Rhizobacteria-Enhanced Drought Tolerance and Post-Drought Recovery of Creeping Bentgrass Involving Modulation of Plant Metabolism

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Drought Stress Has Negative Impacts on Turfgrass

Rapid recovery upon re-watering is an important characteristic of turfgrass to restore turf quality and density after a period of drought stress

Drought Stress

- Reduced turf quality
- Chlorosis, yellowing
- Reduced growth and tillering
- Decline in canopy density
- Ethylene increases

https://www.usga.org/content/usga/home-page/course-care/water-resource-center/bmp-case-studies/2017/transitioning-from-poa-annua-to-creeping-bentgrass-putting-green.html

Recovery

- Turf quality increases
- Ethylene levels are reduced
- Root viability for water and nutrient uptake
- Formation of new tillers
- Increased canopy density

https://www.usga.org/content/usga/home-page/course-care/regional-updates/central-region/2017/how-much-water-is-needed-to-flush-a-usga-putting-green-.html

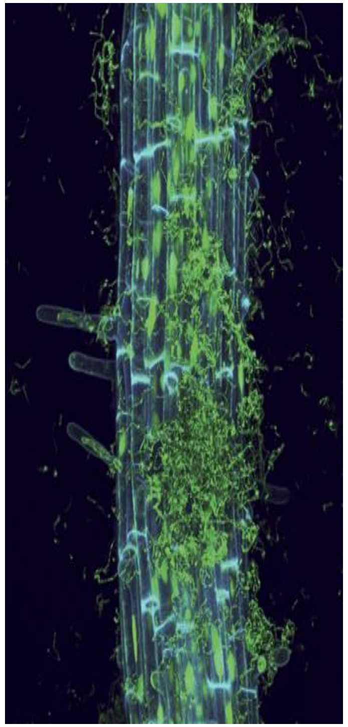
Suppressing Ethylene Production by ACC Deaminase Producing Bacteria may Improve Drought Tolerance

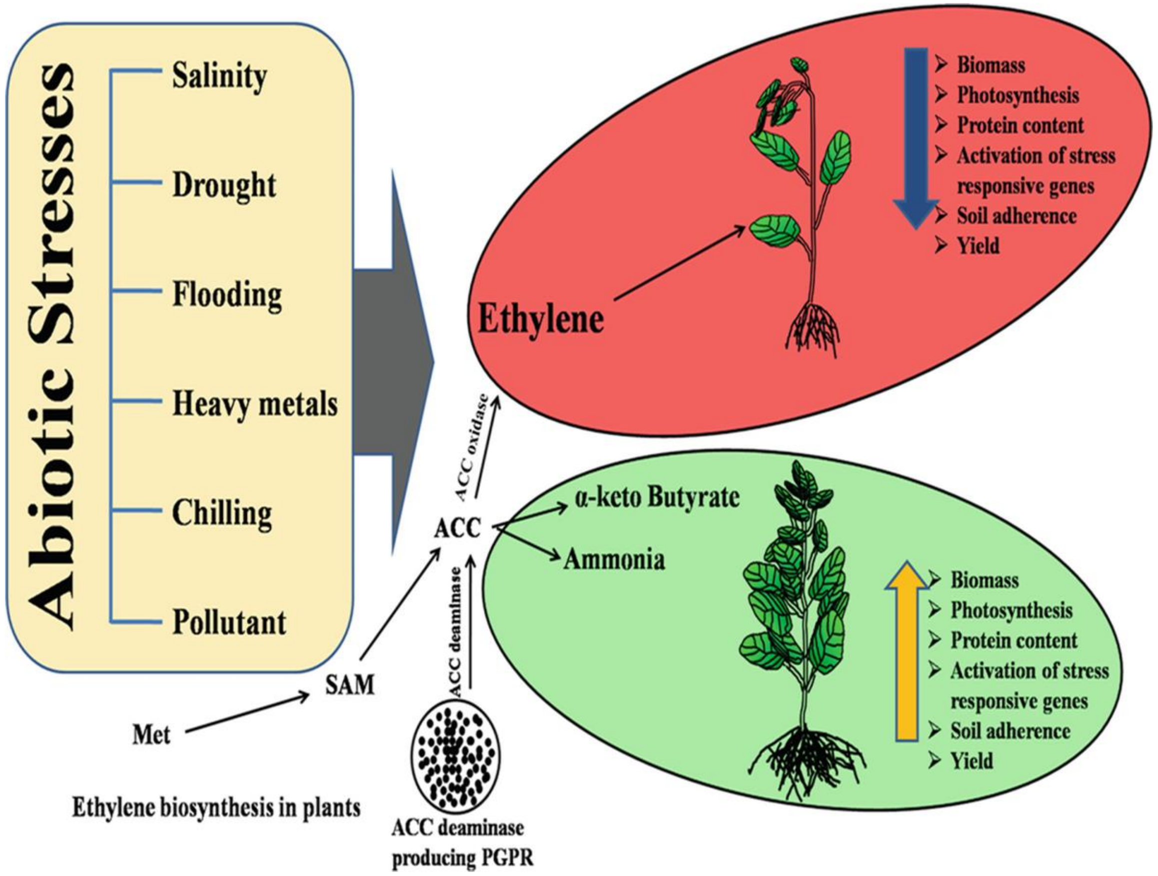
- 1-Aminocyclopropane-1-carboxylic acid **(ACC) – precursor of ethylene**.

