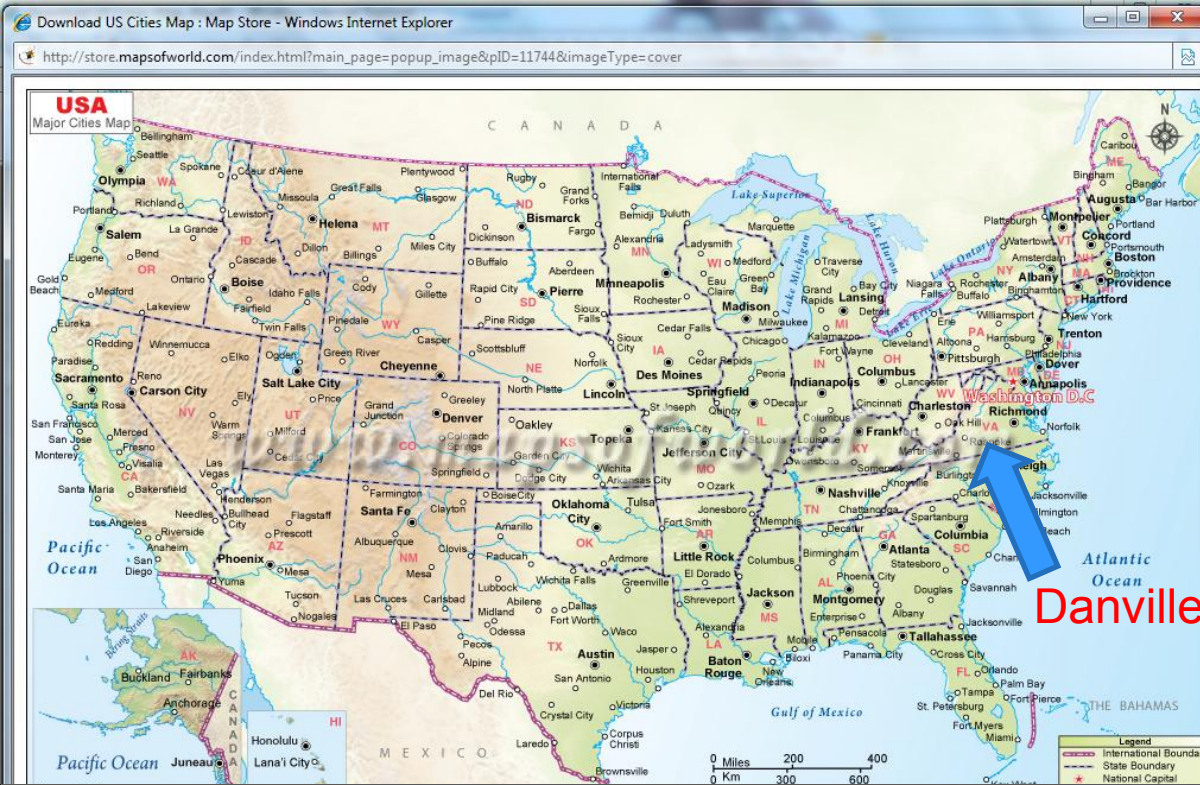


Developing a low input and sustainable switchgrass feedstock production system utilizing beneficial bacterial endophytes

Dr. Chuansheng Mei

**Institute for Sustainable and Renewable Resources
Institute for Advanced Learning and Research**



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

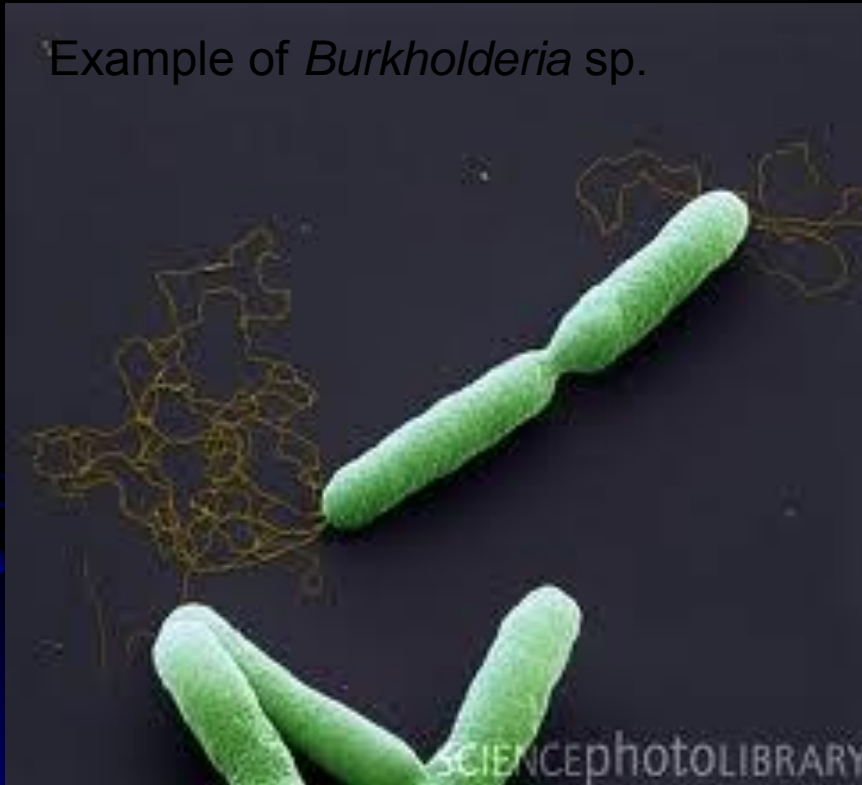
- **Develop a low input and sustainable switchgrass feedstock production system utilizing beneficial endophytes, especially on marginal lands**

