Developing a low input and sustainable switchgrass feedstock production system utilizing beneficial bacterial endophytes

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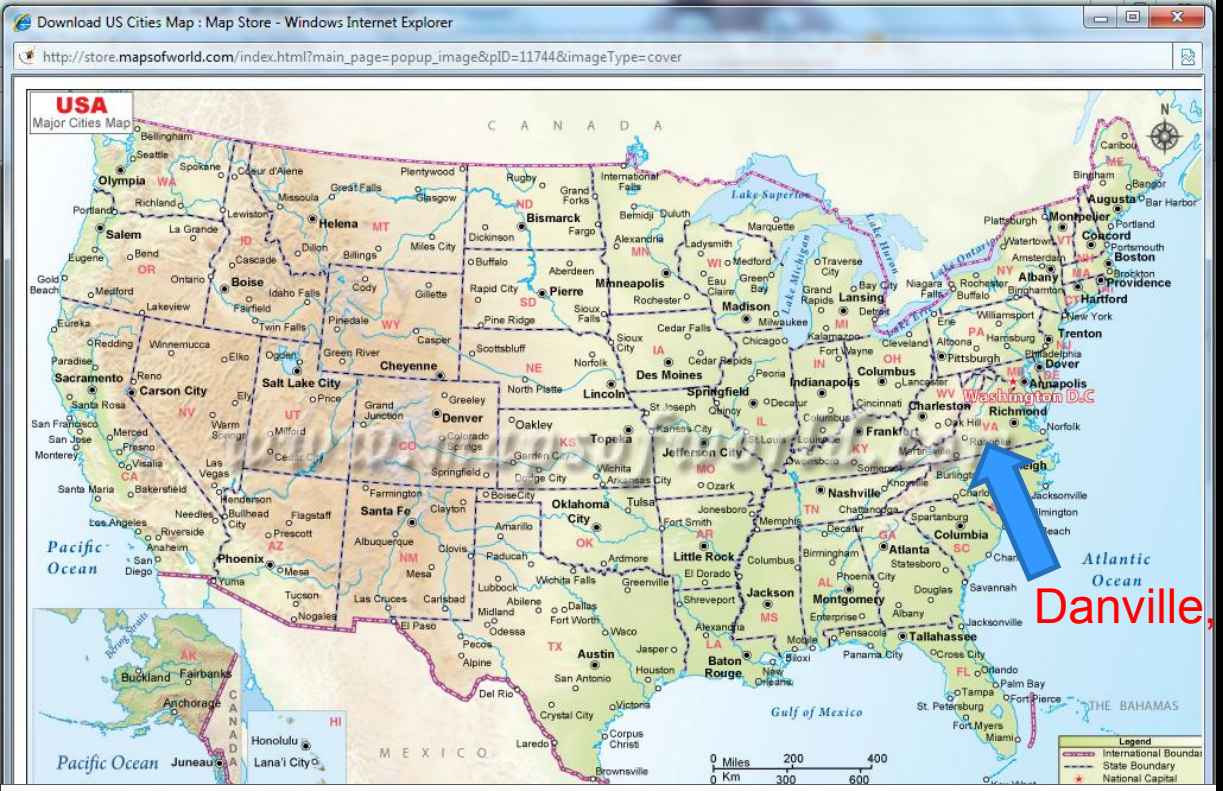


IALR

The Institute for Advanced Learning and Research is a state-supported, Virginia Tech-affiliated research and education center focused on the development and use of technology and education to enhance the development of the economically-depressed Southside Virginia region.

Switchgrass

- A perennial, warm-season grass, the native, highly productive in North America
- Can grow in poor soil and marginal lands
- Requires much less fertilizers and pesticides
- Can be harvested as a cash crop for 10 years or more once established
- Sustainable and renewable crop
- The net energy input/output 1:5

Traits Need to Be Improved

- Poor stand establishment in first year
- Resistance to abiotic and biotic stresses

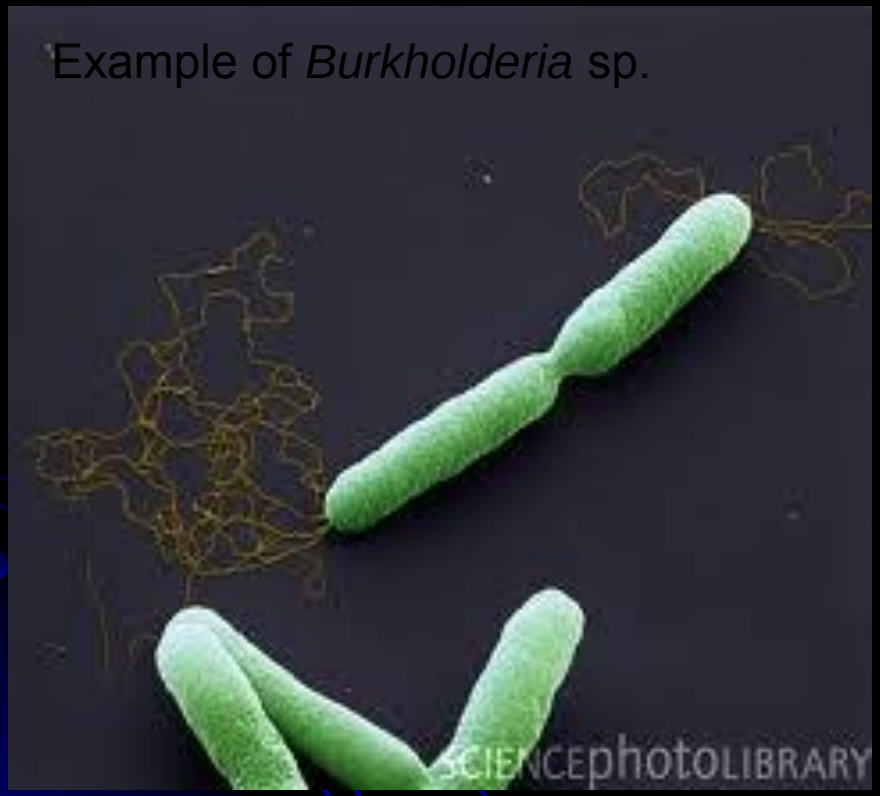
Our Goal

- Develop a low input and sustainable switchgrass feedstock production system utilizing beneficial endophytes, especially on marginal lands

Beneficial Endophytes

- An endophyte is an endosymbiont that lives within a plant for at least part of its life without causing apparent disease.
- Endophyte benefit the host plants by promoting plant growth, increasing nutrient acquisition, stress tolerance, and pathogen resistance.
- Endophytes are naturally existing microorganisms.

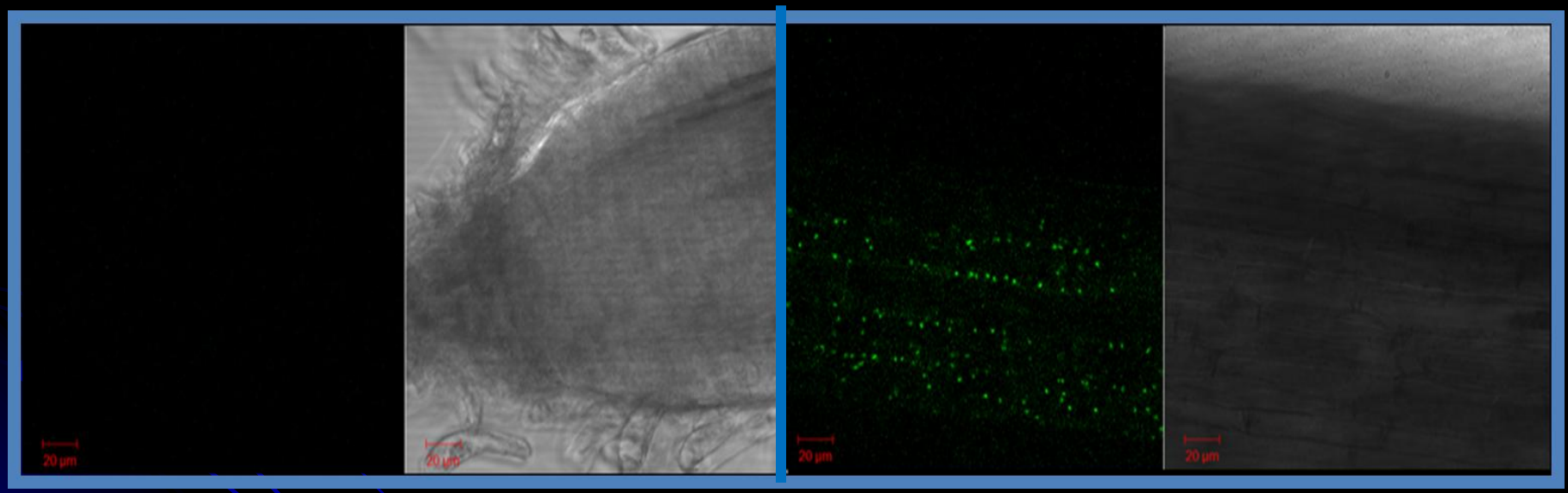
Burkholderia phytofirmans strain PsJN



- Gram negative beneficial bacterial endophyte isolated from onion roots in 1987 by Dr. Jerzy Nowak
- Plant growth promotion has been found in many species.
- Mechanism:
  - ACC deaminase activity
- Complete genome has been sequenced

* (DOE) Sequencing Program (http://www.jgi.doe.gov/CSP/index.html)

Confocal Images



Non-infected Control PsJN-GFP Inoculated

The images were taken three days after inoculation with PsJN-GFP (Appalachian State University)