- 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

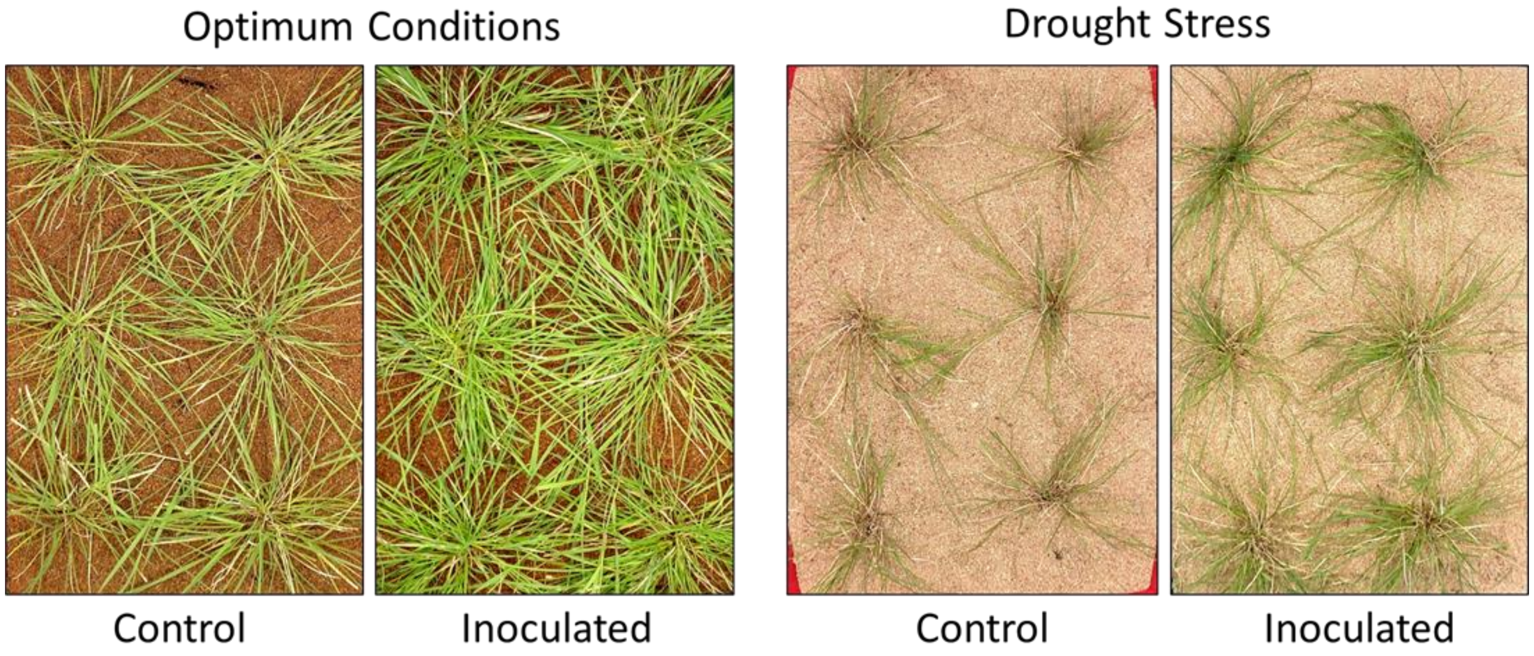


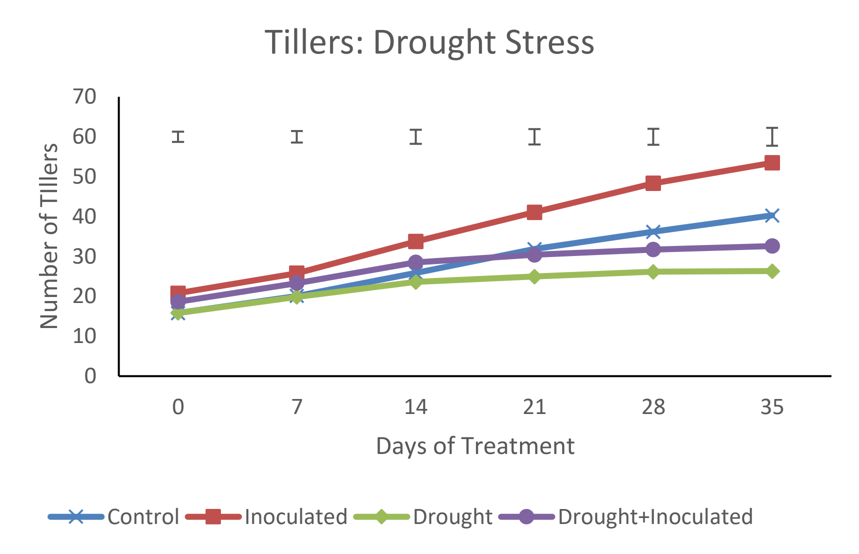


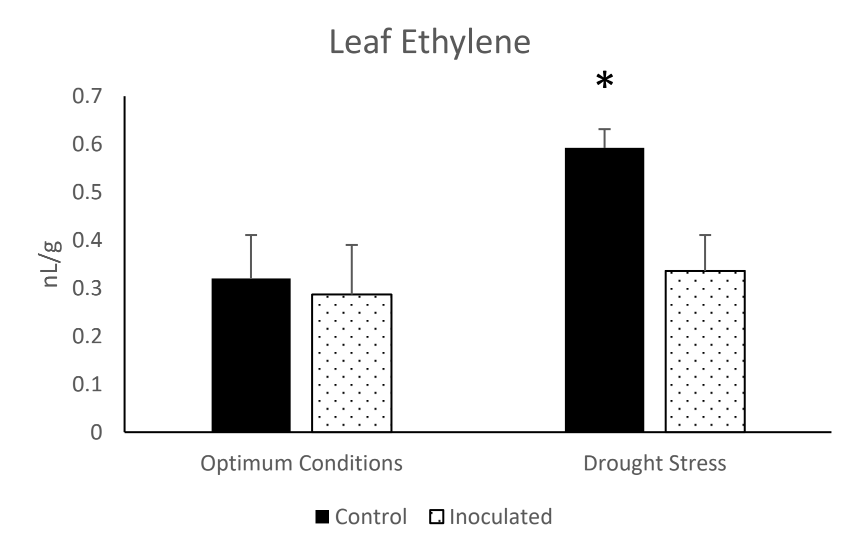
https://link.springer.com/chapter/10.1007/978-981-13-7553-8_14

ACCd bacteria *Burkholderia* enhanced tiller production by reducing ethylene concentrations during drought stress in creeping bentgrass

(Errickson and Huang, 2021 unpublished)







Research Questions

How do ACCd bacteria regulate tiller development and improve drought tolerance and post-drought recovery?

Which metabolic processes may be regulated by ACCd bacteria?