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

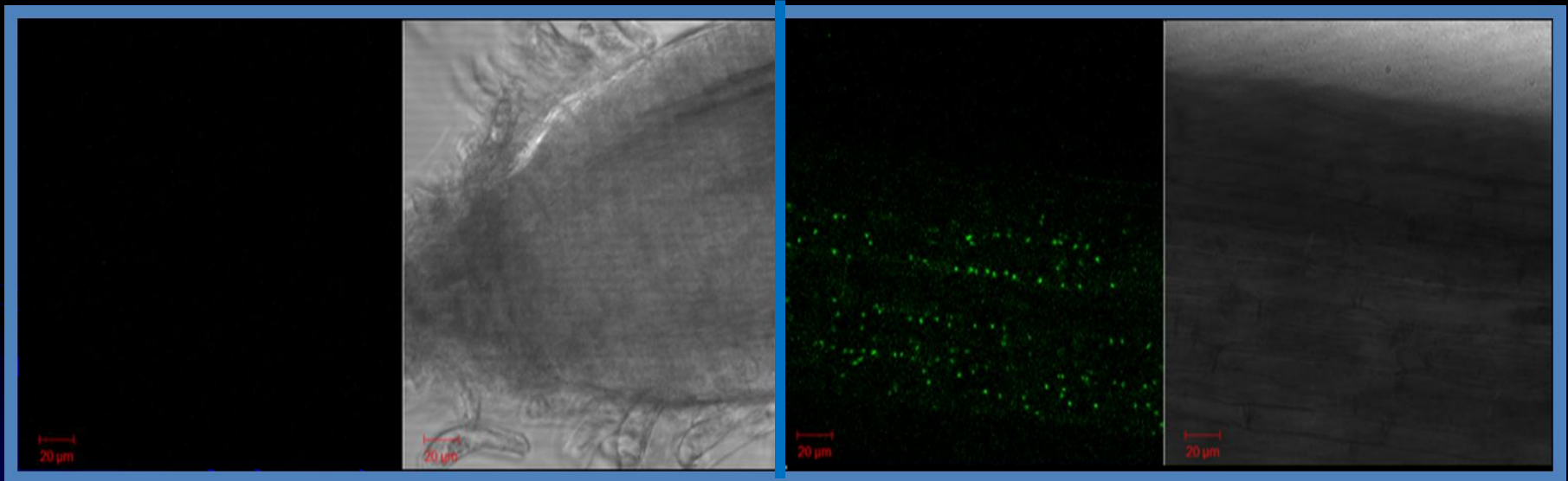
Example of *Burkholderia* sp.



- 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

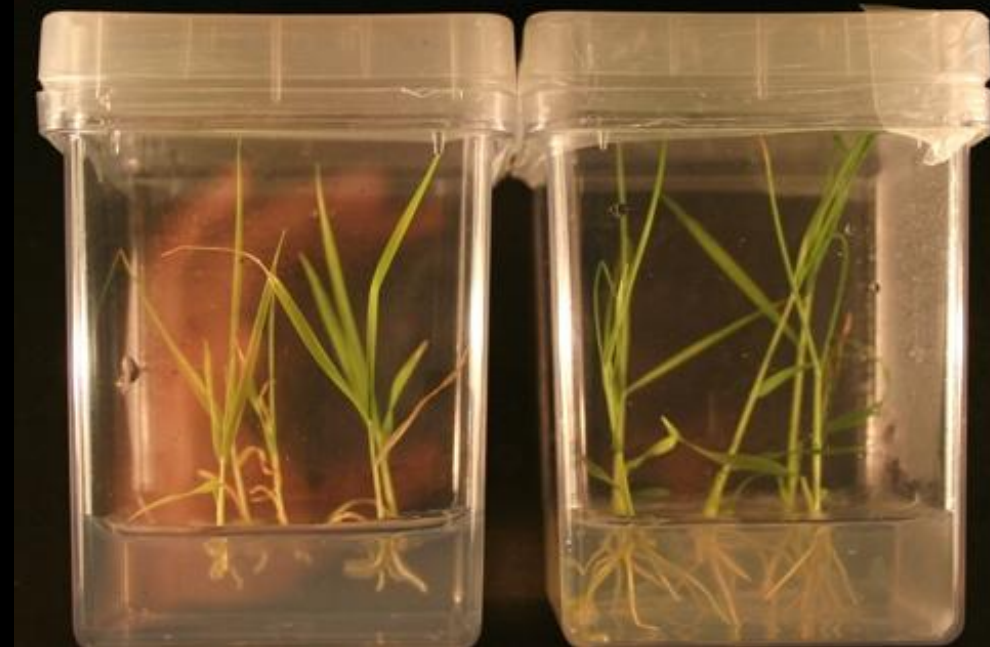
The images were taken three days after inoculation with PsJN-GFP (Appalachian State University)

