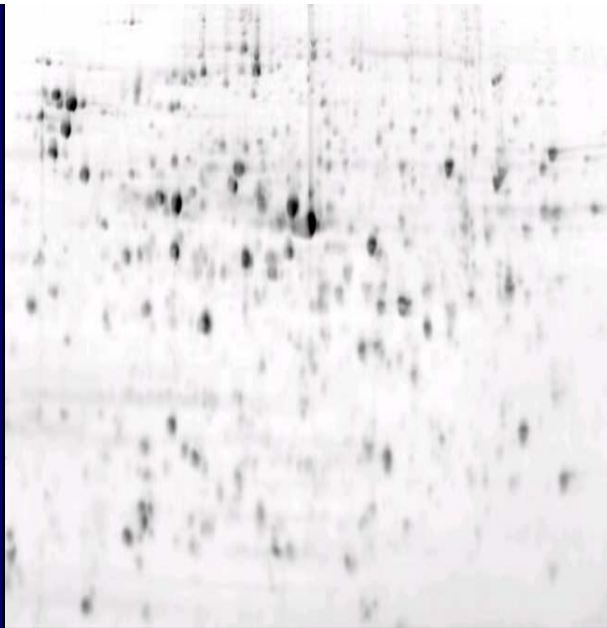
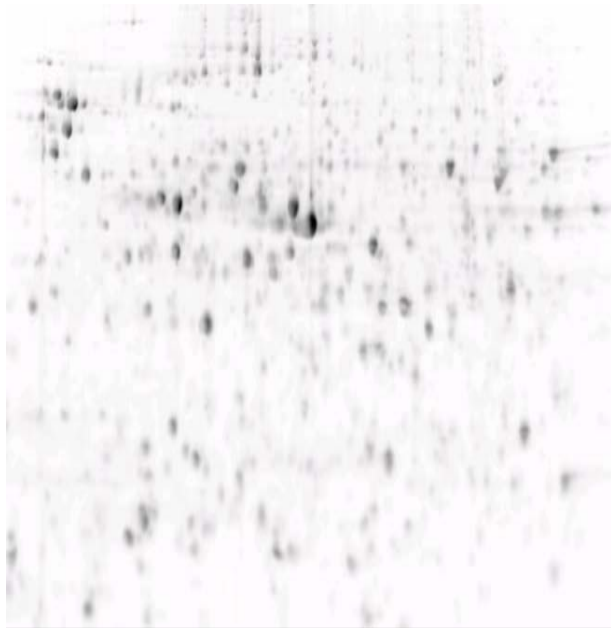
- 2D-PAGE Analysis of Seed Vigour in *Brassica*
- Extensive germination and physiological tests
- Tested LT, Salt, ABA in various combinations with different aged seed
- Used germination data to determine which samples to conduct proteomic analysis

# Proteomics

- 2D-PAGE Analysis of Cold Acclimation in *Brassica*
  - Identified over 150 proteins involved in cold acclimation
  - Both increase and decrease in response to LT
  - Typically accumulate at 10/7 °C or at 0/-2 °C
  - 1% of proteins identified at 10/7 °C and 0/-2 °C are the same
  - Winter and spring cultivars show same response

# Proteomics

- 2D-DIGE (Differential Gel Expression)
- Cold Acclimation and Shock in *Brassica*
- Gel based approach
- Differentially dyed samples run simultaneously on one gel
- Provides ability to confidently quantify protein accumulation differences