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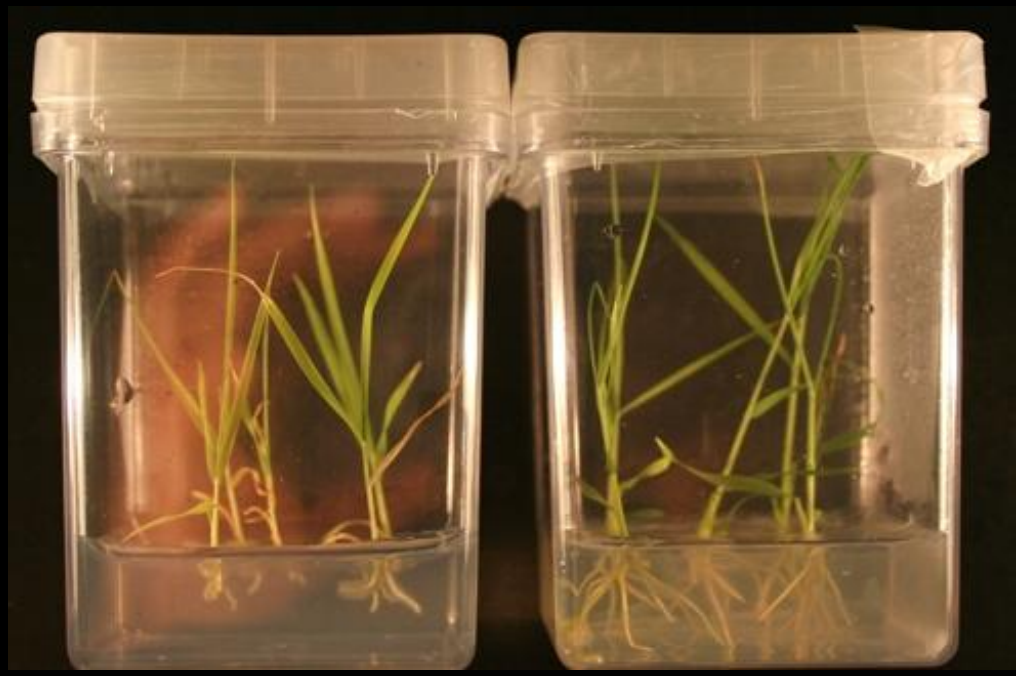
Growth Promotion by PsJN

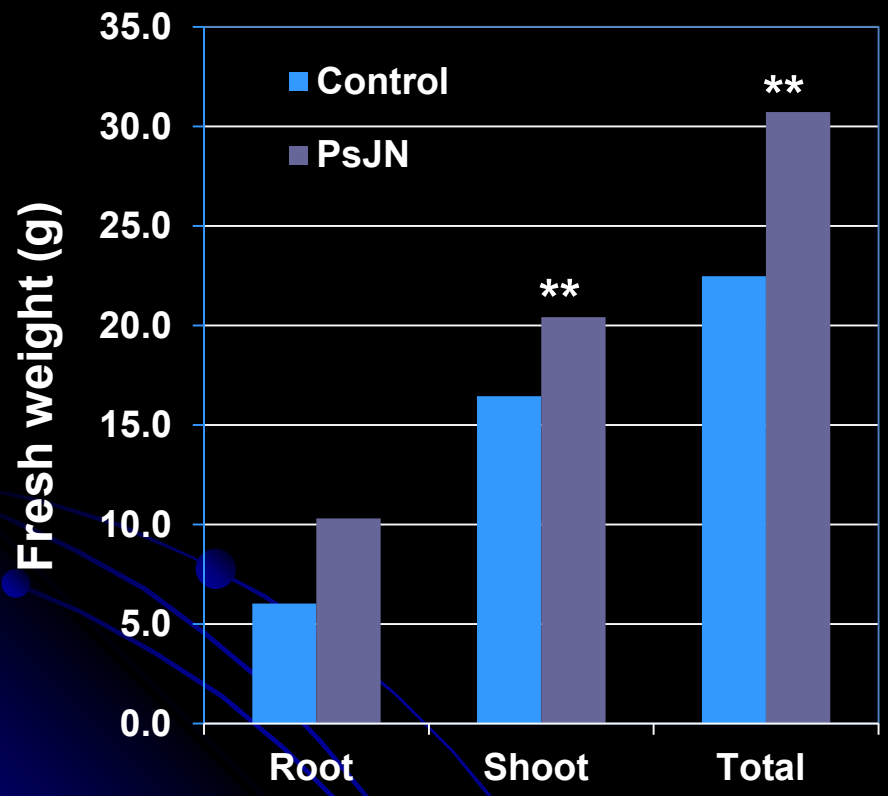
Control PsJN

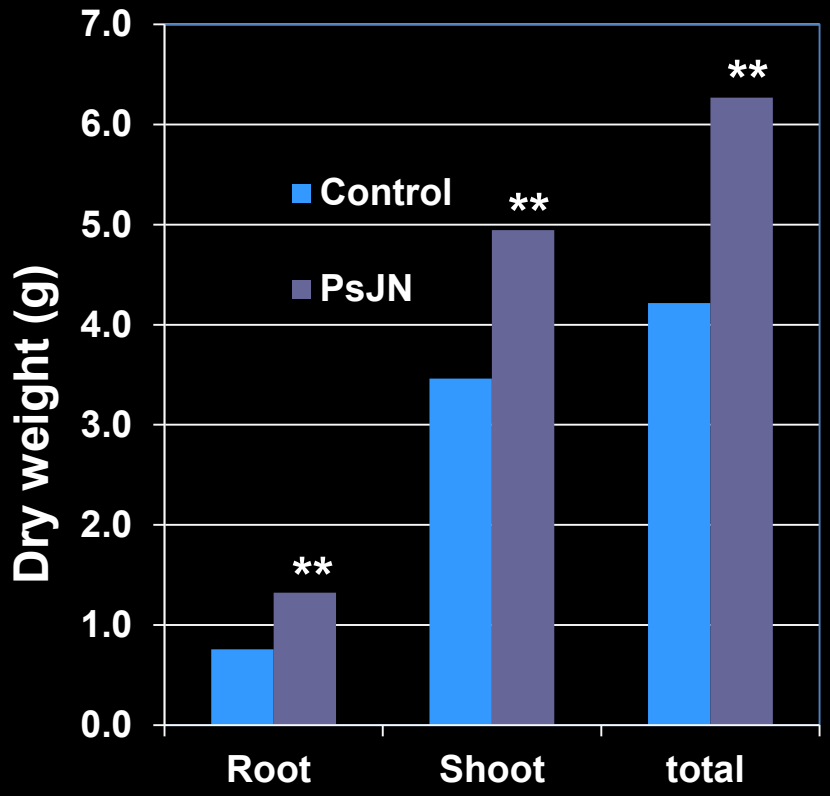


Control PsJN

Pictures were taken one month after Alamo inoculated with PsJN.

Greenhouse Experiment





Growth promotion persistence of Alamo in greenhouse by PsJN inoculation. ** means significant difference at 0.01 level between PsJN and control using student T-test.