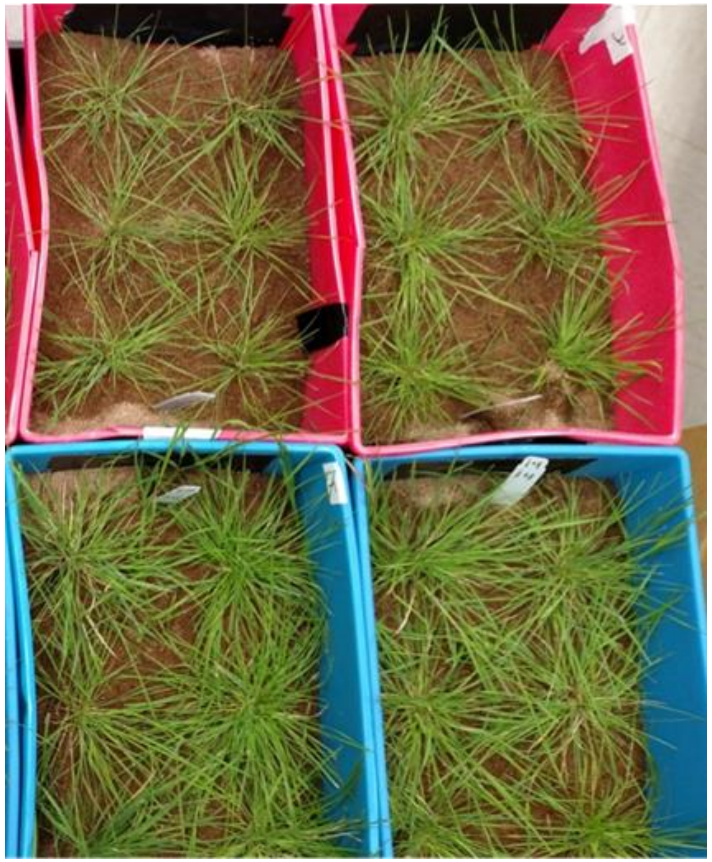
Research Objectives

To understand which key metabolites in leaf tissue are regulated by ACCd bacteria to promote tiller development during drought stress and post-stress recovery

To identify the major metabolic pathways involved in ACCd bacteria regulation of tiller development 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

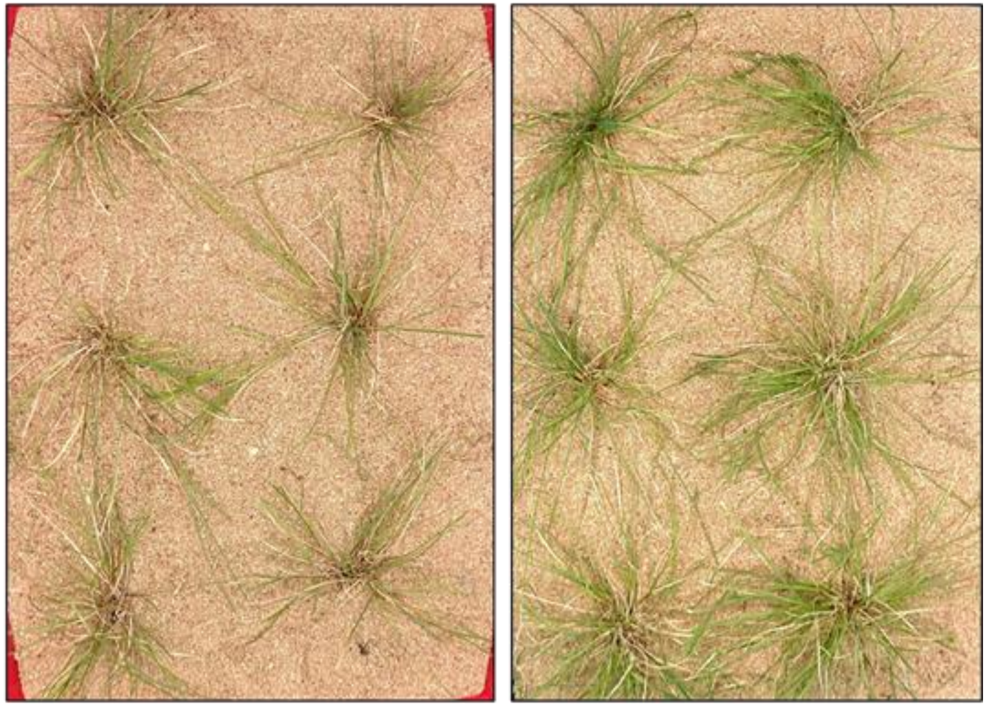
- Fresh leaf tissue samples were frozen in liquid N and stored at -80°C
- Samples were freeze dried (3 days) then ground in liquid N
- 20.0 mg of each ground sample was analyzed by LC-MS

