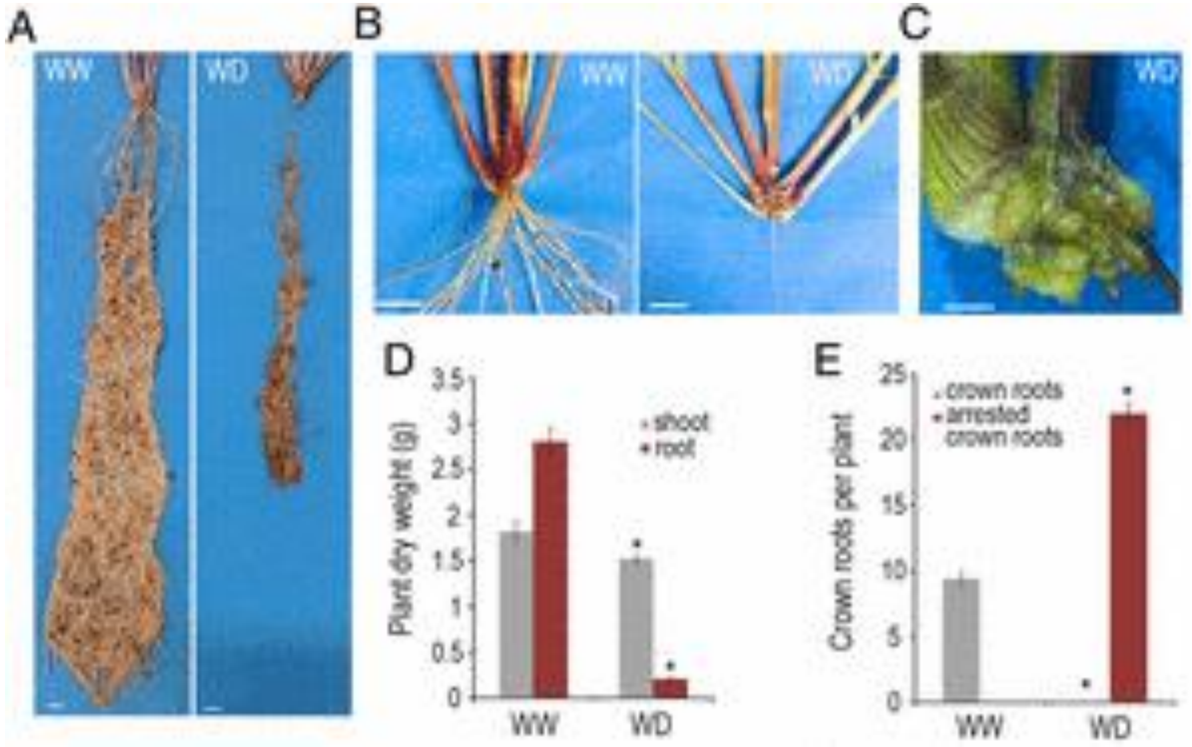
# Rhizobacteria-Promoted Root Growth in Creeping Bentgrass through Metabolic Regulation for Improving Drought Tolerance and Post-Drought Recovery

William Errickson, Bingru Huang, Ning Zhang

RUTGERS
UNIVERSITY | NEW BRUNSWICK

# Drought stress has negative impacts on the growth and quality of cool season grasses such as creeping bentgrass (*Agrostis stolonifera)*

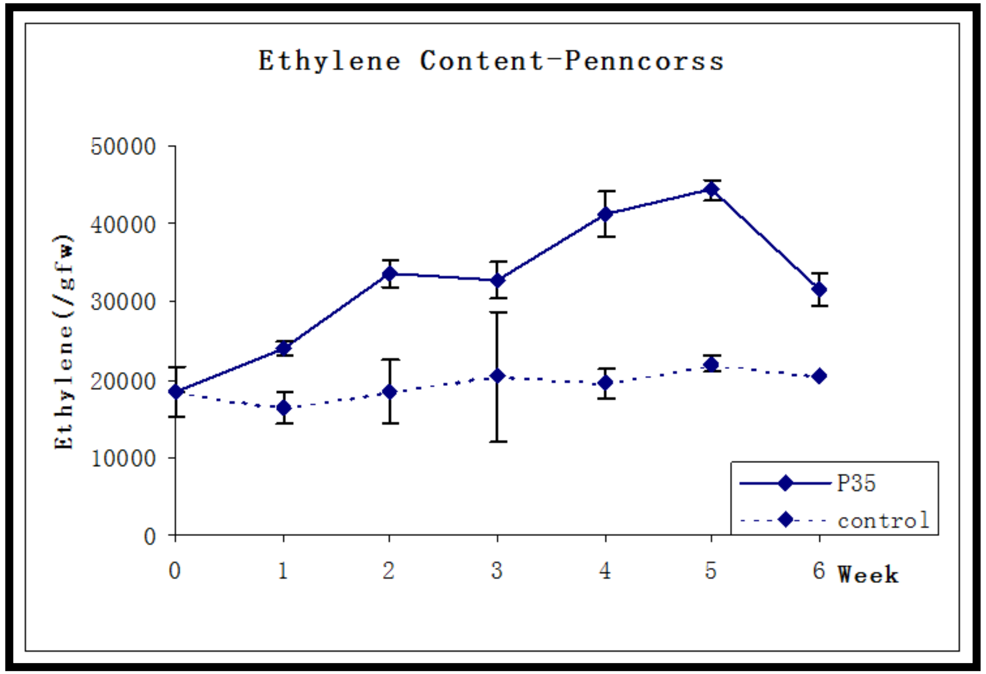



Grasses suppress shoot-borne roots to conserve water during drought (Sebastian et al., 2016 PNAS)

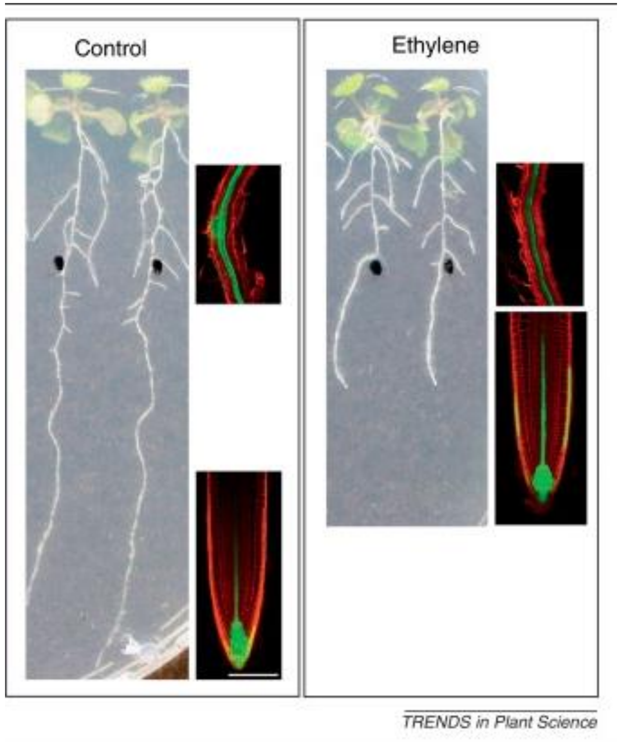
- Reduced turf quality
- Canopy thinning
- Leaf senescence

With the effects of climate change expected to exacerbate drought in many regions where cool season grasses are grown, sustainable turf management strategies that conserve resource use, while adapting to the changing climate deserve further investigation.