Growth Promotion by PsJN



Control

PsJN

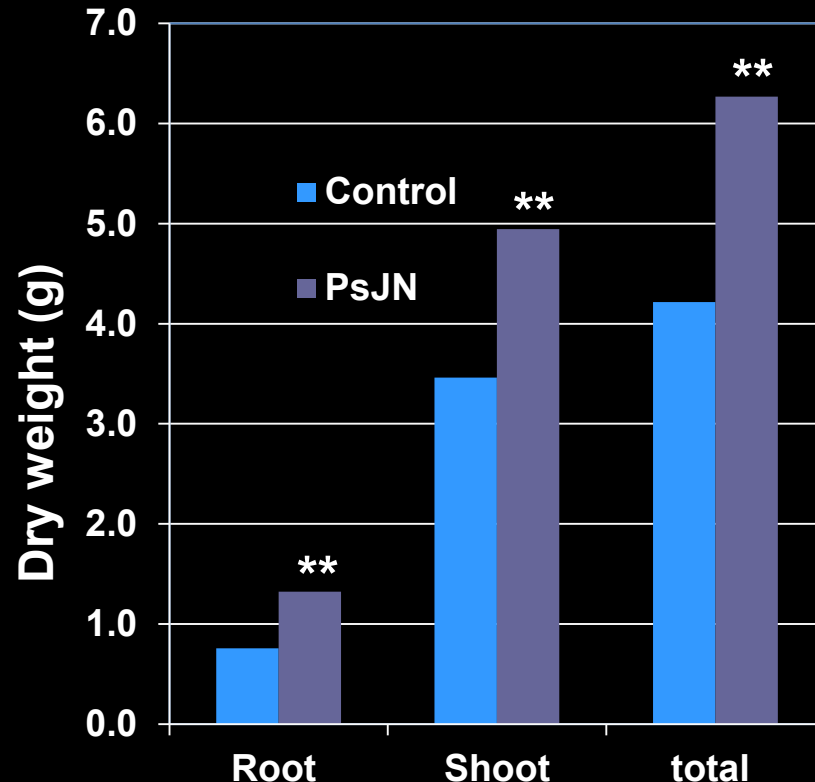
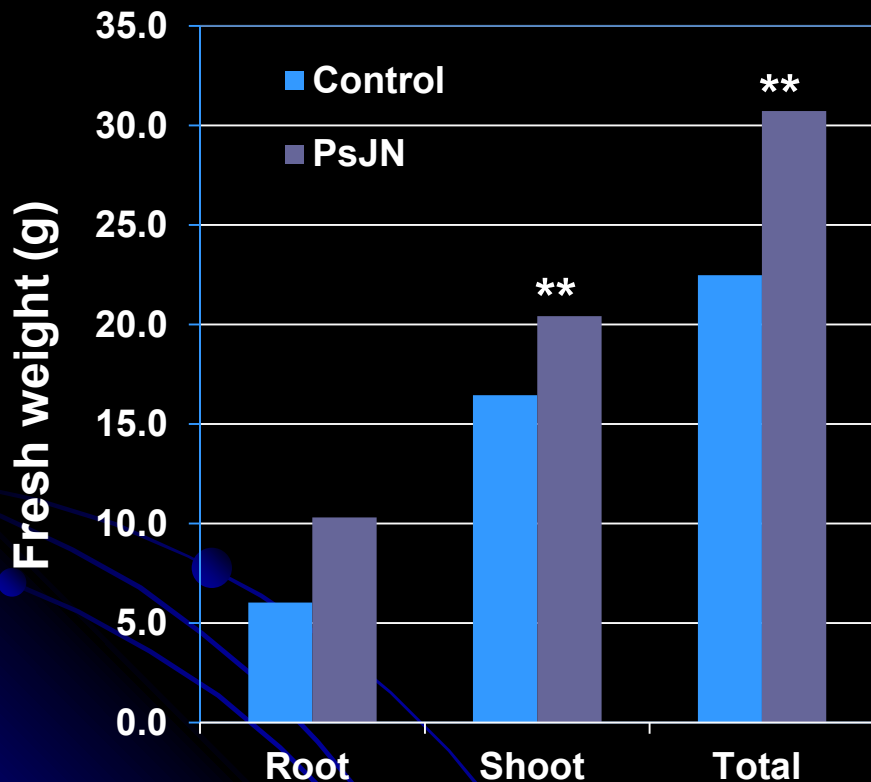