Data Analysis

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

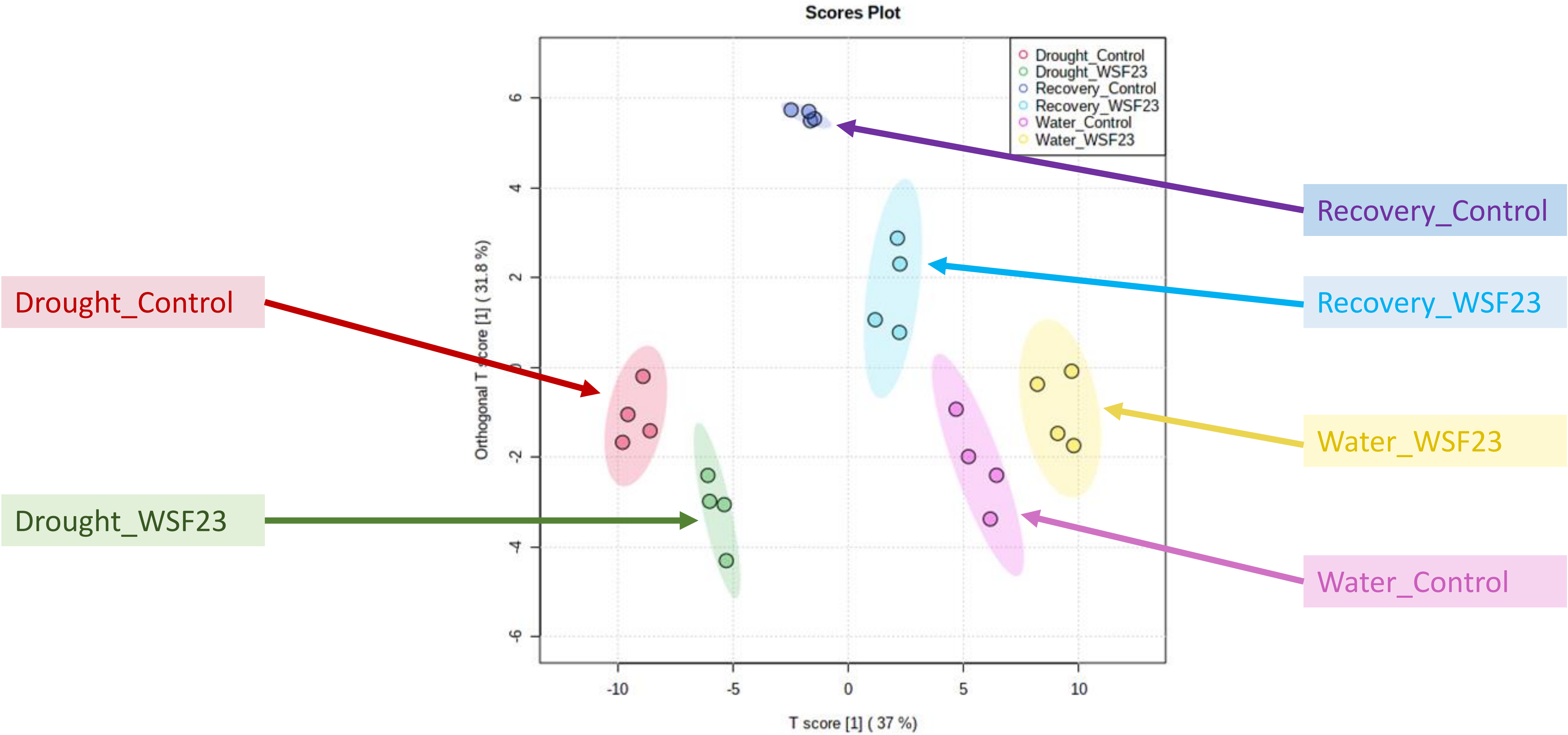
Results

Drought Stress



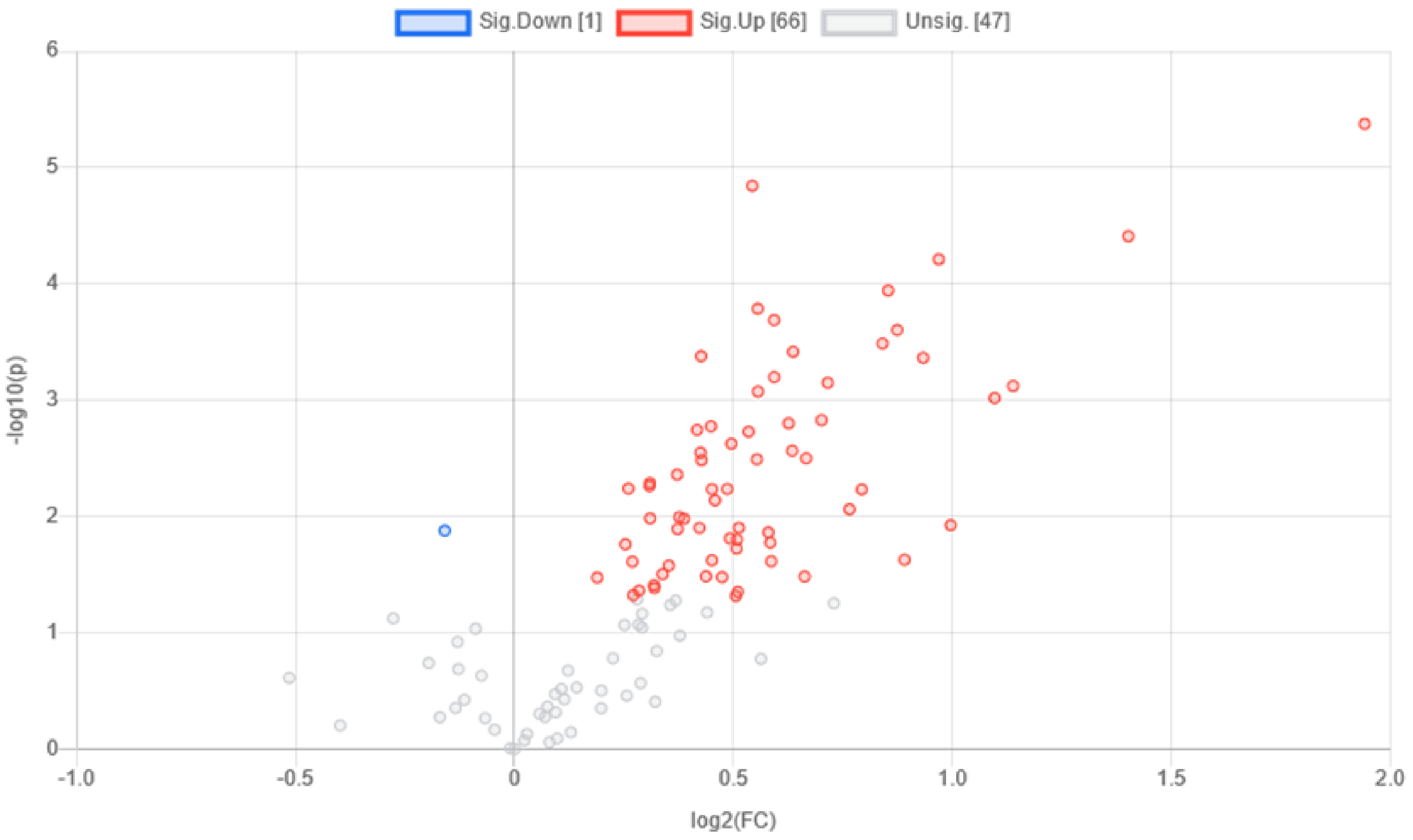
Control Inoculated

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

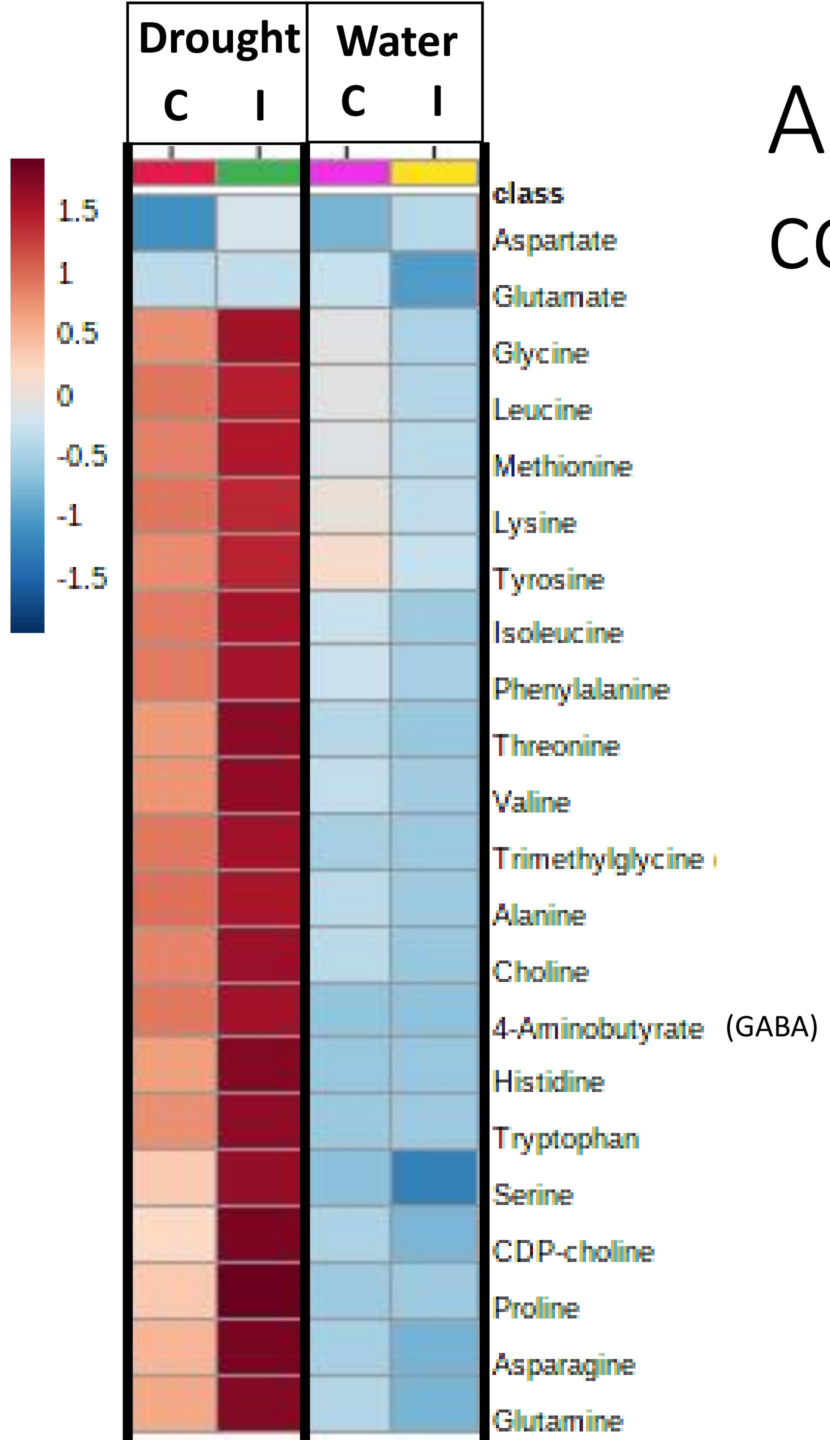


ACCd bacteria inoculation enhanced the accumulation of 66 metabolites under drought stress