# Drought Induced Ethylene Inhibits Root Growth




Xu and Huang, Crop Sci. 2009




- Abiotic stress such as drought causes an increase in ethylene concentrations

- Ethylene causes a reduction in root growth

A comprehensive understanding of the mechanisms involved in improving drought stress tolerance and post-drought recovery will help to facilitate the development of novel approaches for mitigating drought stress in cool season grasses.

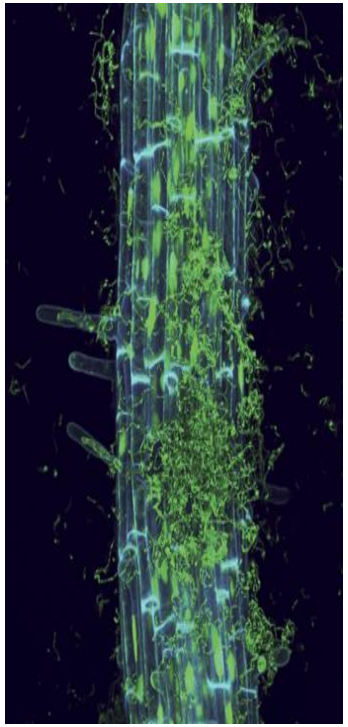
# Suppressing Ethylene Production by ACC Deaminase Producing Bacteria may Improve Drought Tolerance

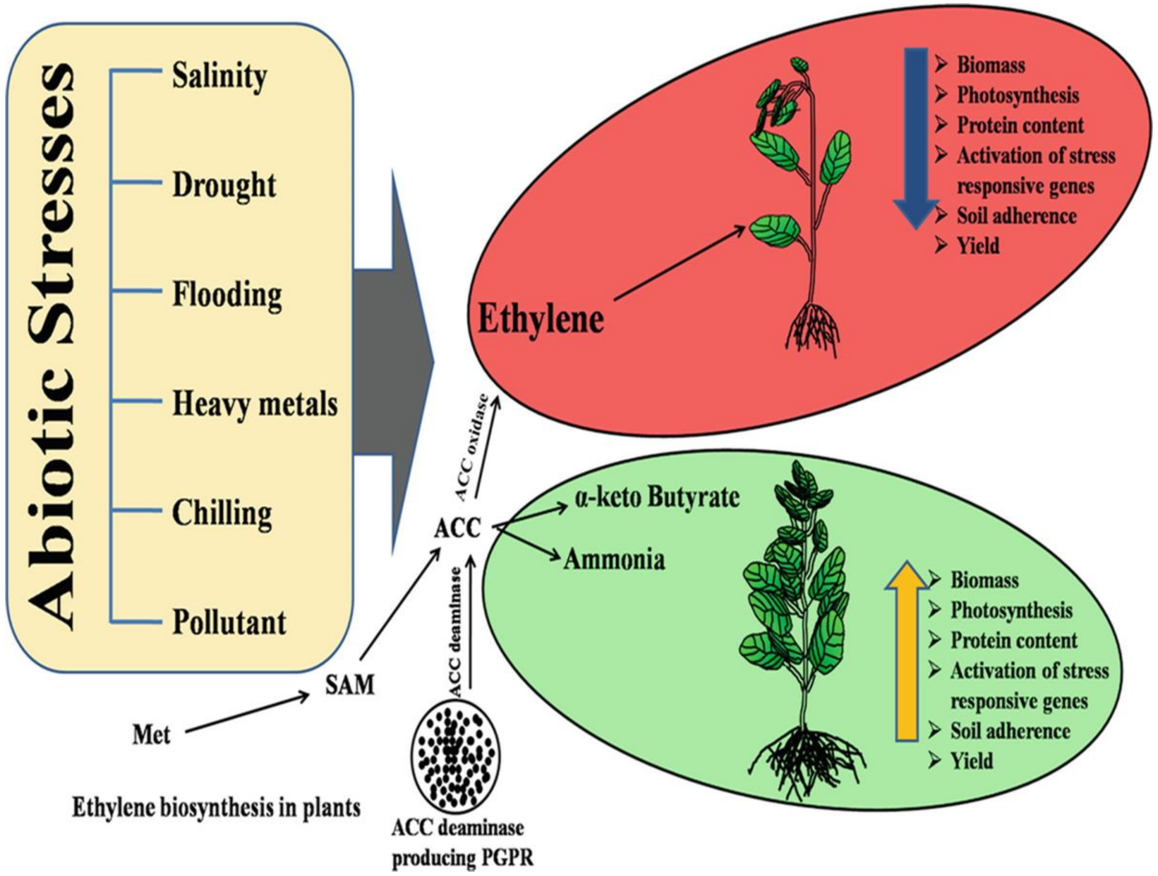
- 1-Aminocyclopropane-1-carboxylic acid **(ACC) – precursor of ethylene**.

- Plant Growth Promoting Rhizobacteria (PGPR) **with ACC Deaminase (ACCd)** enzyme break down ACC into ammonia and a-keto butyrate before ACC becomes ethylene.

- ACCd rhizobacteria utilize the nitrogen from ACC while plant roots benefit from the reduction in ethylene production.