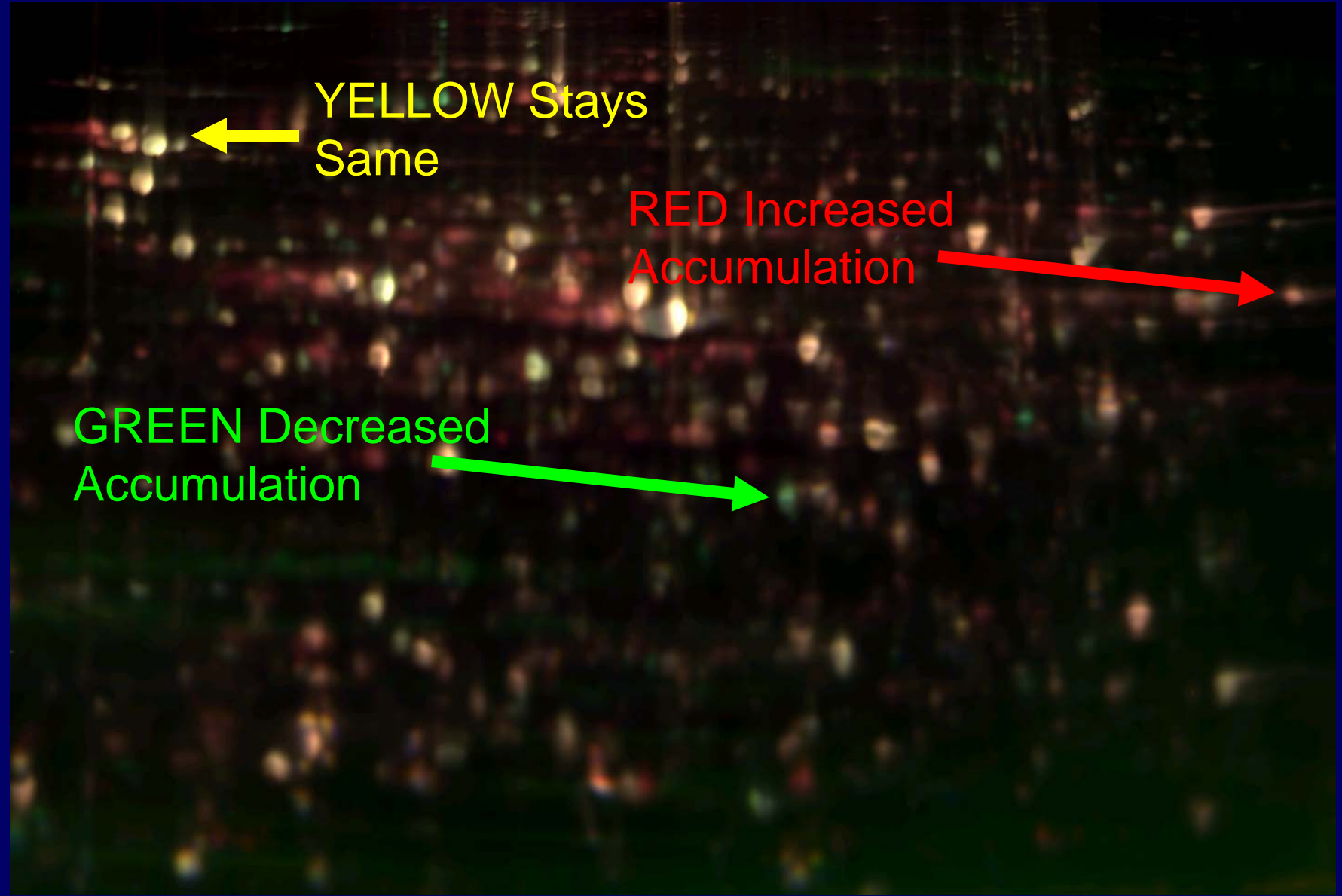
Cy2 (Control Treatment Pool)

Cy3 (Control)

Cy5 (LT Treatment 1)

- When viewed individually look like traditional 2D-GE
- When viewed in combination real differences evident

2D-DIGE Analysis Conducted by GE Healthcare (Amersham Biosciences)



Cy2 (pooled std.), Cy3 (Control) Cy5 (Treatment) Overlay

# Metabolomic Analysis of Acclimation in Brassica

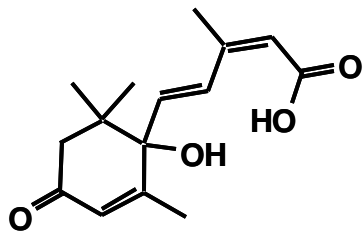
- “analysis of small molecules, or metabolites, in biological samples”
- Measure levels of thousands of metabolites (known and unknown)
- Currently analyzing following LT treatments:
  - Natural vs. Artificial
  - Non-acclimated vs. Acclimated
  - Acclimated vs. Re-acclimated

# Hormonics

- Measure Hormone level in response to stress
- Quantitative due to addition of internal standard
- Measured via LC MS/MS
- Can quantify the following hormones:

# Hormonics

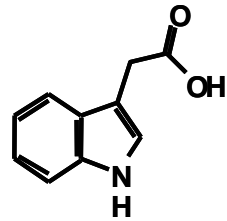
## Abscisic acid



ABA

PA  
DPA  
ABA-GE  
7'OH-ABA  
neo-PA

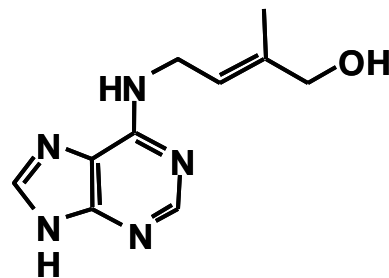
## Auxins



IAA

IAAsp

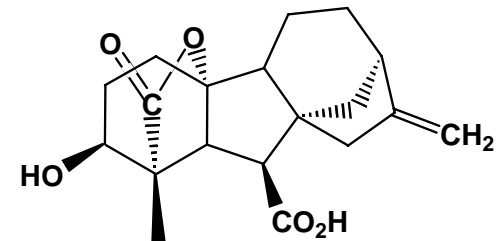
## Cytokinins



Zeatin (Z)

Z riboside  
2iP  
IPA  
DHZ  
DHZR  
Z-O-Glu

## Gibberellins



GA<sub>4</sub>

GA<sub>7</sub>  
GA<sub>1</sub>  
GA<sub>3</sub>

# Bioinformatics

- Utilize multiple software programs to analyze data generated from each activity (genomics, proteomics, metabolomics)
- Vast quantities of data; Synonymous to “drinking from a fire hose”
- Currently **NEED** software that can take data from all activities and can correlate results

# Conclusions

- Large number of genes, proteins, metabolites and hormones change in response to abiotic stress
- Physiological Analysis required
  - To makes conclusions based on all data
  - To determine what comparisons to make
- Tremendous amounts of data that needs to be correlated
  - Need to combine results from different activities to even have a chance at determining what is going on

# Support

- University of Saskatchewan
- Genome Prairie – Functional Genomics of Abiotic Stress
- NSERC Strategic