66 metabolites were **up-regulated** and **1** metabolite was **down-regulated** in the **leaf tissue** of inoculated plants during drought stress



Metabolite	Fold Change (log2)	Function
Proline	+1.4	Osmotic adjustment
Allantoin	+1.14	N mobilization, ROS scavenging
Folic Acid	+1.10	DNA synthesis
SAM	+1.0	Methylation, precursor for polyamines, biotin, ACC
Glycero-phosphocholine	+0.93	Cell membrane stability
Stachyose	+0.89	Osmoregulant, ROS scavenging, membrane stability



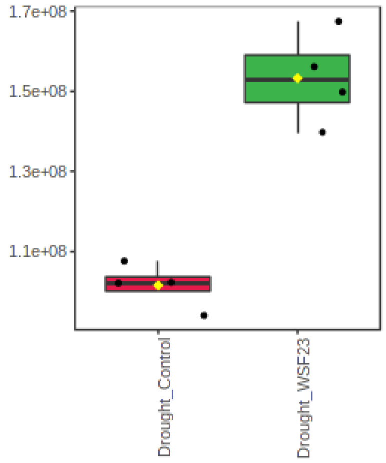
ACCd bacteria increased amino acid content in leaves under drought stress

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

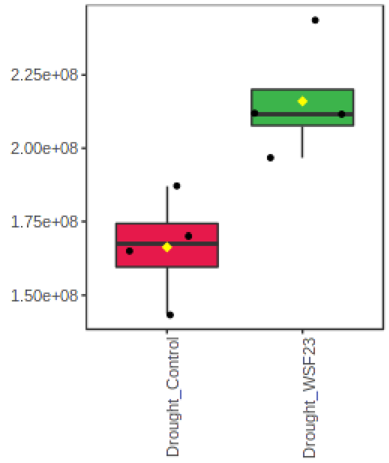
ACCd bacteria increased carbohydrate content in leaves under drought stress