- Reduced ACC → Reduced Ethylene → Reduced Stress Damage

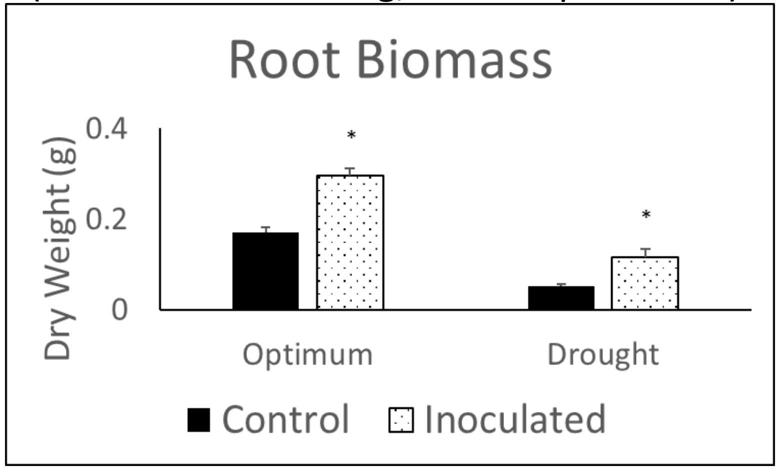


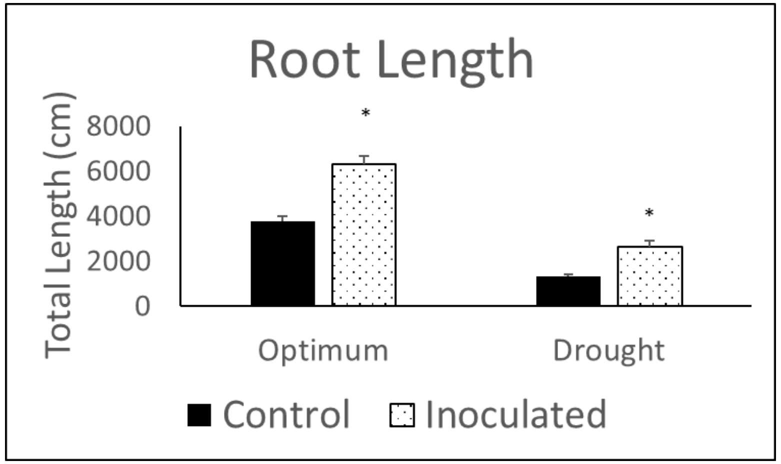


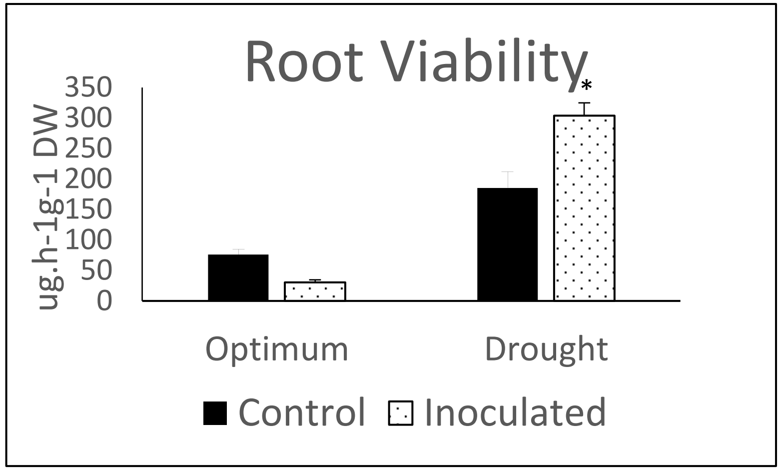

https://link.springer.com/chapter/10.1007/978-981-13-7553-8_14

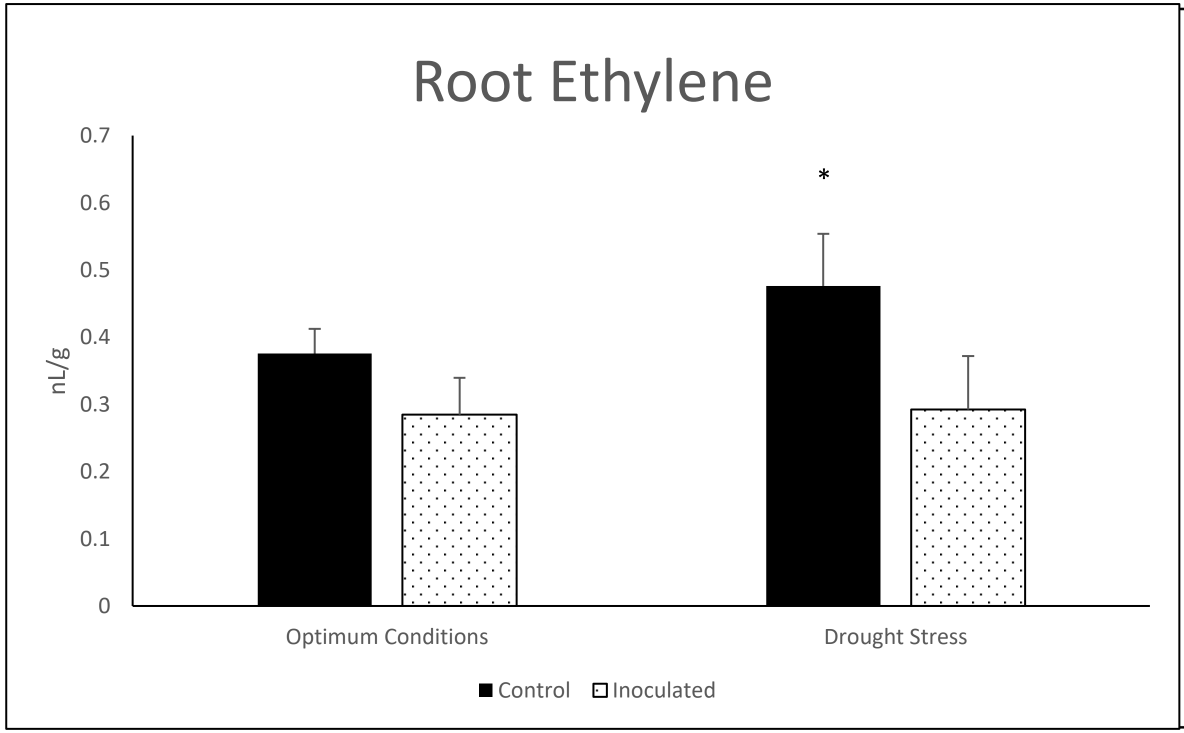
# ACCd bacteria *Burkholderia* enhanced root growth by reducing ethylene production during drought stress in creeping bentgrass

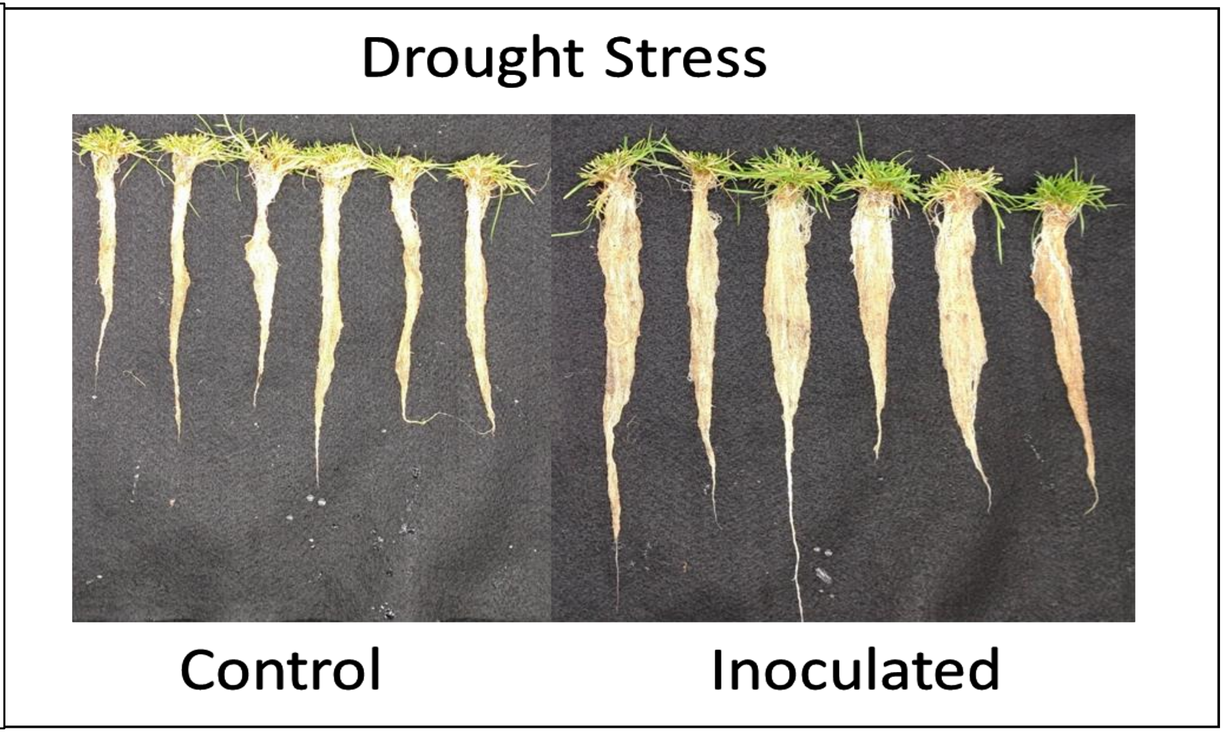
(Errickson and Huang, 2021 unpublished)