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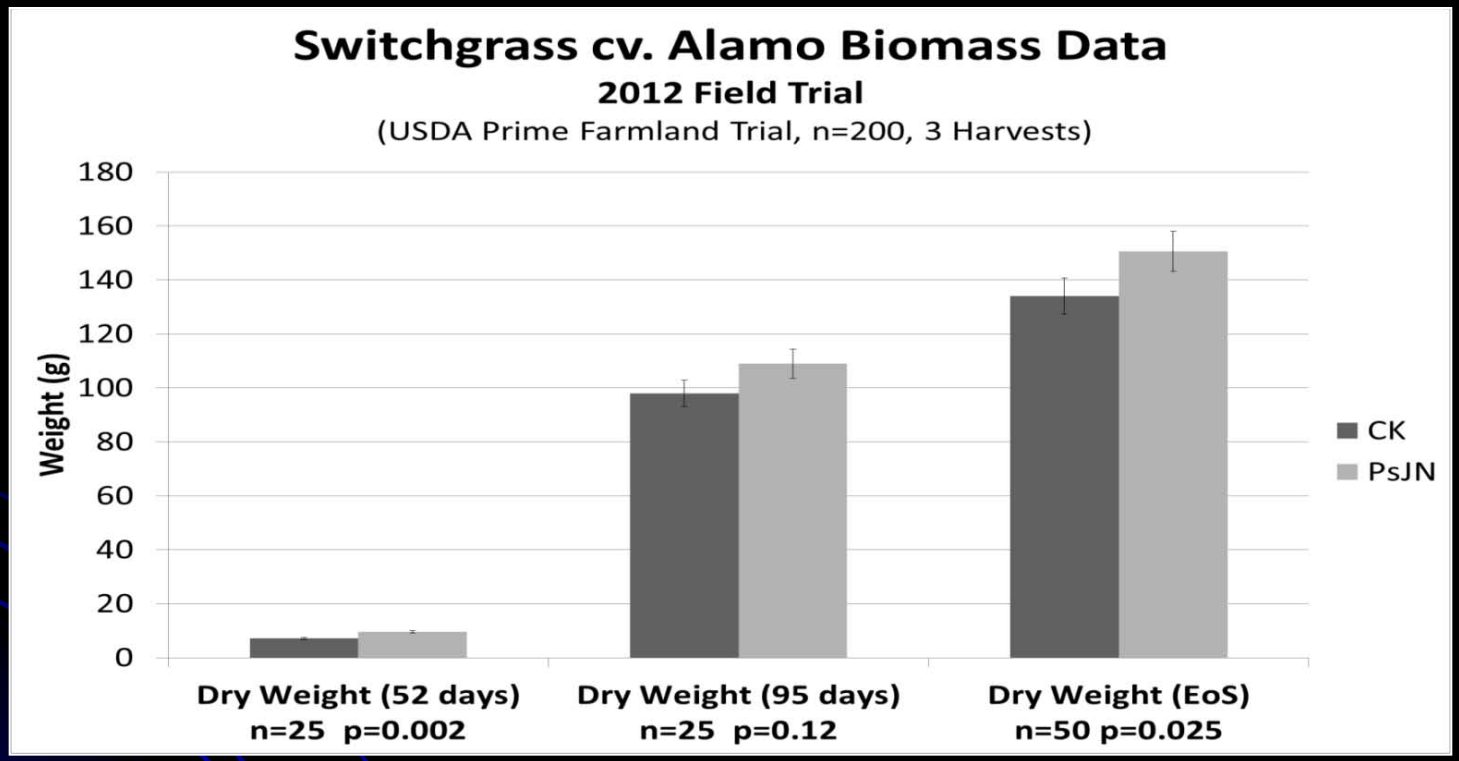
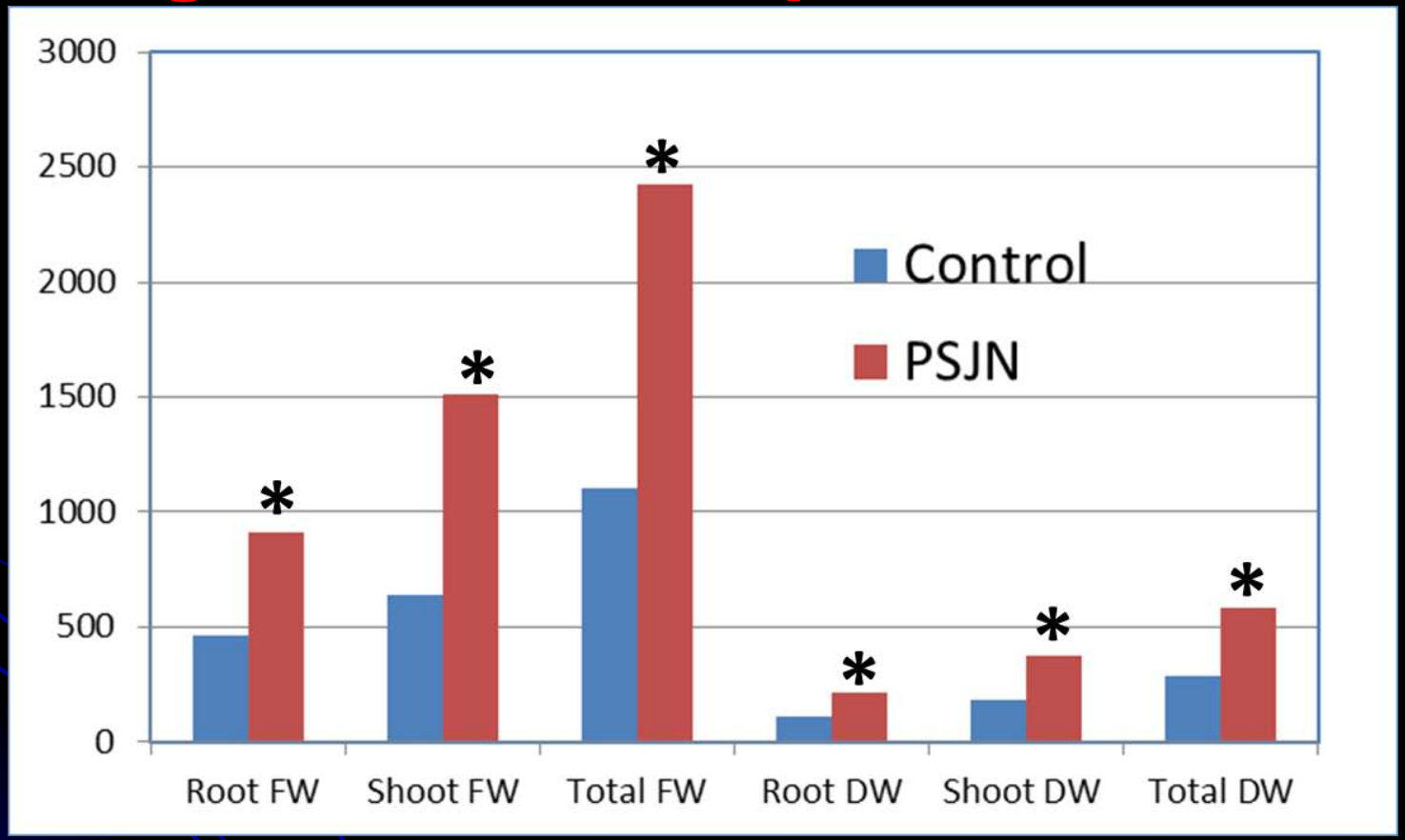
Field Experiment



Dry weight of aboveground in Alamo inoculated with PsJN and control plants grown in USDA prime soil. Data collected during vegetative growth (52 days), at flowering (95 days), and at the end of the season (EoS).

Plants grown in suboptimal conditions



Plants were grown in field soil without fertilization in a greenhouse for 2.5 months in Fall 2010 under suboptimal temperature. * Indicates a p-value of less than 0.005.

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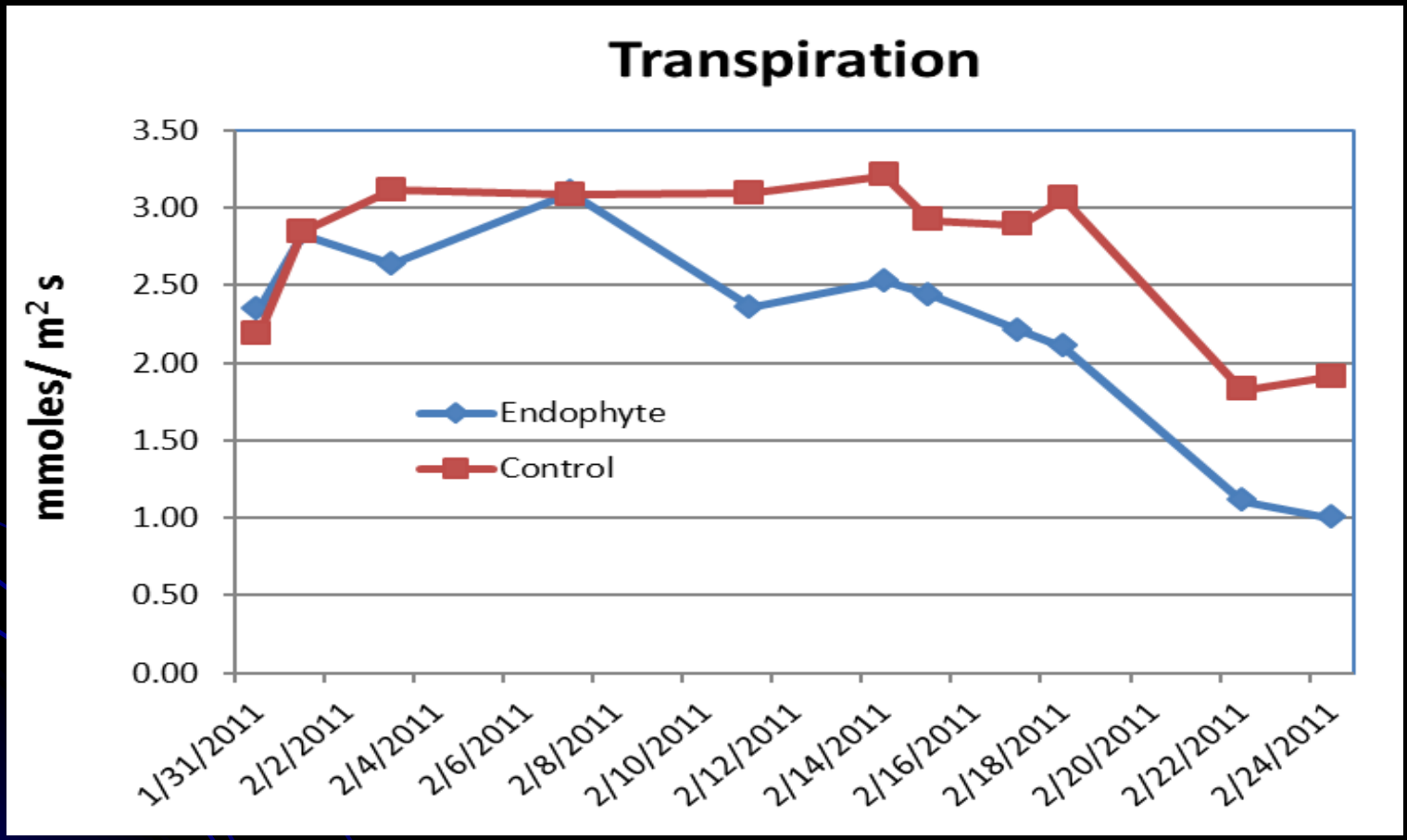
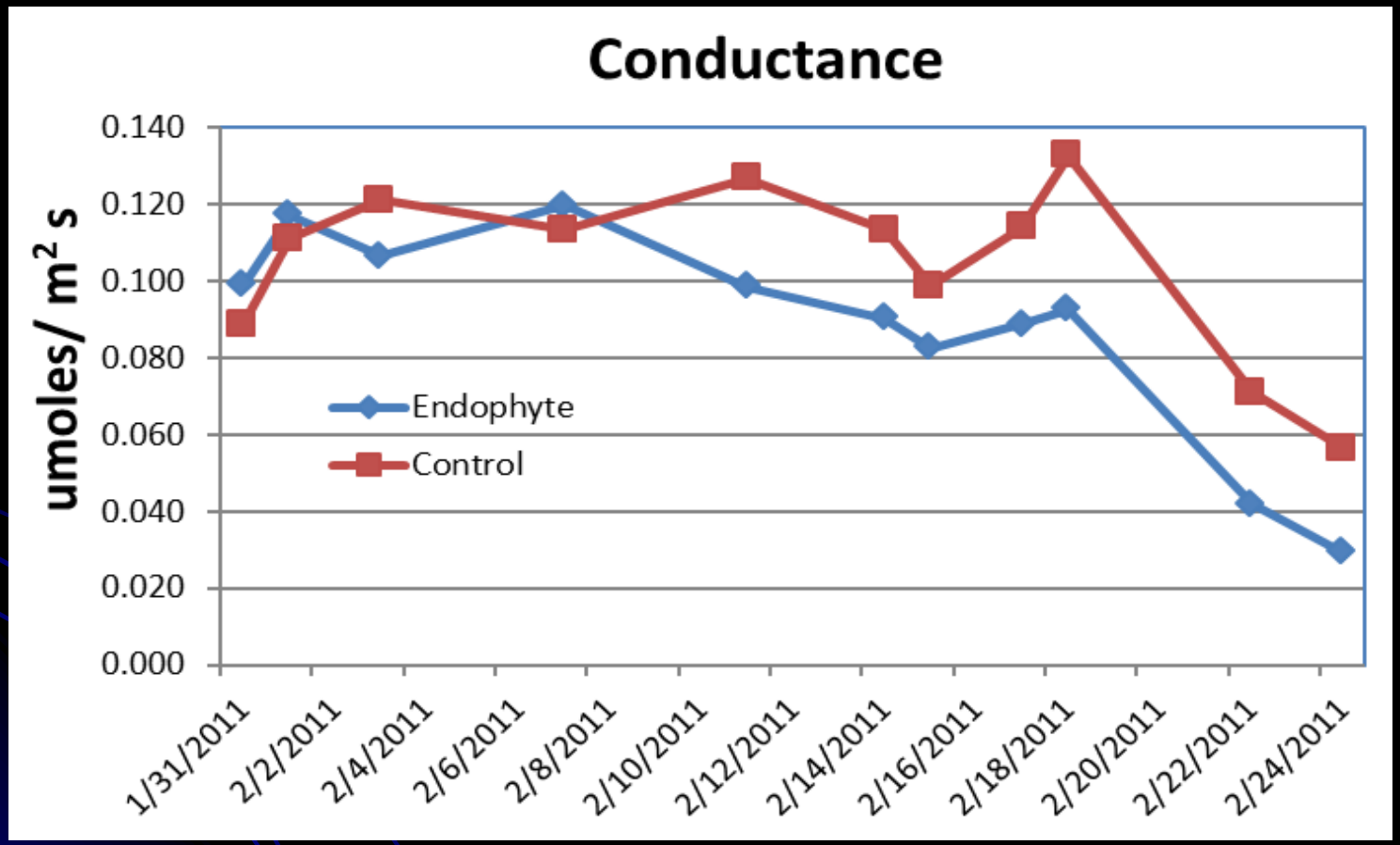
Plant Physiology Parameters