Control

PsJN

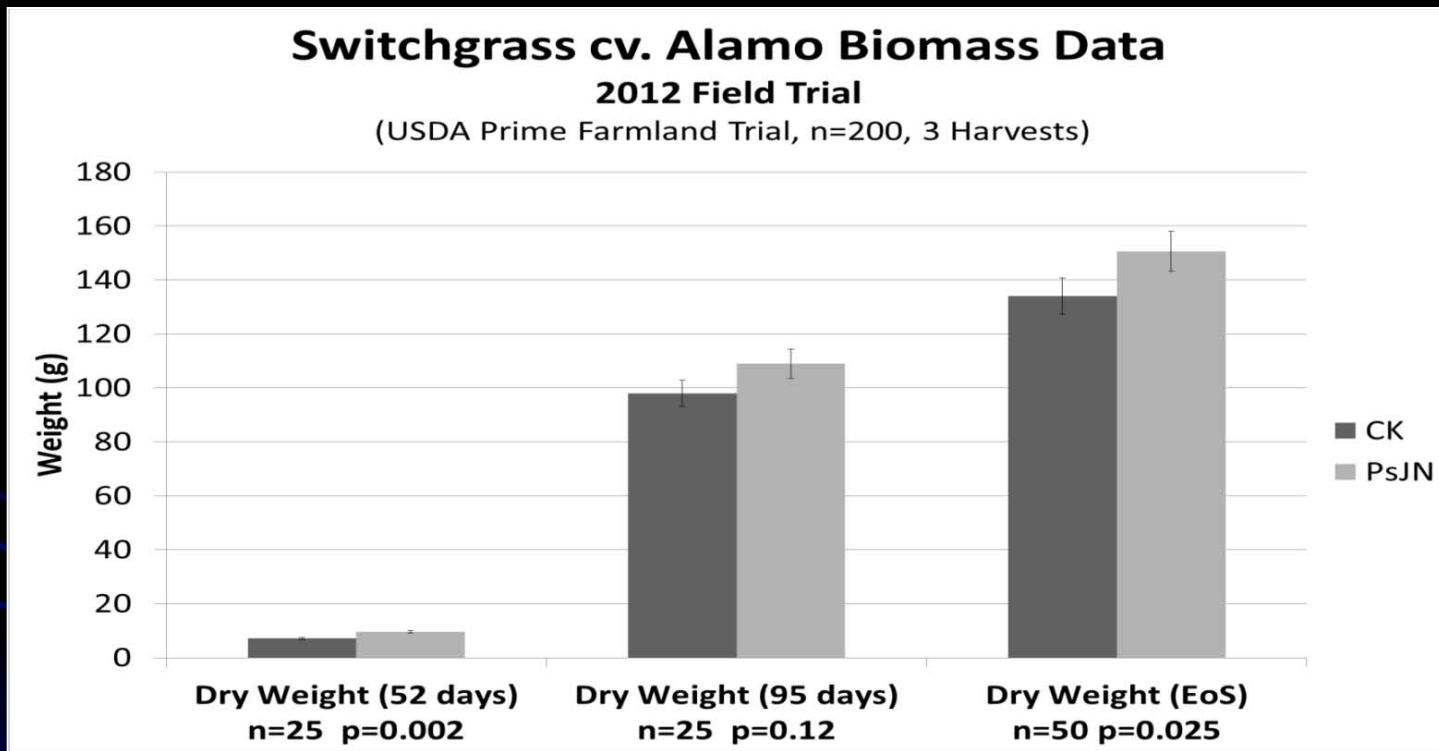
Pictures were taken one month after Alamo inoculated with PsJN.

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.

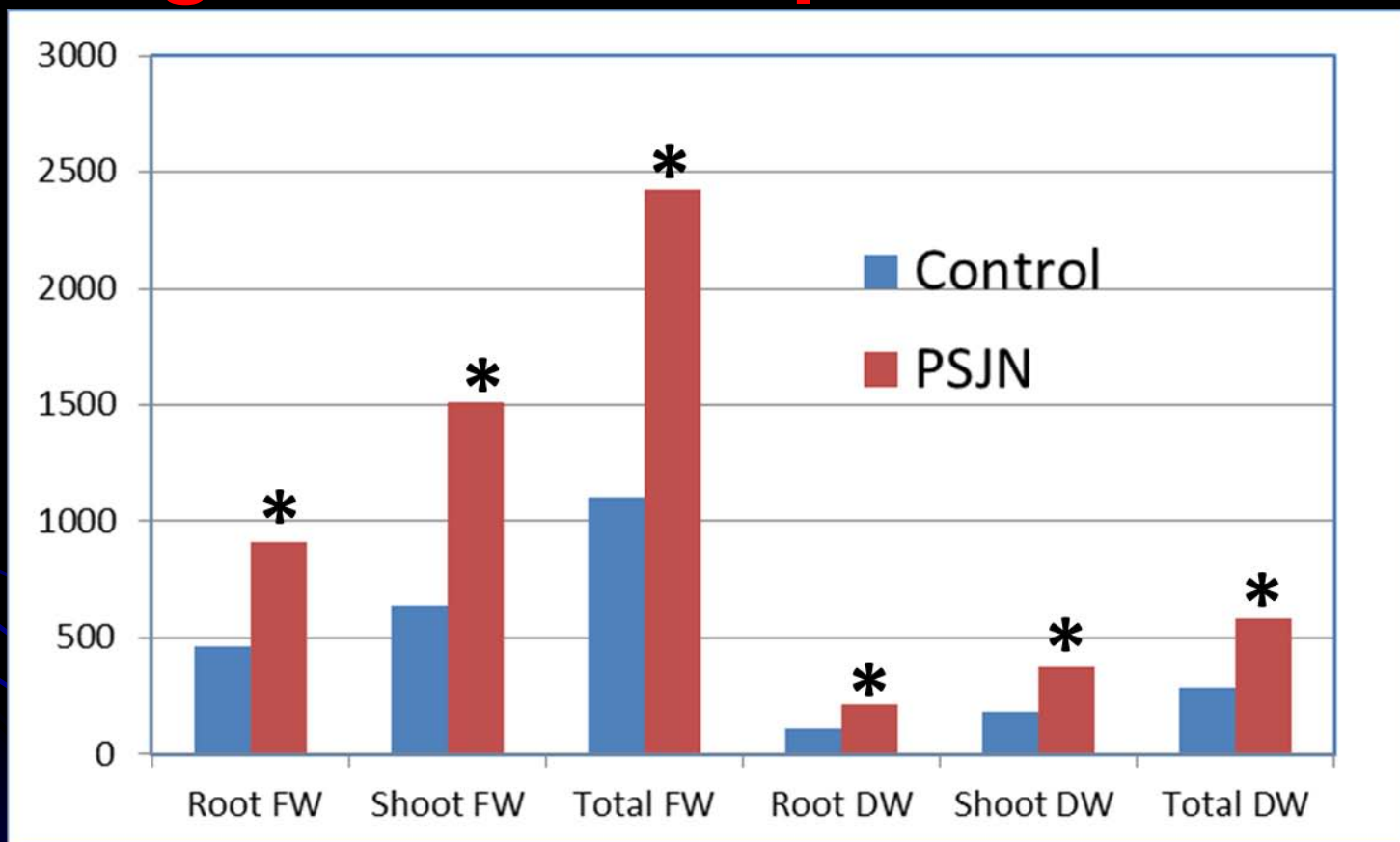
Field Experiment



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).