# Research Questions

How do ACCd bacteria regulate root growth and improve drought tolerance and post-drought recovery?

Which metabolic processes may be regulated by ACCd bacteria?

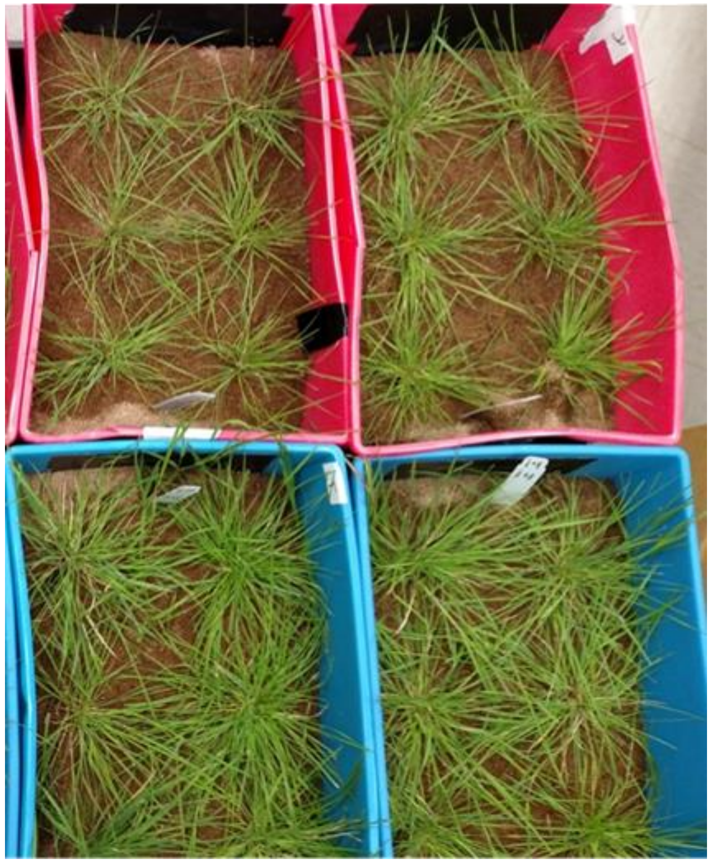
# Research Objectives

To understand which key metabolites in root systems are regulated by ACCd bacteria to promote root growth during drought stress and post-stress recovery

To identify the major metabolic pathways involved in ACCd bacteria regulation of root growth under drought stress and during post-stress recovery

# Materials & Methods

- <u>Plant Materials & Growth Conditions</u>
  - Creeping bentgrass (*Agrostis stolonifera cv. Penncross*) was established from tillers in bins (20 cm x 30 cm) filled with fritted clay. Each bin contained 6 sets of plants and each treatment was replicated in 8 bins in controlled environment growth chambers.
- <u>Inoculation Treatments</u>
  - ACCd bacteria *Burkholderia aspalathi* WSF23 was used to inoculate creeping bentgrass plants via soil drench method
  - Non-inoculated control plants were used for comparison
- <u>Irrigation Treatments</u>
  - Control: Plants were well watered
  - Drought Stress: Irrigation was withheld for 35 days
  - Re-watering to evaluate post-stress recovery: Drought-stressed plants were re-watered for 15 days



# Metabolomic Analysis

## Metabolite extraction and analysis

- Fresh leaf and root tissue samples were frozen in liquid N and stored at -80$^{o}$C
- Samples were freeze dried (3 days) then ground in liquid N
- 20.0 mg of each ground sample was analyzed by LC-MS

## Data Analysis

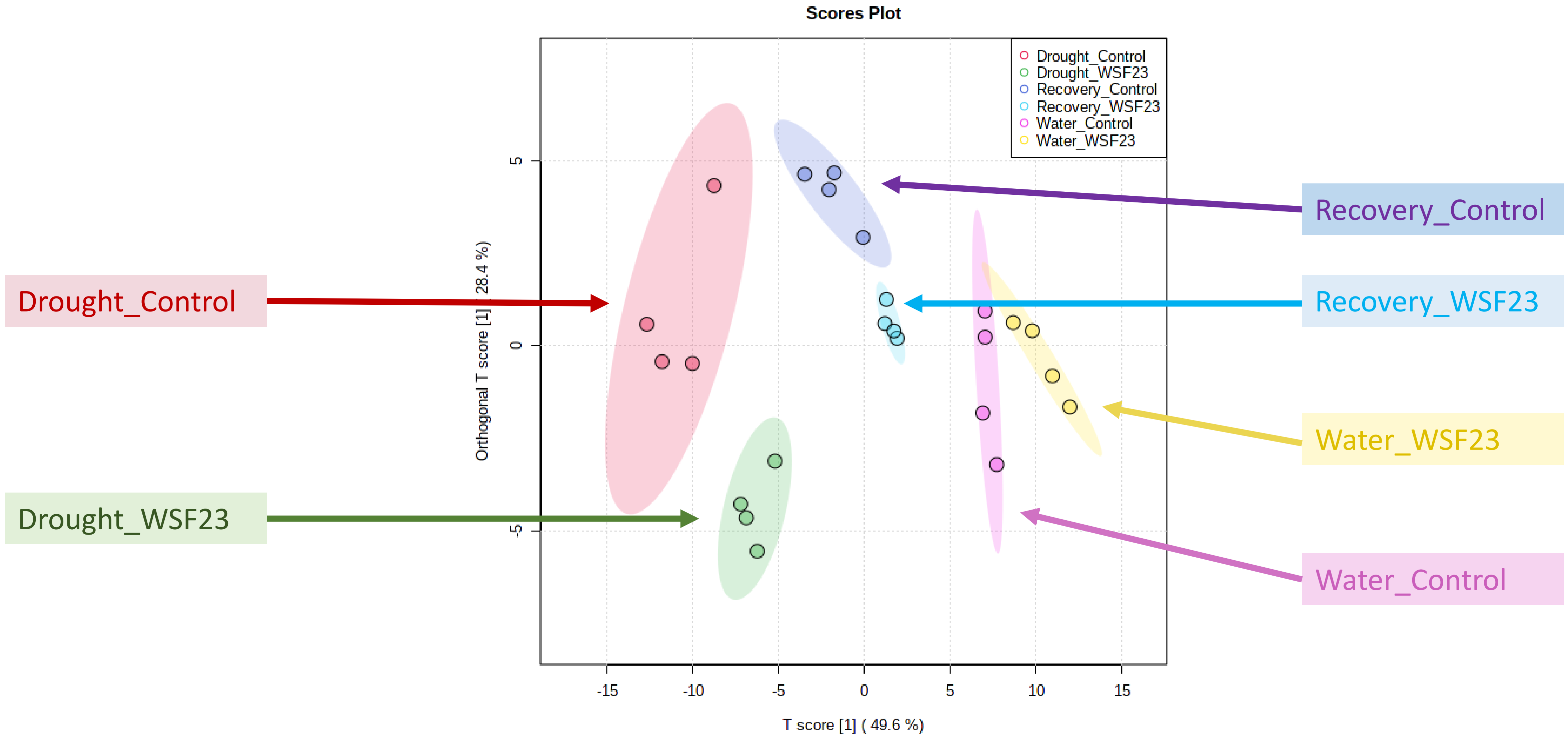
- Data analysis was performed using the **Metaboanalyst Program**
- **Analysis of Variance and Least Significance Test (P = 0.05)**