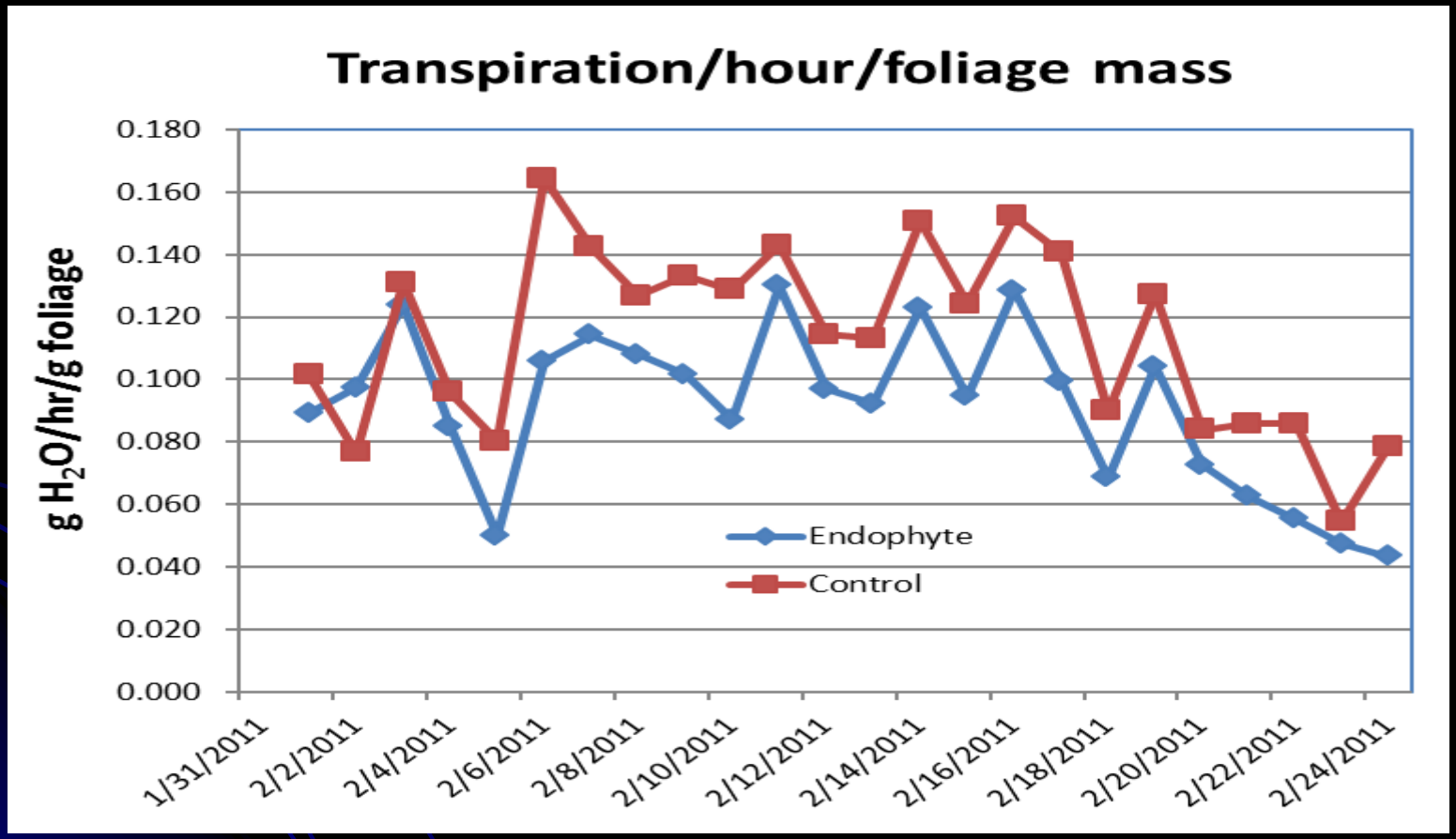
Comparison of transpiration of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



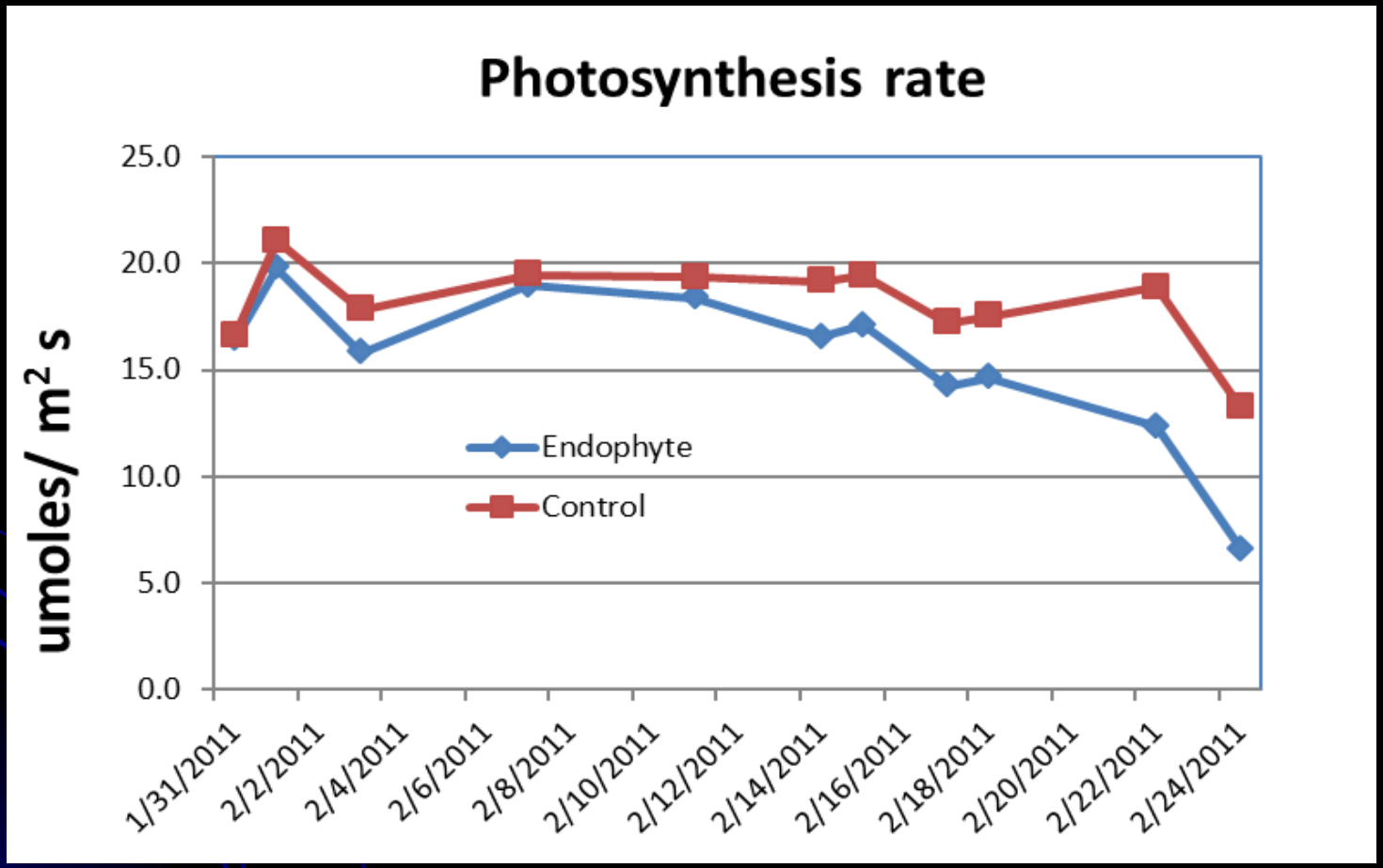
Comparison of conductance of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