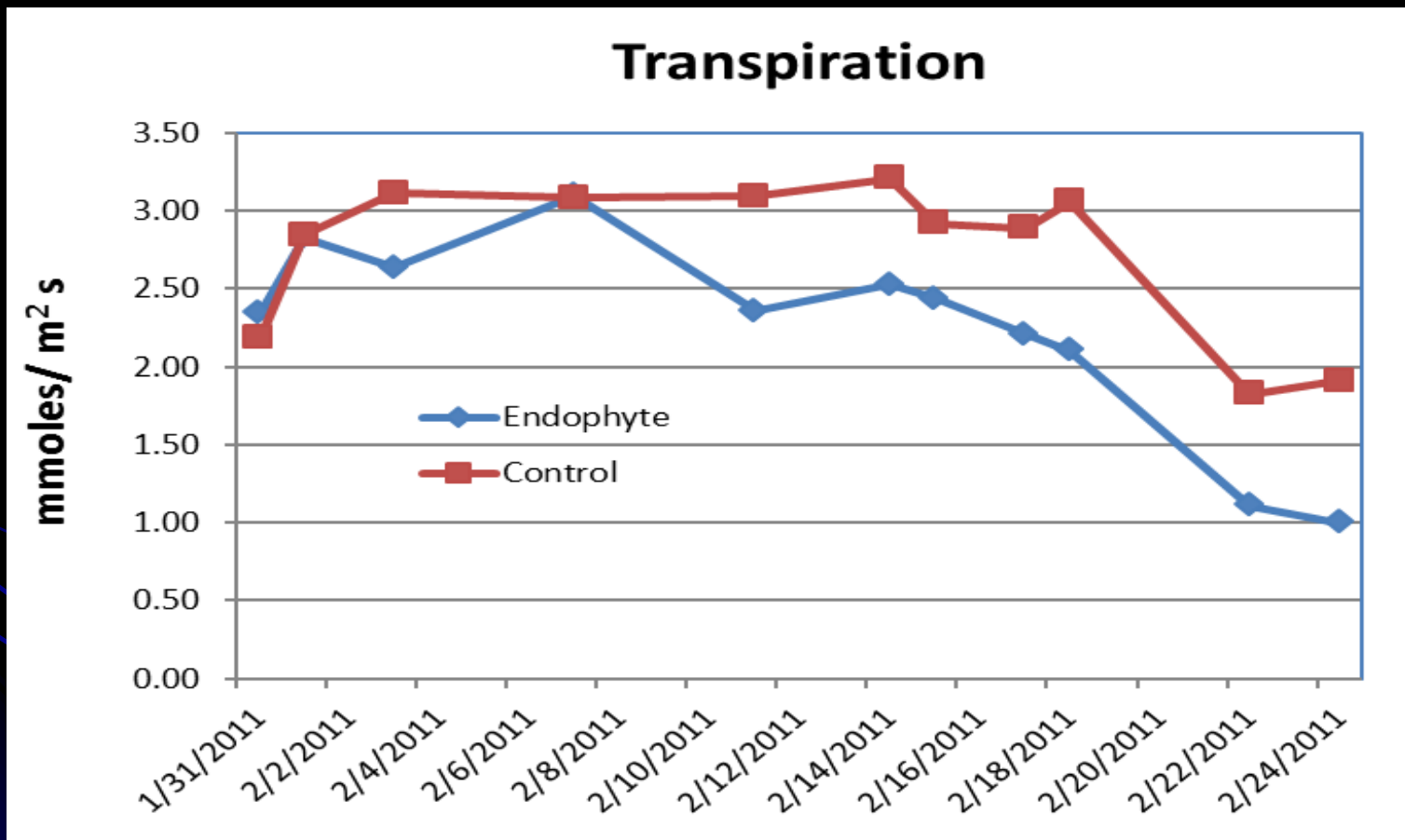
Plants grown in suboptimal conditions

Weight (mg/plant)