# Results

Drought Stress

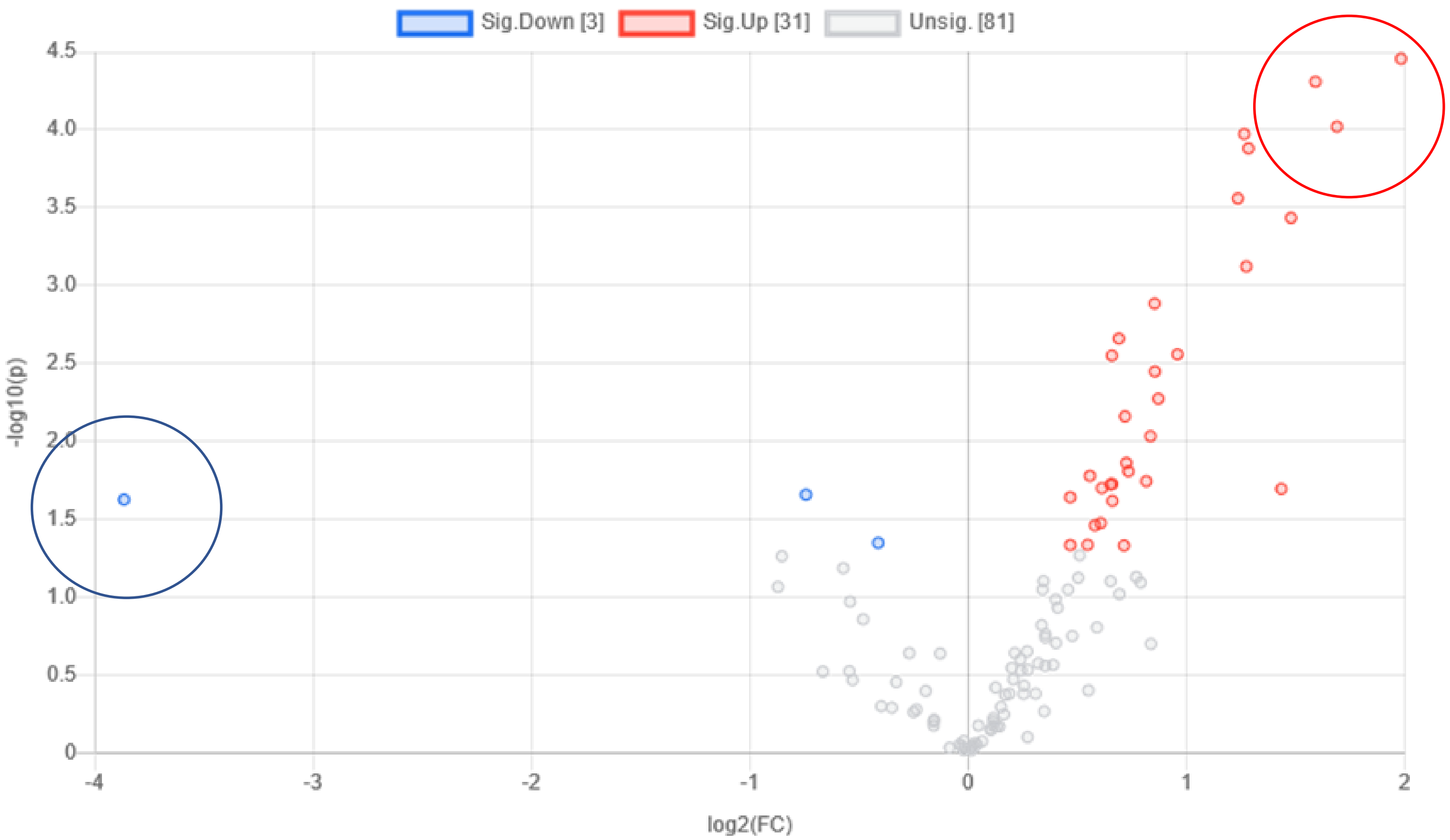
Control Inoculated

# Distinct metabolite clusters in ACCd bacteria inoculated plants from non-inoculated plants under drought stress, re-watering, and well-watered conditions among 115 metabolites by OPLS-DA analysis