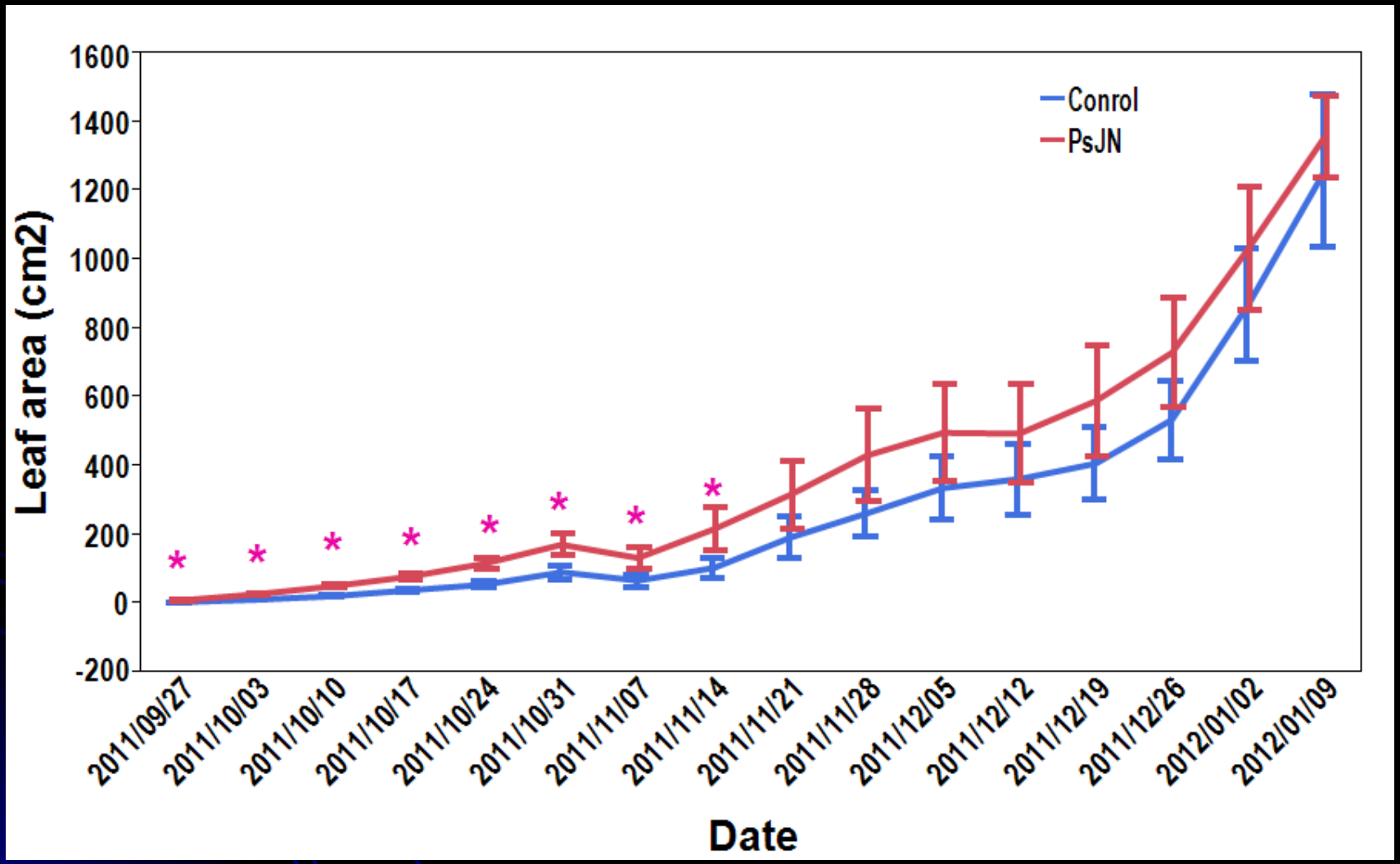
Comparison of water use efficiency of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



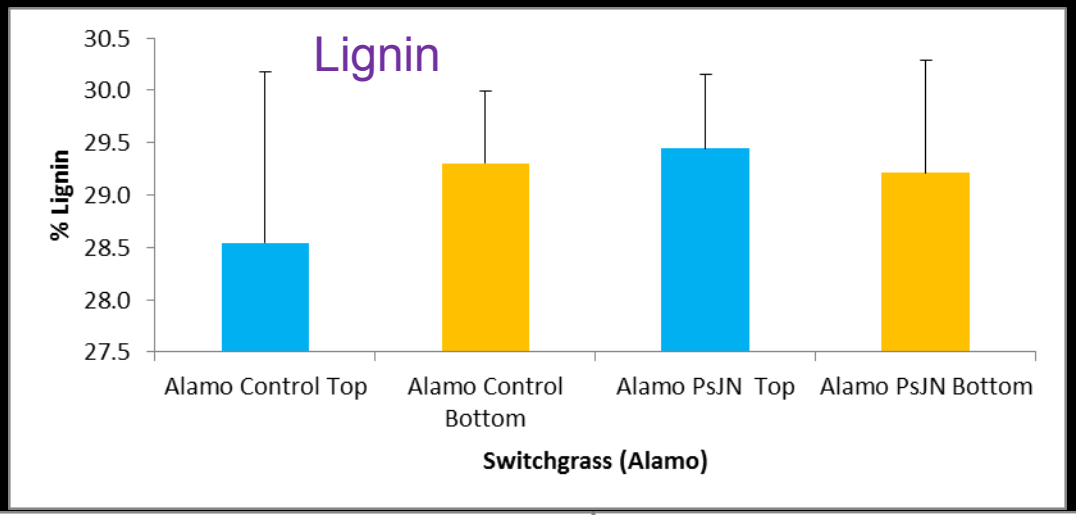
Comparison of photosynthesis rate of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

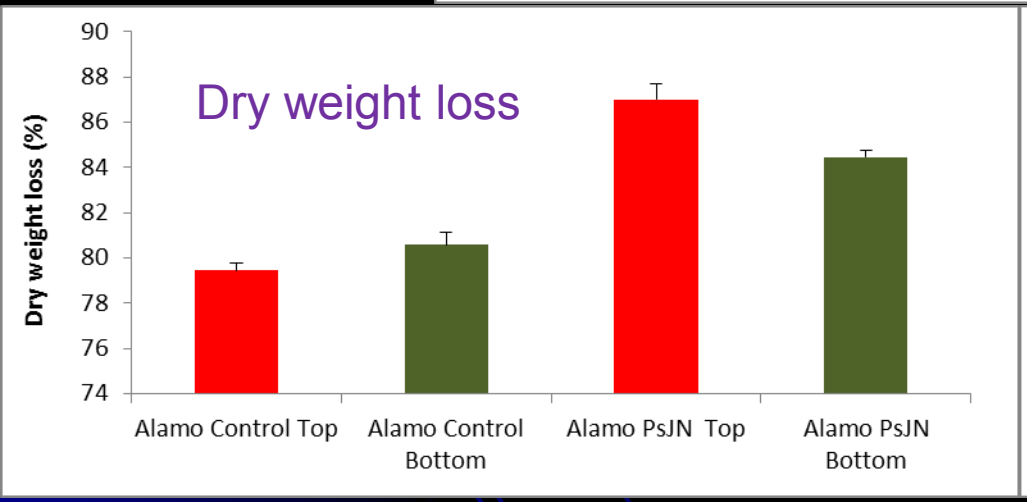
Leaf area

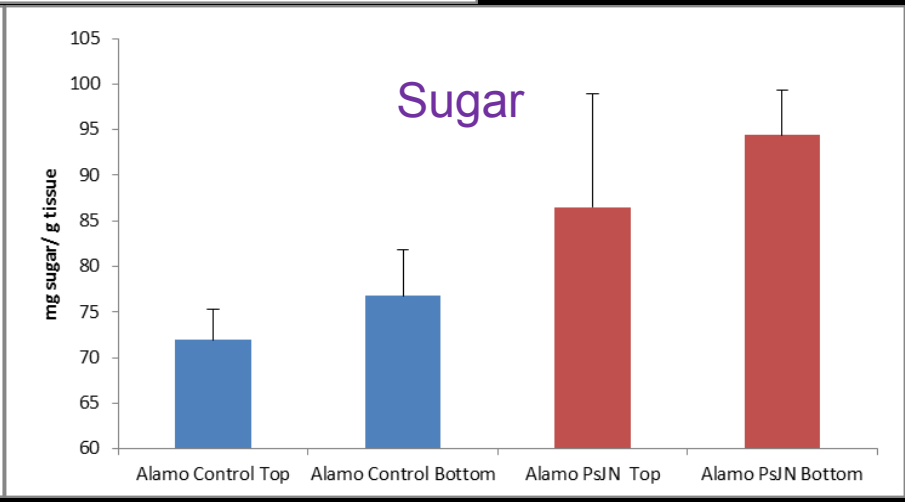


Leaf area was recorded every week from 09/27/2011 to 01/09/2012 with Photoshop CS5 (Adobe Systems Inc.).