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

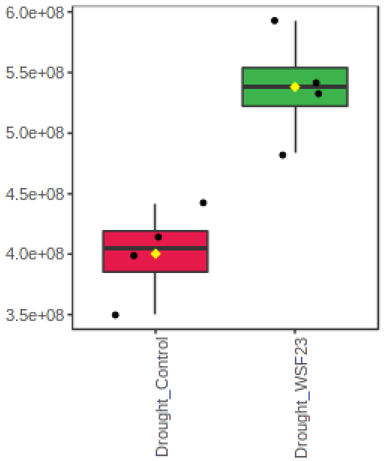
Glucose



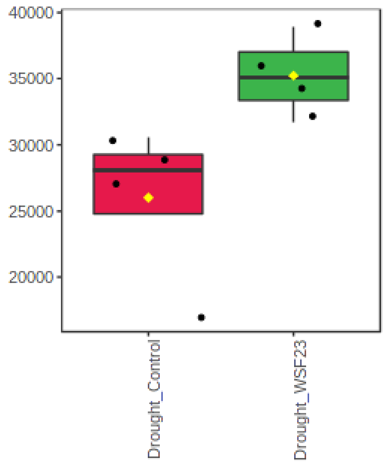
Fructose



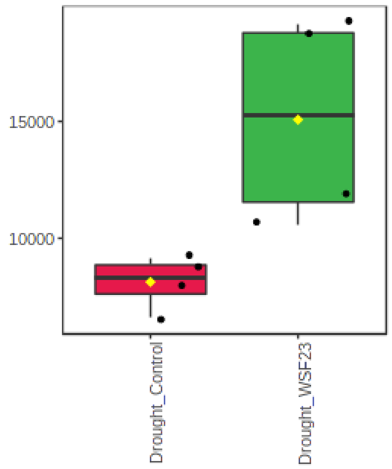
Sucrose



Raffinose



Stachyose

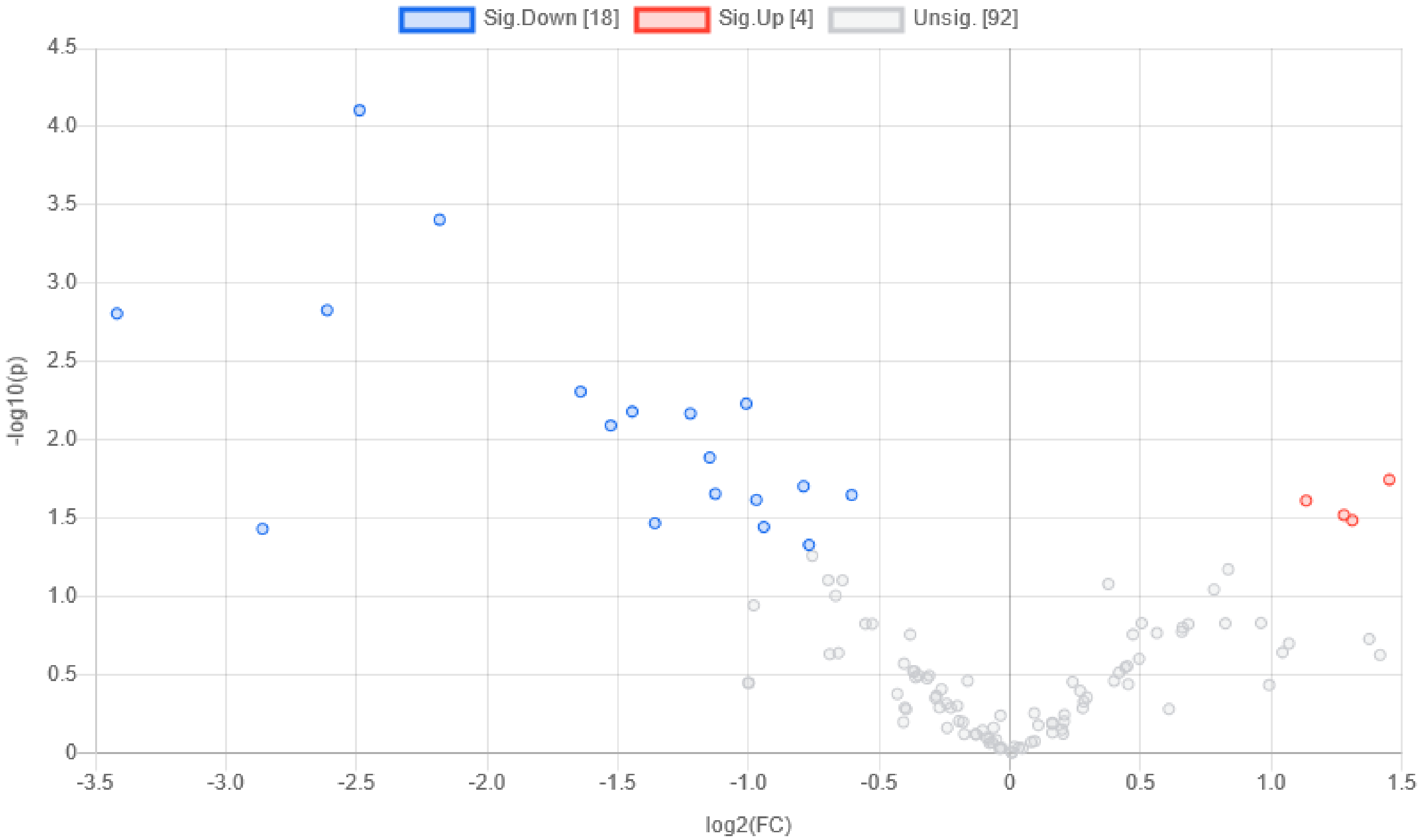


Metabolic pathways regulated by ACCd bacteria in leaves exposed to drought stress by KEGG Analysis

Leaf Drought	Total	Expected	Hits	Raw p	Impact
Alanine, aspartate and glutamate metabolism	22	1.0506	8	3.34E-06	0.47123
Aminoacyl-tRNA biosynthesis	46	2.1967	11	4.57E-06	0
Glycine, serine and threonine metabolism	33	1.5759	7	0.000656	0.14384
Nicotinate and nicotinamide metabolism	13	0.62081	4	0.002454	0.23636
Galactose metabolism	27	1.2894	5	0.007608	0.13734
Arginine biosynthesis	18	0.85959	4	0.00876	0.06019
Glyoxylate and dicarboxylate metabolism	29	1.3849	5	0.010391	0.20645
Citrate cycle (TCA cycle)	20	0.9551	4	0.012904	0.14776
Pyrimidine metabolism	38	1.8147	5	0.031492	0.10696
Arginine and proline metabolism	28	1.3371	4	0.040929	0.09361