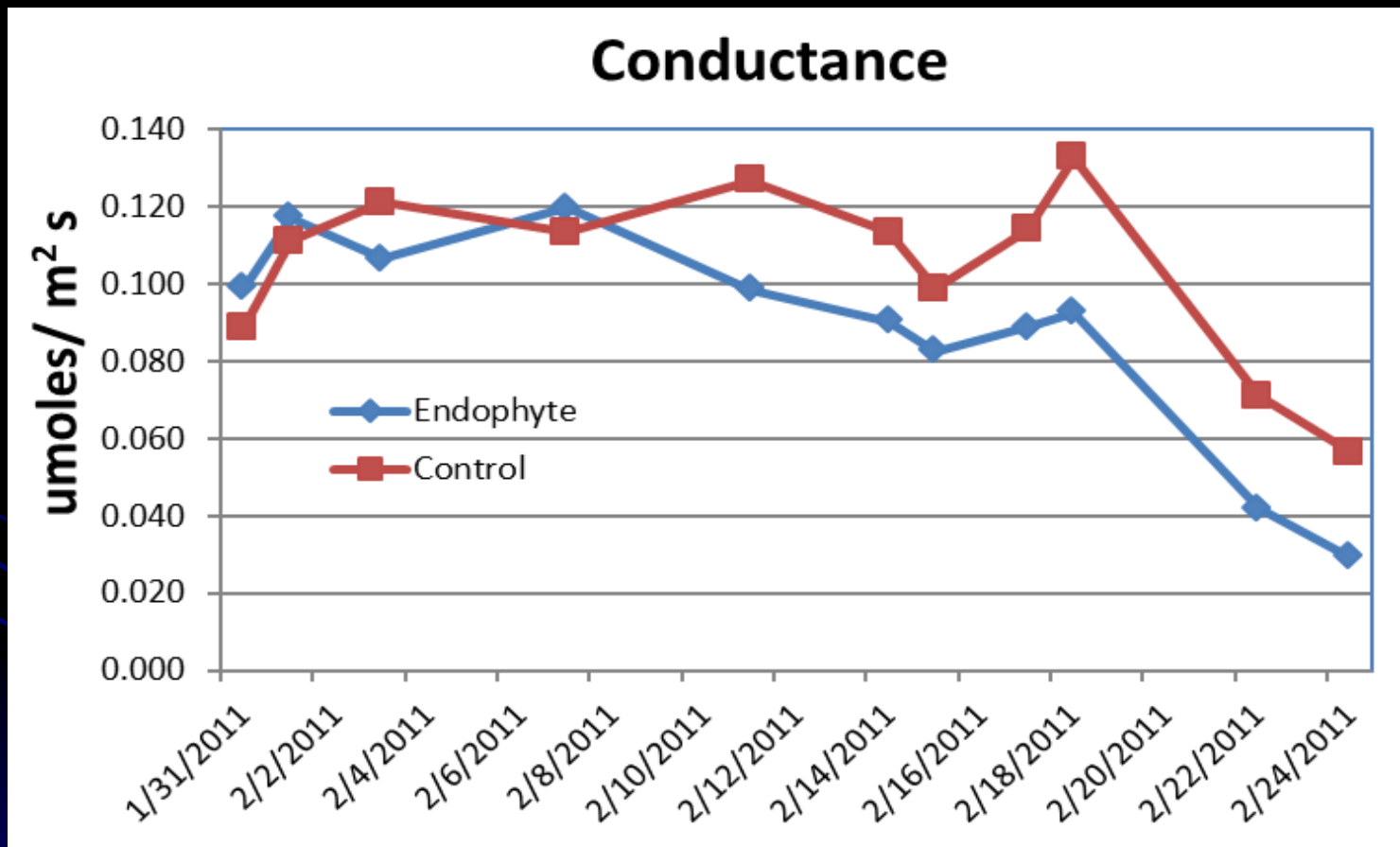
Plants were grown in field soil without fertilization in a greenhouse for 2.5 months in Fall 2010 under suboptimal temperature. * Indicates a p-value of less than 0.005.

Plant Physiology Parameters



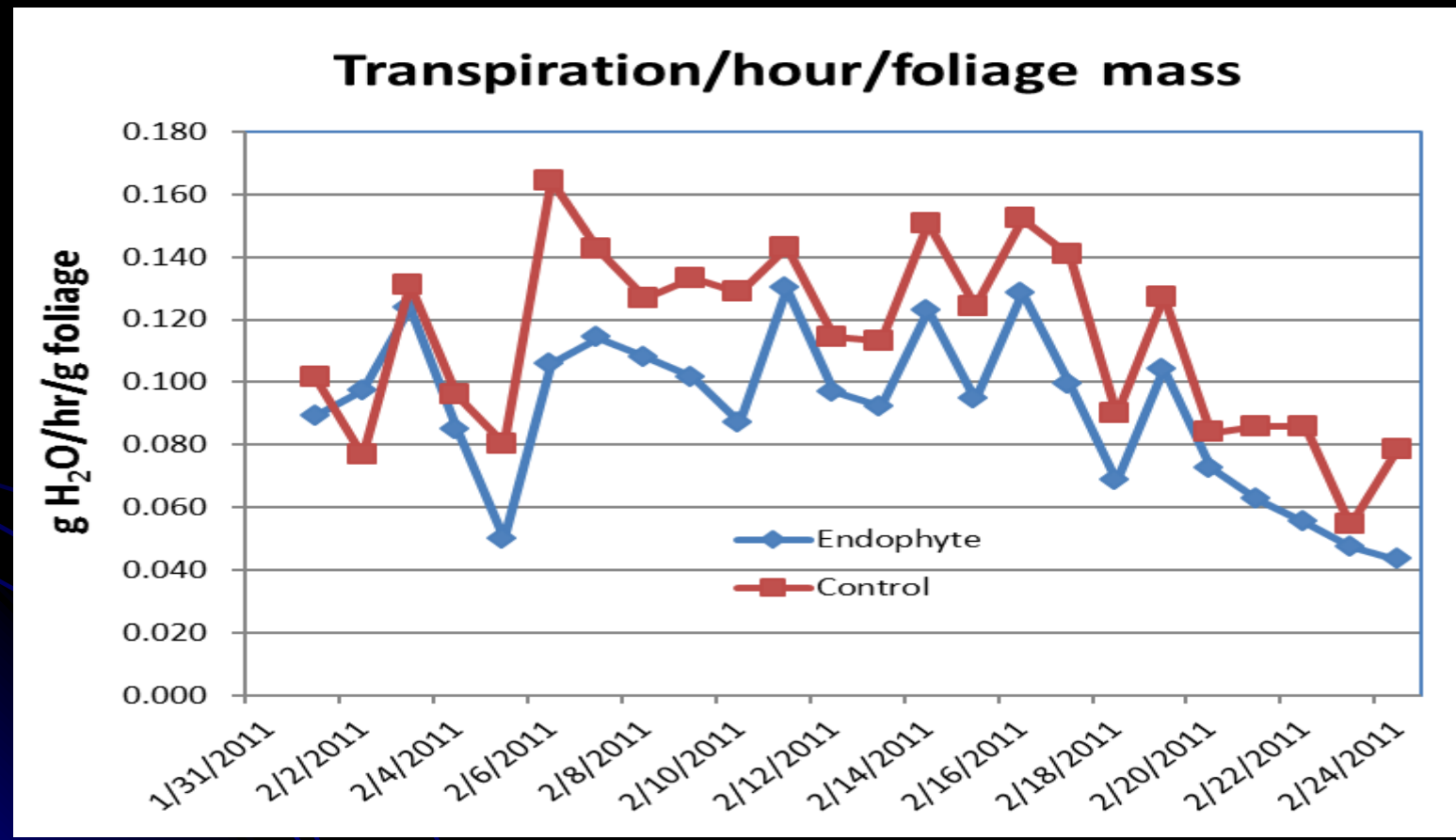
Comparison of transpiration of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