# ACCd bacteria inoculation enhanced the accumulation of 31 metabolites under drought stress

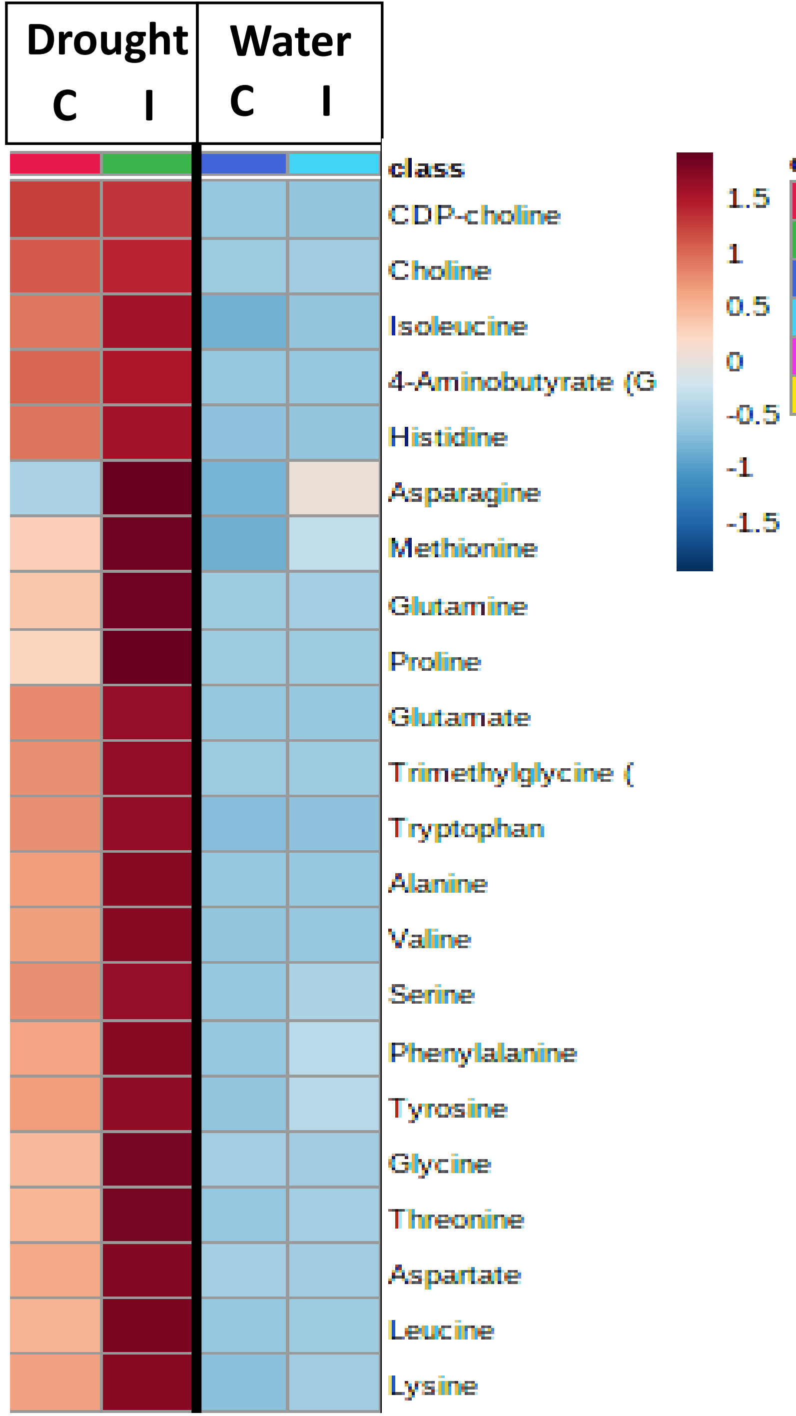
**31** metabolites were **up-regulated** and **3** metabolites were **down-regulated** in **roots** of inoculated plants during drought stress



| Metabolite | Fold Change (log2) | Function |
|---|---|---|
| Asparagine | +1.98 | N storage and transport |
| Arabinose | +1.69 | Cell wall elasticity |
| Proline | +1.59 | Osmotic adjustment |
| Allantoin | +1.48 | N mobilization, ROS scavenging |
| Riboflavin | +1.43 | Antioxidant activity |
| ADP-ribose | -3.86 | ROS formation, cellular damage |

# ACCd bacteria increased amino acid content in roots under drought stress

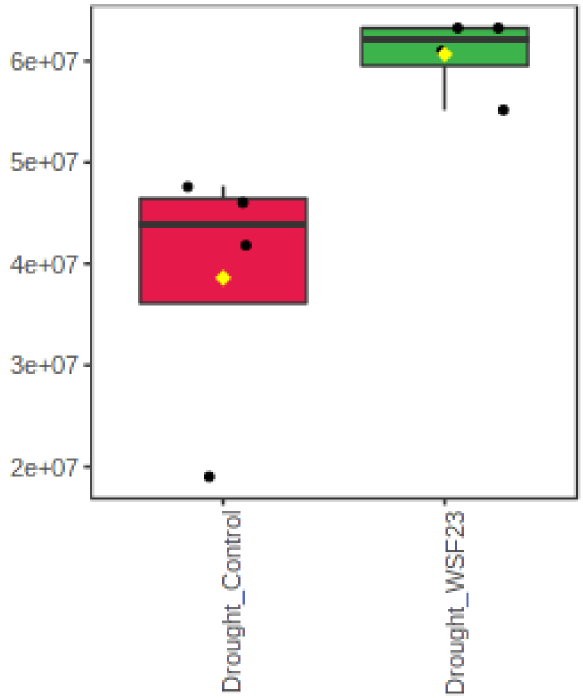
- Choline → Glycine Betaine
  - Increased antioxidant activity (SOD, CAT, POD)
- GABA
  - Maintenance of membranes, upregulation of antioxidants
- Glutamate, Glutamic Acid
  - Guard cell function, chlorophyll synthesis
- Proline
  - Improved cell turgor and membrane stability; reduced ROS
- Asparagine
  - N storage and transport



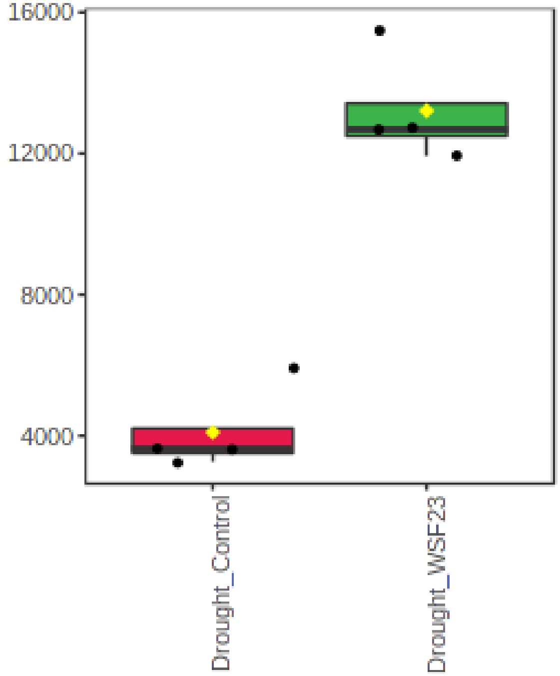
# ACCd bacteria increased carbohydrate content in roots under drought stress

Carbohydrates function as compatible solutes that help maintain cell turgor pressure by affecting osmotic adjustment in water limiting situations