ACCd bacteria inoculation enhanced the accumulation of 4 metabolites for post-drought recovery

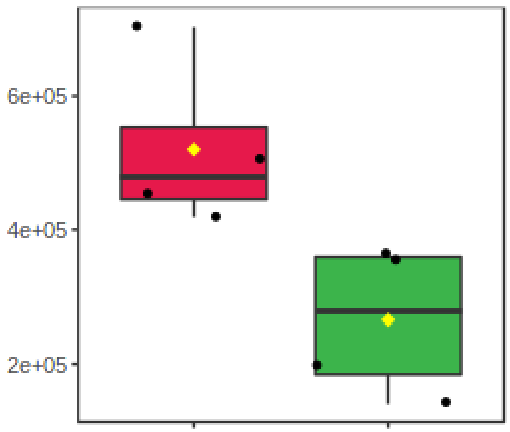
4 metabolites were **up-regulated** and **18** metabolites were **down-regulated** in the **leaf tissue** of inoculated plants during recovery



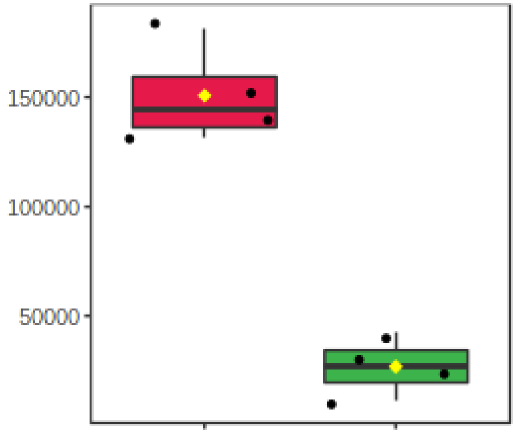
Metabolite	Fold Change (log2)	Function
Phenethylamine	+1.45	Indication of bacterial activity
Arginino-succinate	+1.31	Arginine biosynthesis
Riboflavin	+1.28	Antioxidant activity
Thylamine	+1.13	Pyrimidine metabolism, N cycling
NAD	-3.42	Regulation of stress-induced accumulation of ABA and Proline

ACCd bacteria reduced carbohydrates and osmoregulants in the leaves for post-stress recovery

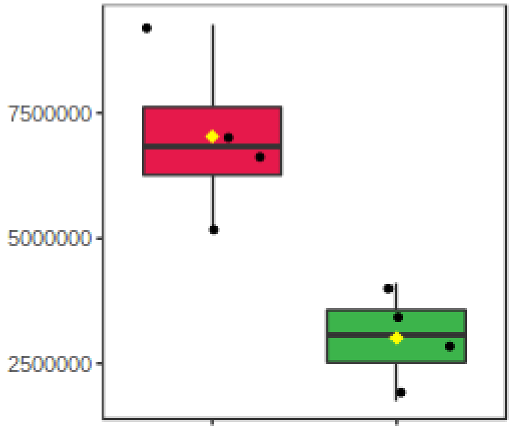
Arabitol



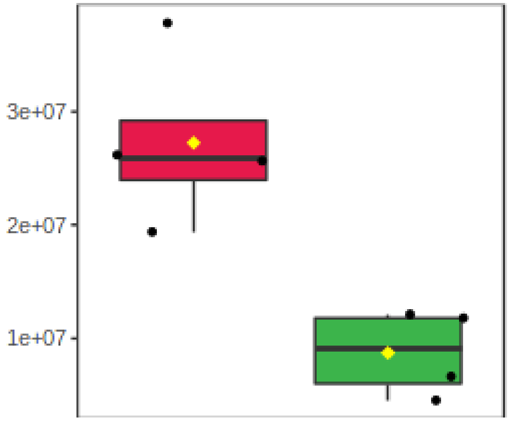
UDP-Glucose



Proline



Sucrose



1. Inoculated plants are returning to non-stress conditions
2. Leaves of inoculated plants are metabolizing the sugars for active respiration

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

Leaf Recovery	Total	Expected	Hits	Raw p	Impact
Starch and sucrose metabolism	22	0.34498	3	0.004252	0.29252
Galactose metabolism	27	0.42338	3	0.007675	0.06411
Pyrimidine metabolism	38	0.59587	3	0.01979	0.13243
Arginine biosynthesis	18	0.28225	2	0.030862	0.14563
Zeatin biosynthesis	21	0.32929	2	0.041176	0.0271
Alanine, aspartate and glutamate metabolism	22	0.34498	2	0.044868	0.34173

Cytokinin levels were increased and ABA was decreased by inoculation with ACCd bacteria

Drought Stress

- 34% Higher C-Zeatin

Recovery

- 40% Higher t-ZR
- 38% Higher C-Zeatin
- 50% Lower ABA

	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

Cytokinins promote tillering and preservation of chlorophyll

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

Drought

- ↑ Osmoregulation
- ↑ Cell wall stability
- ↑ Carbohydrates
- ↑ N mobilization
- ↑ ROS scavenging
- ↑ Cytokinins

Recovery

- ↑ Carbohydrate metabolism
- ↑ Pyrimidine metabolism
- ↑ Cytokinins → Tillering
- ↓ ABA → Stomatal regulation

Thank You

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- USDA SARE

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