Saccharification

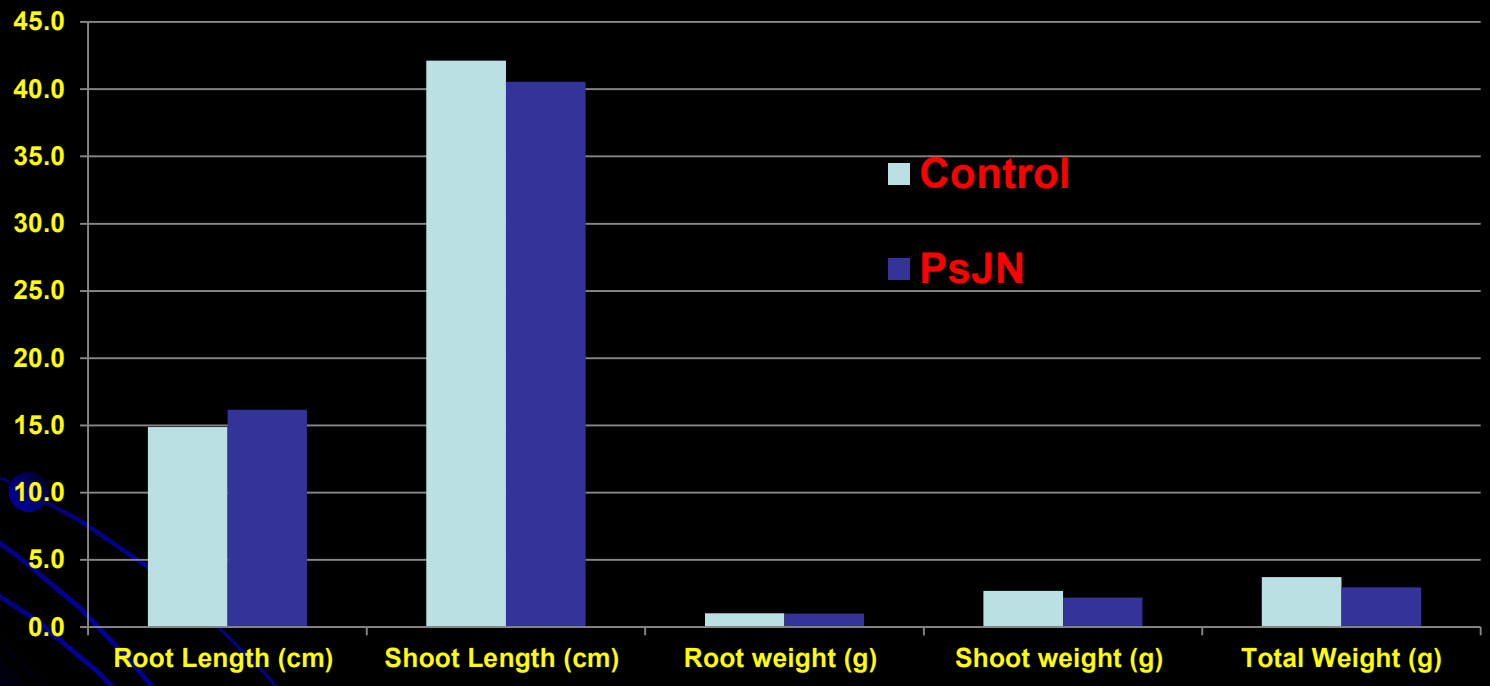






a) Lignin concentration in PsJN-inoculated Alamo and control samples determined with the acetyl bromide method; b) Dry weight loss after cellulase enzyme treatment for 72 hours of PsJN-inoculated and control plants; and c) Sugar amount released after cellulase enzyme treatment.

Genotype Specificity of PsJN



PsJN does not have growth promotion on Cave-in-Rock

Transcriptional Profiling

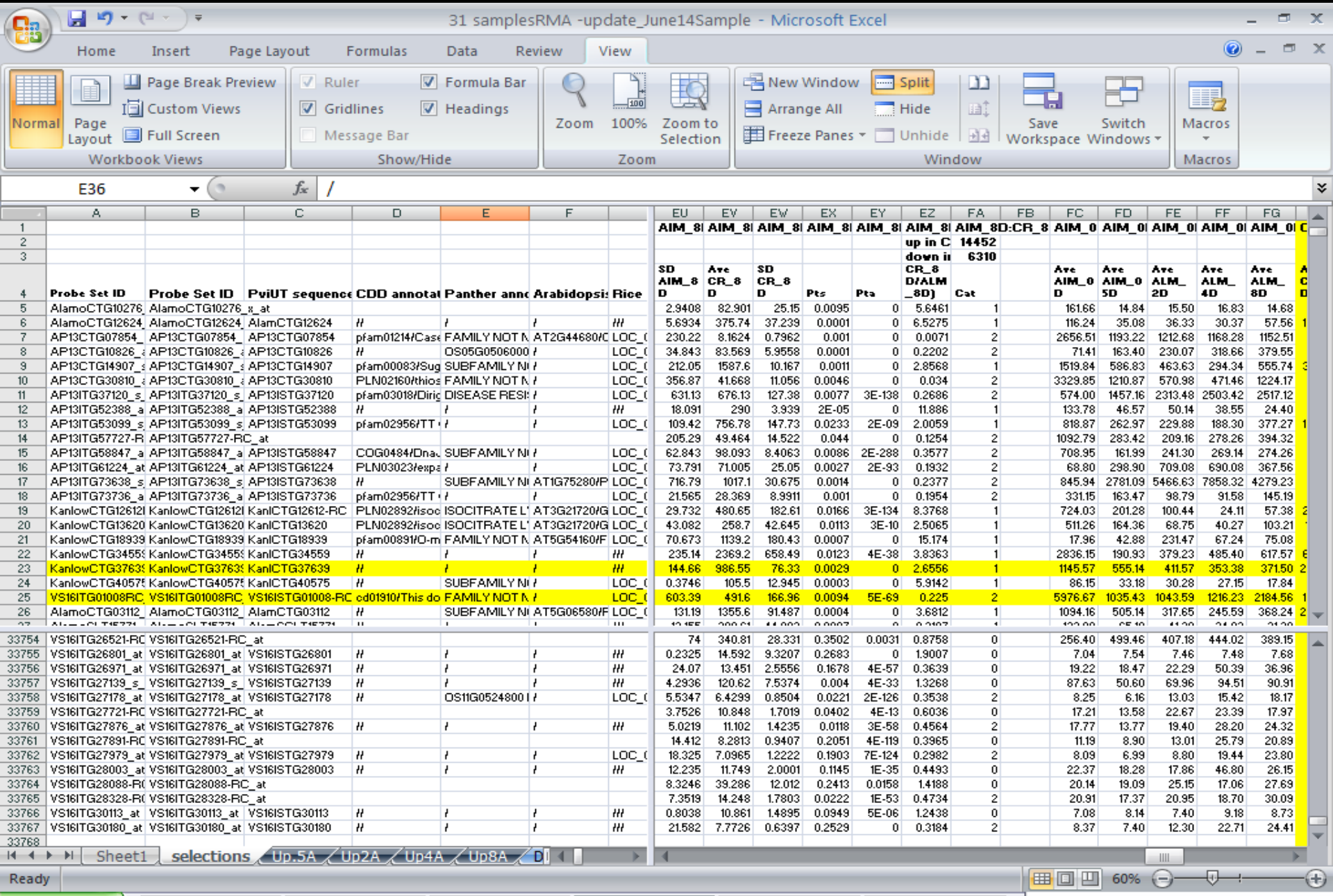
Collect and isolate RNA from days 0, 0.5, 2, 4, and 8 of Alamo and Cave-in-Rock post-inoculation (3 biological replicates)