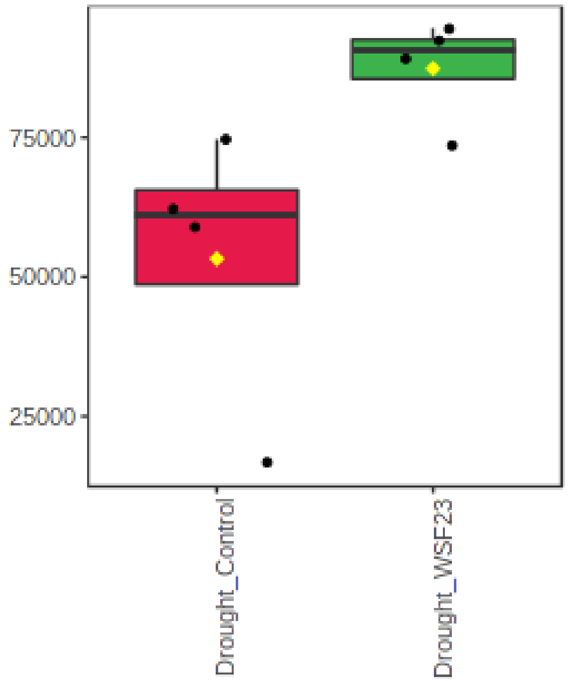
Glucose




Arabinose




Raffinose

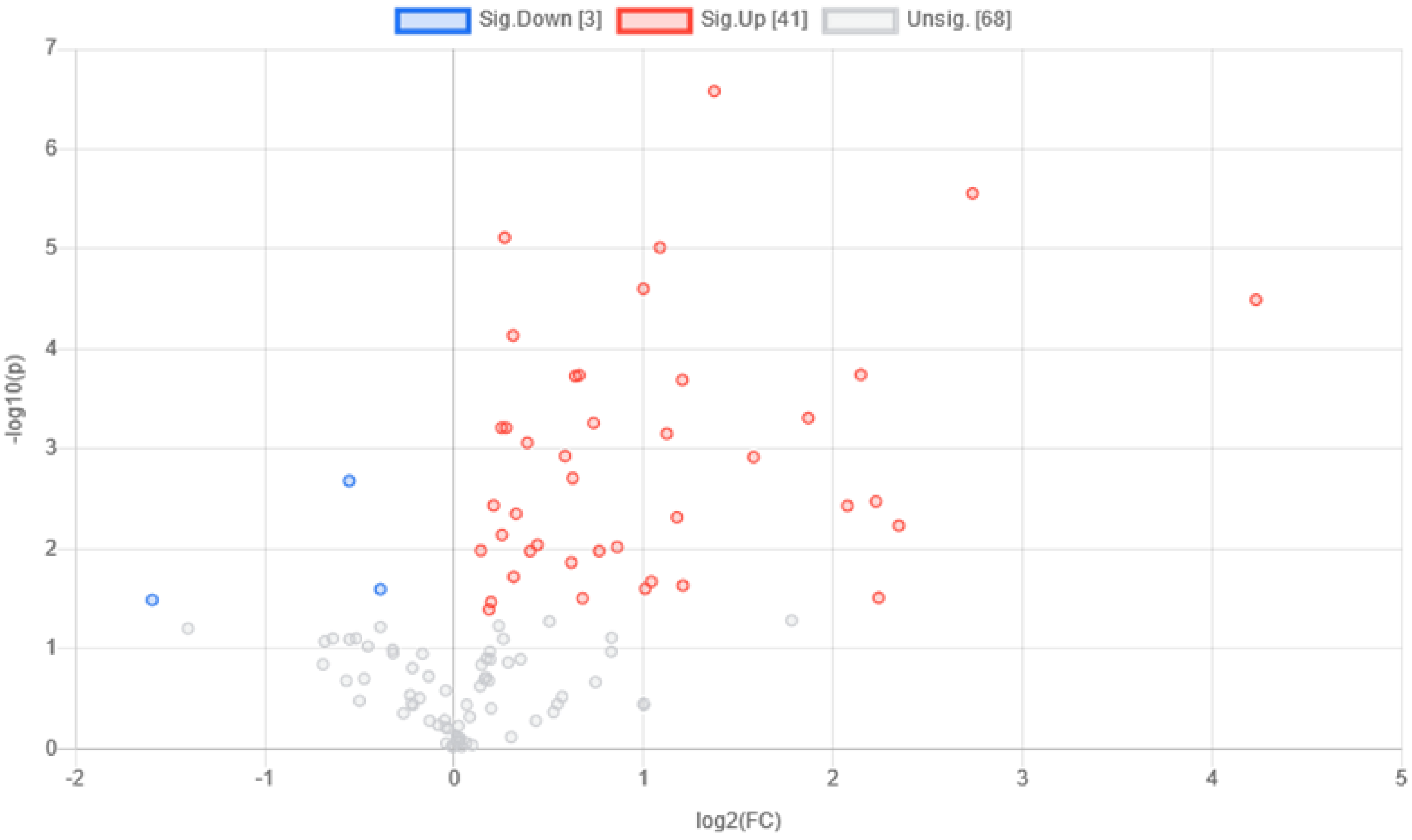


Metabolic pathways regulated by ACCd bacteria in roots exposed to drought stress by KEGG Analysis

| **Root Drought** | Total | Expected | Hits | Raw p | Impact |
|---|---|---|---|---|---|
| Aminoacyl-tRNA biosynthesis | 46 | 1.1148 | 15 | 5.56E-15 | 0 |
| Alanine, aspartate and glutamate metabolism | 22 | 0.53314 | 6 | 7.25E-06 | 0.71582 |
| Arginine biosynthesis | 18 | 0.43621 | 4 | 0.000693 | 0.08544 |
| Glycine, serine and threonine metabolism | 33 | 0.79971 | 5 | 0.000901 | 0.33218 |
| Glyoxylate and dicarboxylate metabolism | 29 | 0.70278 | 4 | 0.00445 | 0.19876 |

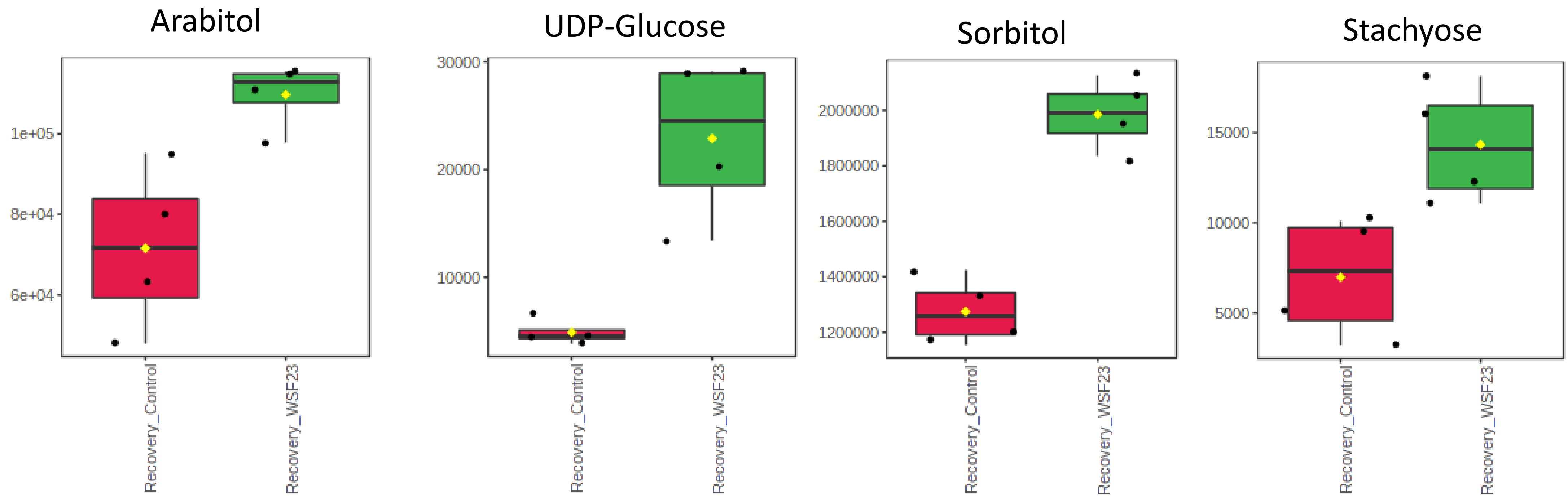
# ACCd bacteria inoculation enhanced the accumulation of 41 metabolites for post-drought recovery