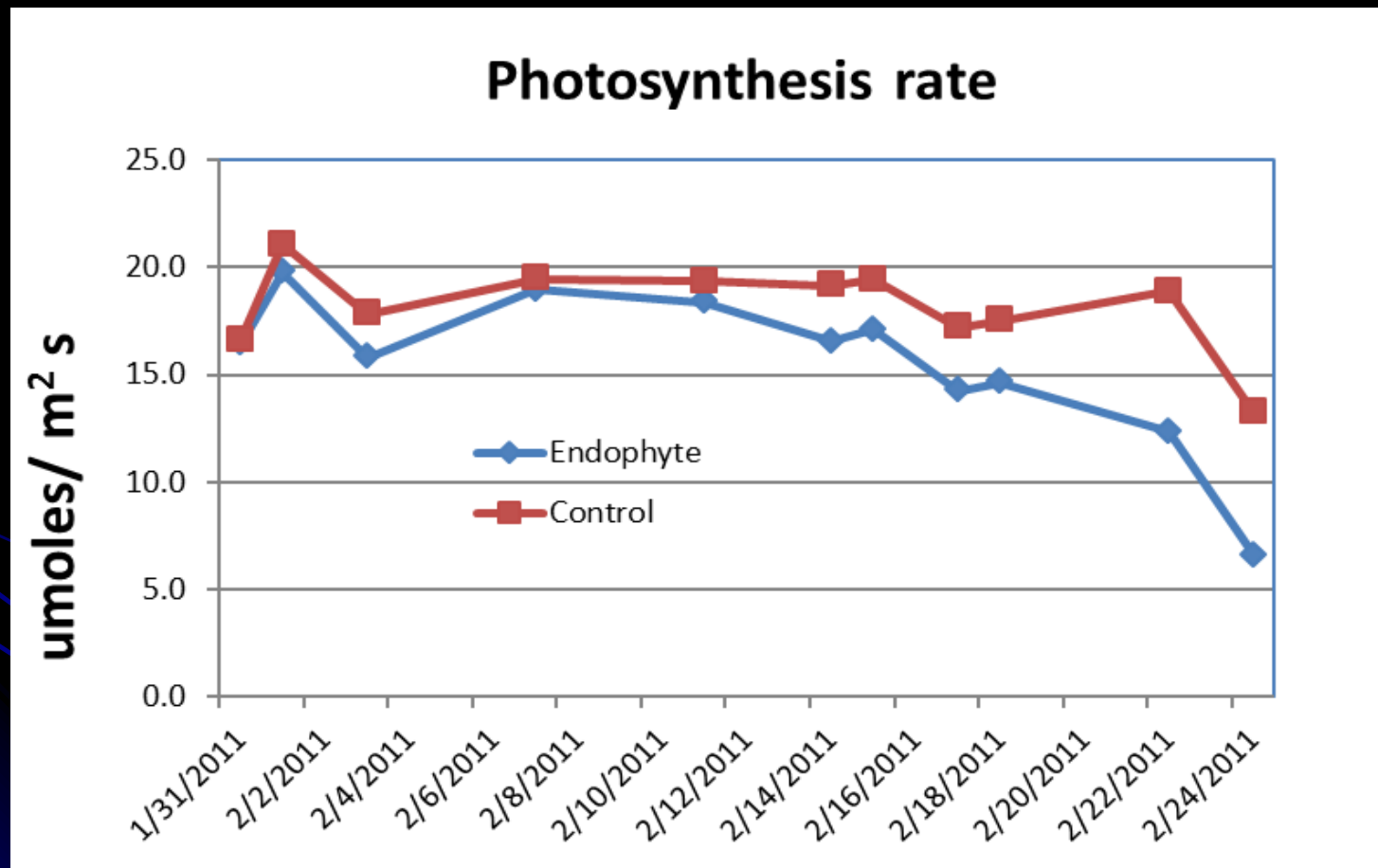
Comparison of conductance of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