Microarray analysis to find out key genes

Study key gene functions via Overexpression and RNAi knockout

Test growth responses of modified plants to PsJN

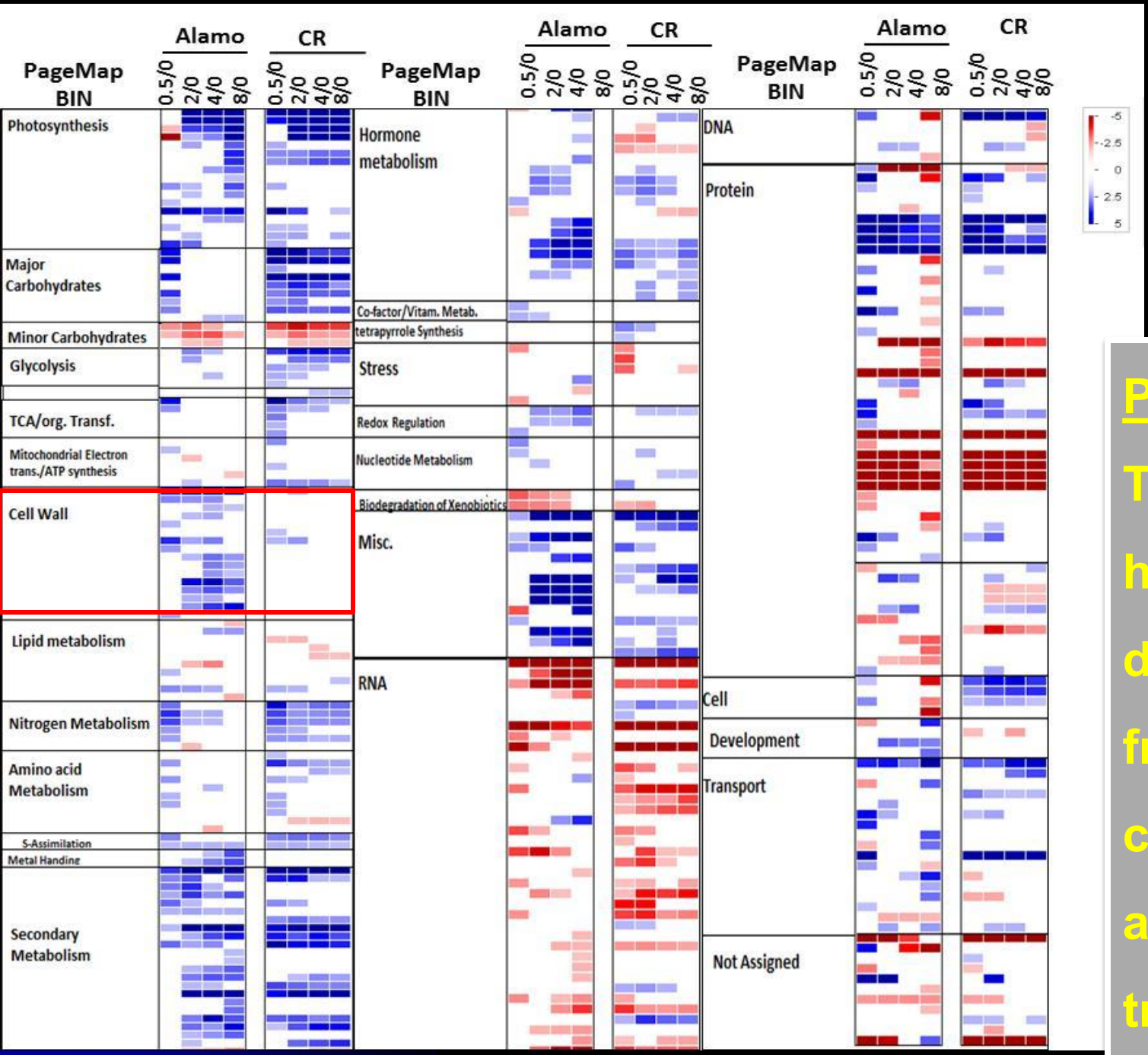
Microarray Data



Transcription Factors

ID probe	Annotation	Alamo (days after PsJN inoculation				Cave-in-Rock (days after PsJN inoculation)			
		0.5	2	4	8	0.5	2	4	8
AP13ITG55712_at	AP2 domain	1.71	1.48	2.14	2.80	0.05	0.05	0.07	0.07
AP13ITG63524RC_s_at		2.27	1.75	2.59	2.29	0.89	0.68	0.79	1.14
AP13CTG22494_at	bZIP	1.88	3.58	3.03	1.80	1.27	1.48	1.51	0.95
AP13ITG54829_at		2.62	2.05	2.79	1.71	1.45	1.40	1.39	1.68
AP13CTG24092_at	MYB family	1.52	1.68	2.06	1.24	0.95	0.94	0.91	0.98
KanlowCTG34263_at		1.24	2.09	5.46	4.58	0.71	0.93	1.42	2.12
KanlowCTG22073_s_at		2.25	0.94	0.57	0.52	1.36	1.37	1.26	1.24
AP13ITG65291_at	F-box domain	1.53	2.03	2.26	2.88	1.15	1.06	0.79	0.98
KanlowCTG42852_s_at		1.20	1.70	2.13	2.15	0.75	0.77	0.66	0.68
AP13ITG41289_at		1.18	1.60	2.07	1.83	0.32	0.27	0.29	0.33
AP13ITG57608_s_at	RING-H2 finger	1.09	2.28	2.49	2.81	0.77	0.95	0.88	0.96
AP13ITG69131RC_at	zinc finger, C3HC4 type	1.56	1.76	2.07	2.28	0.74	0.62	0.67	0.71
AlamoCTG04292_s_at		2.26	1.22	1.16	1.11	1.65	1.95	1.89	1.90
AP13CTG19863_at	TFs having WRKY and zinc finger domain	3.13	1.94	1.70	1.69	0.12	0.11	0.17	0.17
AP13CTG44559_s_at		1.68	2.53	4.58	4.20	0.03	0.04	0.05	0.06
AP13.12336.m00003_s_at	No apical meristem	3.60	1.55	0.83	0.90	3.80	4.31	4.85	3.94
KanlowCTG46205_s_at	Transcription elongation factor	3.71	2.02	1.88	1.17	0.77	0.70	1.29	1.67
AP13CTG09371_s_at	zinc finger	2.53	1.30	0.89	0.73	1.15	1.82	1.96	1.95
AP13ITG48832_s_at	AT hook motif	2.73	1.38	0.67	0.45	1.20	1.33	1.35	1.19

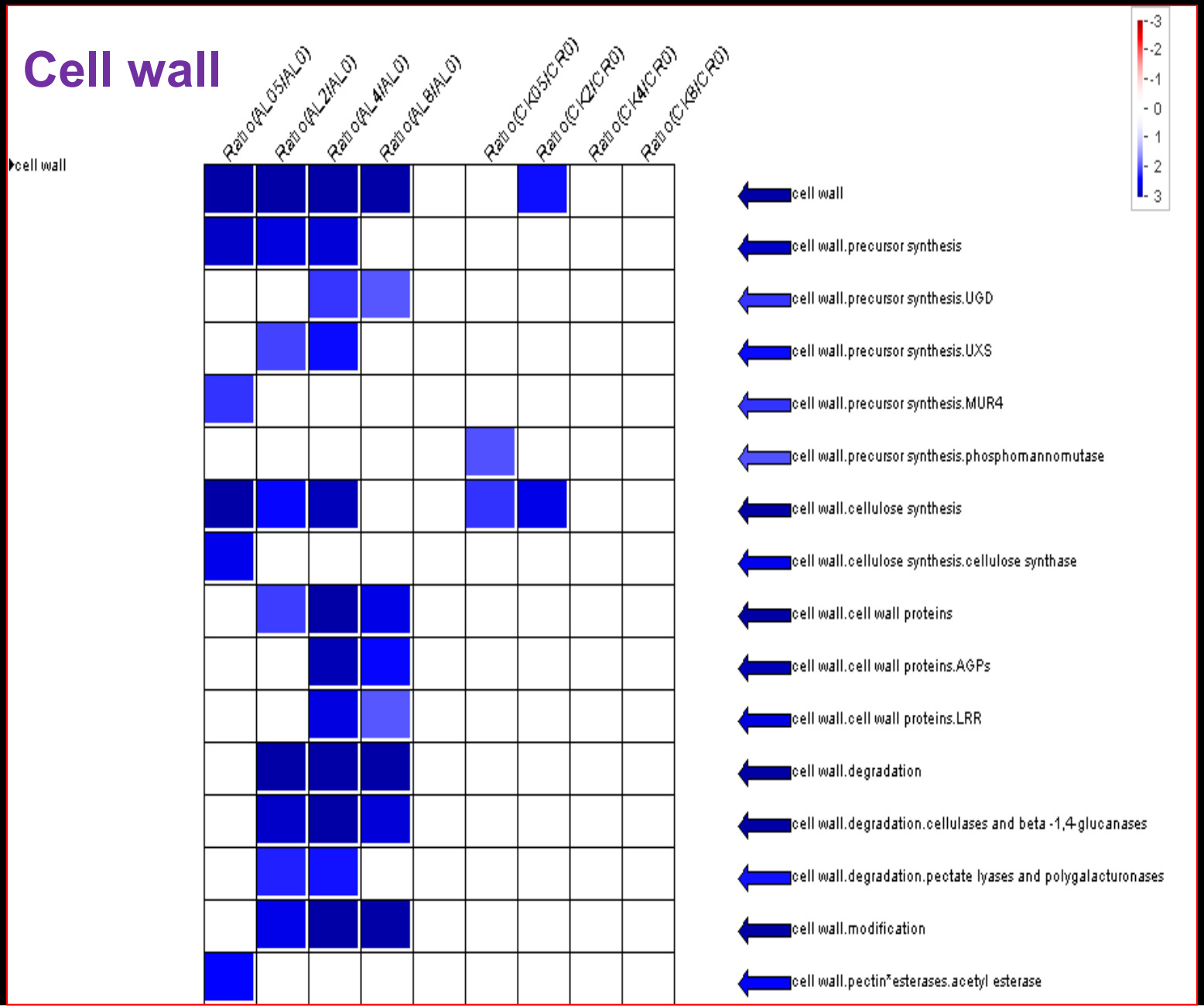
Expression level changes of transcription factor genes of interest in Alamo and Cave-in-Rock at 0.5, 2, 4 and 8 days following inoculation with PsJN, compared with expression level at 0 day, respectively.



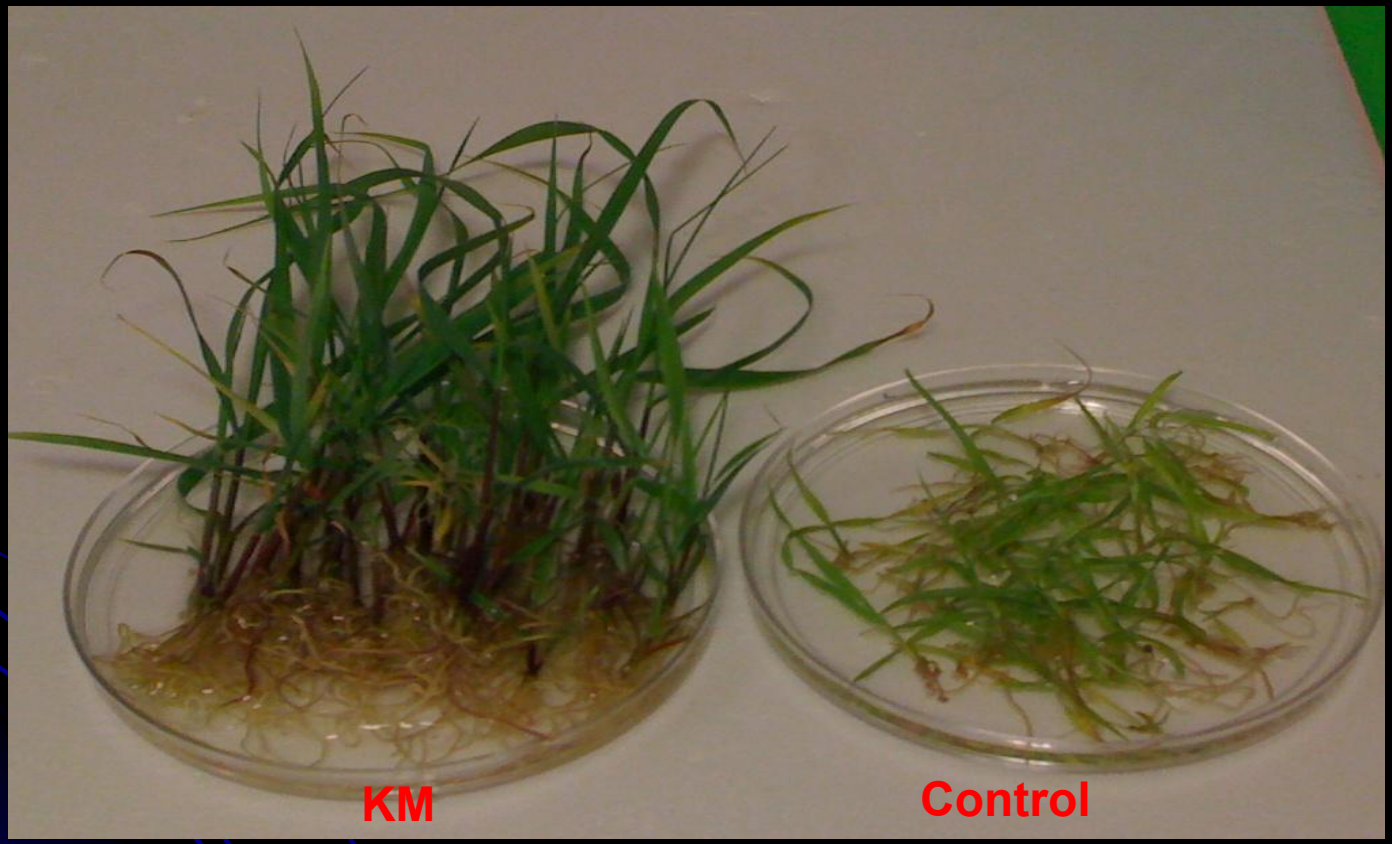
PAGEMAN:

This software helps analyze data series from time courses and/or treatments.