**41** metabolites were **up-regulated** and **3** metabolites were **down-regulated** in the **root tissue** of inoculated plants during recovery



| Metabolite | Fold Change (log2) | Function |
|---|---|---|
| Methionine | +4.23 | Protein synthesis, mRNA translation |
| a-Ketoglutarate | +2.73 | Protein synthesis, TCA cycle |
| SAM | +2.35 | Methylation, precursor for polyamines, biotin, ACC |
| Folic Acid | +1.48 | DNA Synthesis |
| UDP-Glucose | +1.43 | Glycosylation, cell wall synthesis, secondary metabolites, signaling |
| Glycerate | -1.59 | Photorespiration |

# ACCd bacteria increased carbohydrate content in roots for post-stress recovery



Metabolic pathways regulated by ACCd bacteria in roots for post-stress recovery by KEGG Analysis

| **Root Recovery** | Total | Expected | Hits | Raw p | Impact |
|---|---|---|---|---|---|
| Aminoacyl-tRNA biosynthesis | 46 | 1.4098 | 9 | 4.80E-06 | 0 |
| Glyoxylate and dicarboxylate metabolism | 29 | 0.88881 | 6 | 0.000163 | 0.25766 |
| Citrate cycle (TCA cycle) | 20 | 0.61297 | 5 | 0.000235 | 0.25453 |
| Glycerolipid metabolism | 21 | 0.64362 | 4 | 0.003135 | 0.10275 |
| Alanine, aspartate and glutamate metabolism | 22 | 0.67427 | 4 | 0.003747 | 0.26618 |
| Pyrimidine metabolism | 38 | 1.1646 | 5 | 0.00505 | 0.15115 |
| Galactose metabolism | 27 | 0.82751 | 4 | 0.008039 | 0.0566 |
| Zeatin biosynthesis | 21 | 0.64362 | 3 | 0.024275 | 0.0271 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 22 | 0.67427 | 3 | 0.027516 | 0.02152 |
| Starch and sucrose metabolism | 22 | 0.67427 | 3 | 0.027516 | 0.28539 |
| Phenylalanine metabolism | 12 | 0.36778 | 2 | 0.04985 | 0.61539 |

# Cytokinin levels were increased by inoculation with ACCd bacteria

**Drought Stress**

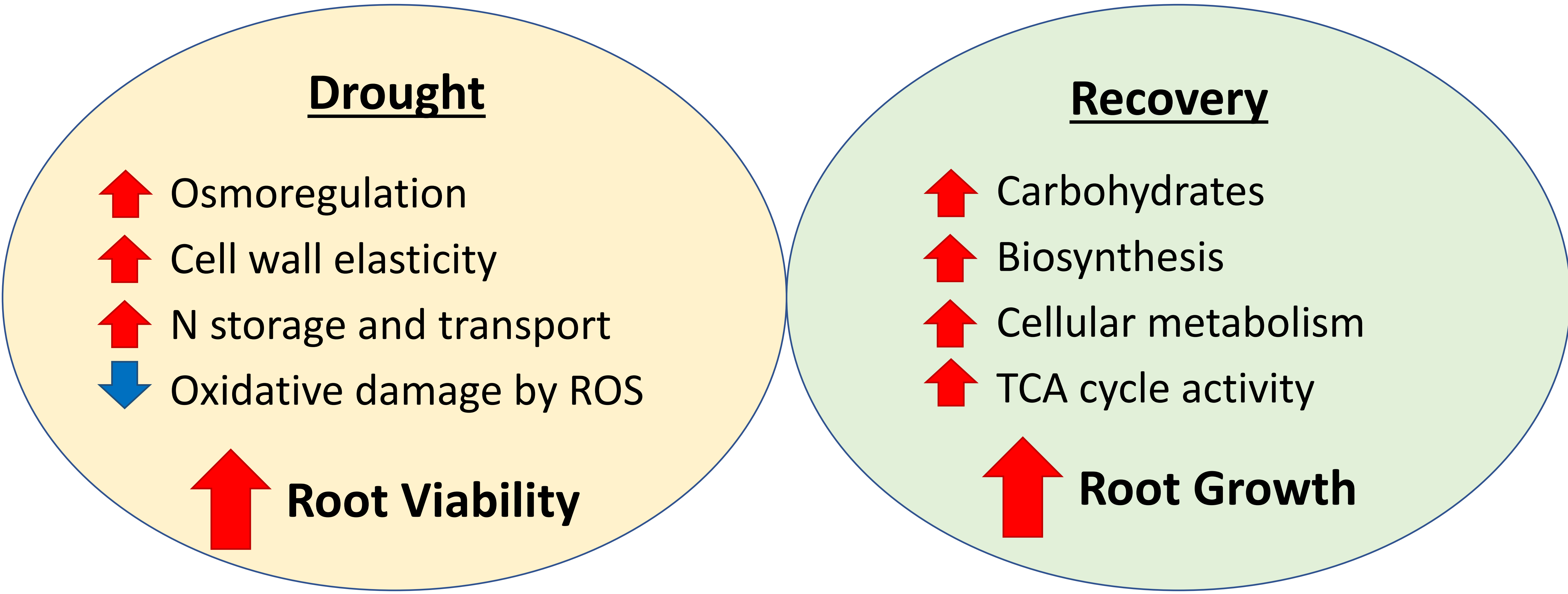
- 34% Higher C-Zeatin

**Recovery**

- 40% Higher t-ZR
- 38% Higher C-Zeatin

| | t-ZRiboside | | JA-Ile | | c-Zeatin | | IAA | | ABA | |
|---|---|---|---|---|---|---|---|---|---|---|
| Well Watered | | | | | | | | | | |
| Control | 1.55 | a | 168.85 | b | 0.78 | a | 16.93 | a | 14.85 | a |
| Inoculated | 1.18 | a | 222.28 | a | 0.72 | a | 16.03 | a | 15.70 | a |
| Drought | | | | | | | | | | |
| Control | 1.38 | a | 52.55 | a | 3.88 | b | 236.03 | a | 296.38 | a |
| Inoculated | 1.65 | a | 51.00 | a | 5.20 | a | 190.33 | b | 279.98 | a |
| Recovery | | | | | | | | | | |
| Control | 0.55 | b | 442.85 | b | 0.71 | b | 97.03 | a | 24.40 | a |
| Inoculated | 0.77 | a | 618.78 | a | 0.98 | a | 100.88 | a | 12.23 | b |

# Inoculation with ACCd bacteria enhanced root viability and growth for improving drought tolerance and post-drought recovery through regulating metabolic pathways



# Thank You


- Dr. Bingru Huang
- Dr. Ning Zhang
- Huang Lab Members
- Rutgers University Center for Turfgrass Science
- USDA SARE


RUTGERS
New Jersey Agricultural Experiment Station
Center for Turfgrass Science

SARE
Sustainable Agriculture Research & Education

william.errickson@rutgers.edu