Cell wall

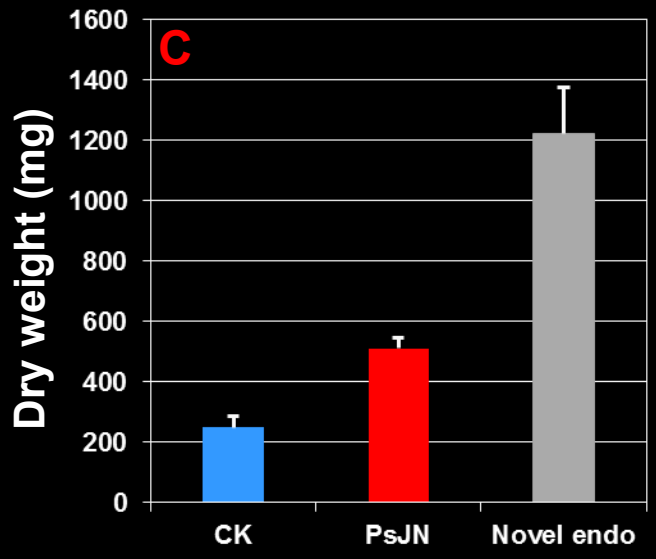

Ratio(AL05/AL0)
Ratio(AL2/AL0)
Ratio(AL4/AL0)
Ratio(AL8/AL0)
Ratio(CK05/CR0)
Ratio(CK2/CR0)
Ratio(CK4/CR0)
Ratio(CK8/CR0)
-3
-2
-1
0
1
2
3
cell wall
cell wall
cell wall.precursor synthesis
cell wall.precursor synthesis.UGD
cell wall.precursor synthesis.UXS
cell wall.precursor synthesis.MUR4
cell wall.precursor synthesis.phosphomannomutase
cell wall.cellulose synthesis
cell wall.cellulose synthesis.cellulose synthase
cell wall.cell wall proteins
cell wall.cell wall proteins.AGPs
cell wall.cell wall proteins.LRR
cell wall.degradation
cell wall.degradation.cellulases and beta -1,4-glucanases
cell wall.degradation.pectate lyases and polygalacturonases
cell wall.modification
cell wall.pectin*esterases.acetyl esterase

Novel Endophyte-KM



KM significantly promotes growth of Alamo

Novel Endophyte (KM) vs PsJN

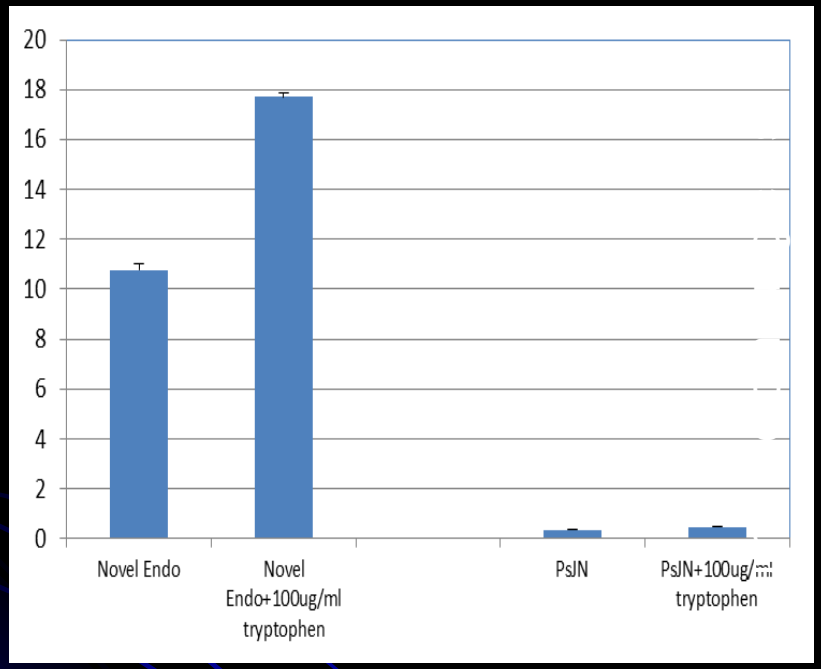


Comparison of KM with PsJN in growth promotion on Alamo under in vitro condition.

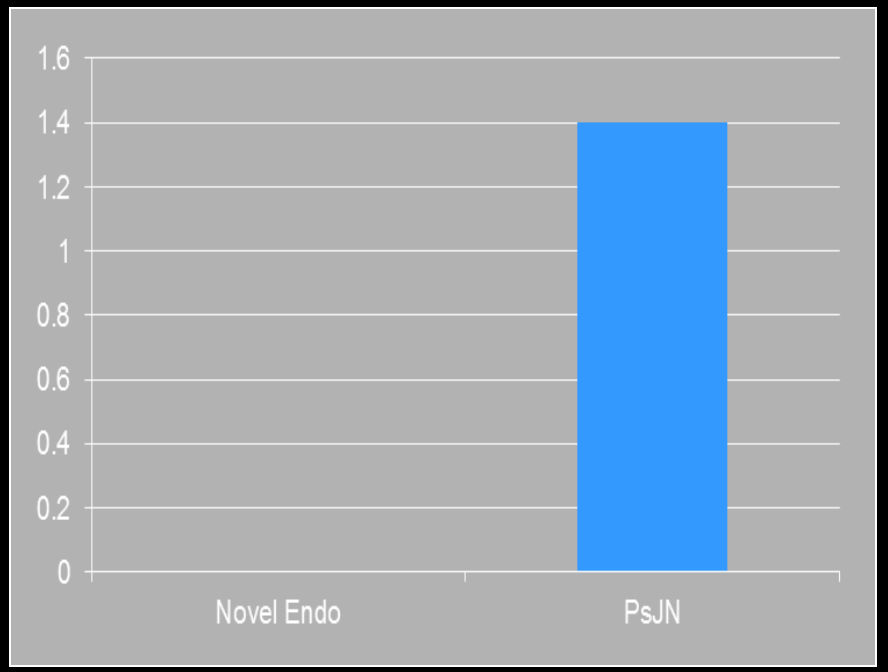
A Broad Spectrum of Promotion by KM

Forestburg				Nebraska				Shawnee				Blackwell			
	Shoot L	Root L	Total W		Shoot L	Root L	Total W		Shoot L	Root L	Total W		Shoot L	Root L	Total W
CK	8.8	2.0	32.6	CK	10.1	1.9	34.8	CK	7.0	2.5	31.6	CK	10.6	2.4	45.9
SE	0.490	0.260	2.330	SE	0.652	0.176	2.718	SE	1.008	0.299	2.478	SE	1.685	0.313	8.048
KM/CK	1.29	0.67	1.77		1.63	1.22	2.47		2.16	1.53	2.68		1.46	1.63	2.67
KM	11.4	1.3	57.7	KM	16.5	2.3	86.1	KM	15.1	3.8	84.7	KM	15.4	3.9	122.4
SE	0.966	0.177	8.990	SE	1.103	0.302	10.876	SE	1.041	0.311	10.705	SE	1.154	0.561	17.189
P-Value	0.01018	0.0239	0.00437		9.4E-06	0.12376	3.6E-05	P-Value	1.8E-06	0.00235	4.5E-05	P-Value	0.010174	0.019722	0.000456633
Shelton				Sunburst				Canthage				Cave in Rock			
	Shoot L	Root L	Total W		Shoot L	Root L	Total W		Shoot L	Root L	Total W		Shoot L	Root L	Total W
CK	12.1923	2.99231	43.6154	CK	9.7857	1.58571	37	CK	8.51	2.14	29.7		2.7	2.3	25.3
SE	1.01087	0.45382	3.81216	SE	1.1602	0.312	6.41341	SE	0.87349	0.16069	2.76506		0.1292	0.1238	1.6605
KM/CK	0.93	1.08	3.24		1.54	2.61	3.39		2.12	1.56	2.90		3.61	1.57	2.97
KM	11.3538	3.23077	141.231	KM	15.043	4.13571	125.429	KM	18	3.34	86	KM	8.3	3.6	75.2
SE	1.01087	0.45382	3.81216	SE	1.2774	0.43911	21.4547	SE	1.34371	0.37895	13.0461	SE	1.2693	0.5511	11.4658
P-Value	0.31226	0.34774	0.01285	P Value	0.0026	3.4E-05	0.00027	P-Value	6.6E-06	0.00462	0.00026	P-value	7.8E-19	7.5E-07	2.5E-11

IAA and ACC Deaminase



IAA concentration



ACC deaminase activity

KM produces higher level of IAA and does not have ACC deaminase activity

Future Research

- Growth promotion persistence by endophytes in the field
- Sustainability for biomass production
- Environmental effects - Soil C and N dynamics
- Systems biology – Metagenomics: Soil microbial community, especially AMF
- Genetic engineering of microbes
- Multi-functional bio-inoculants (Podile and Kishore 2007)

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Dr. Shuijin Hu, North Carolina State University
Dr. Bingyu Zhao, VT

USDA United States Department of Agriculture National Institute of Food and Agriculture

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http://genomicscience.energy.gov/research/DOEUSDA/

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