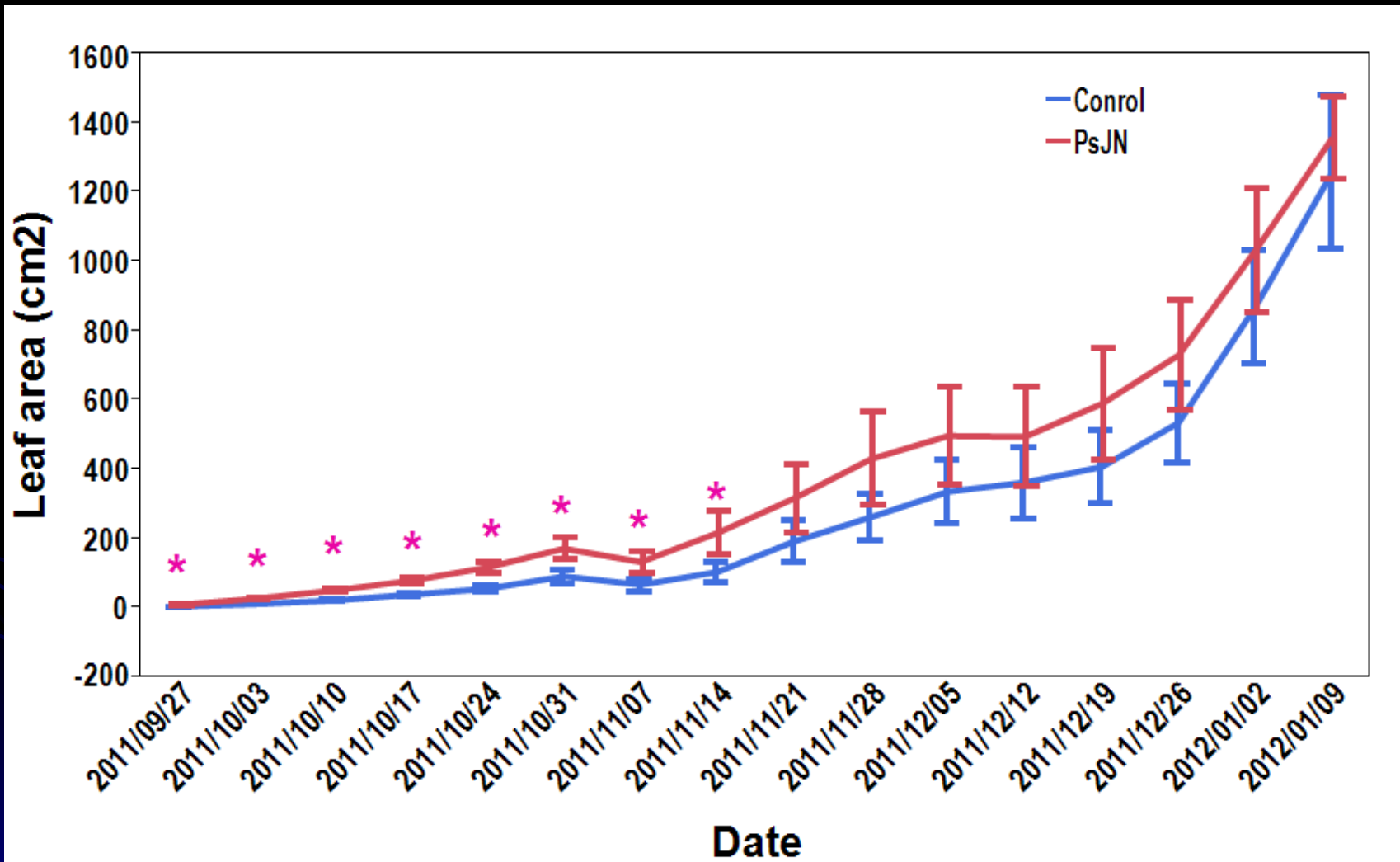
Comparison of water use efficiency of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



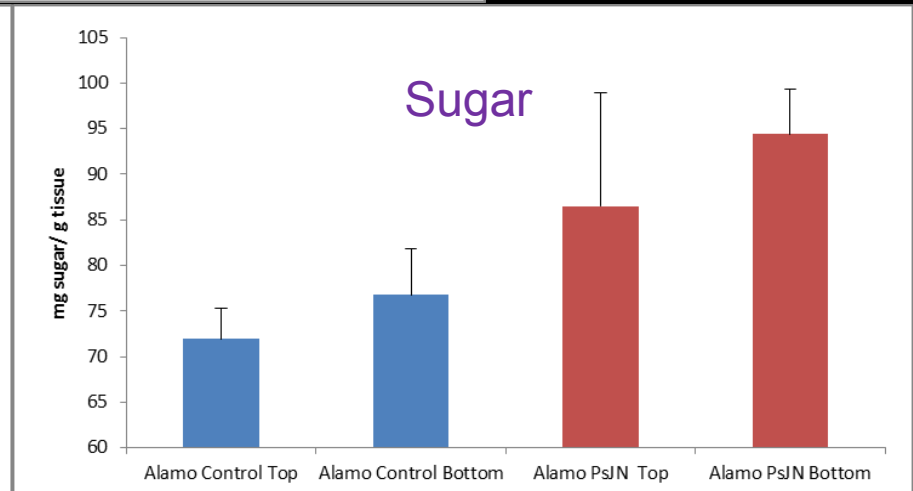
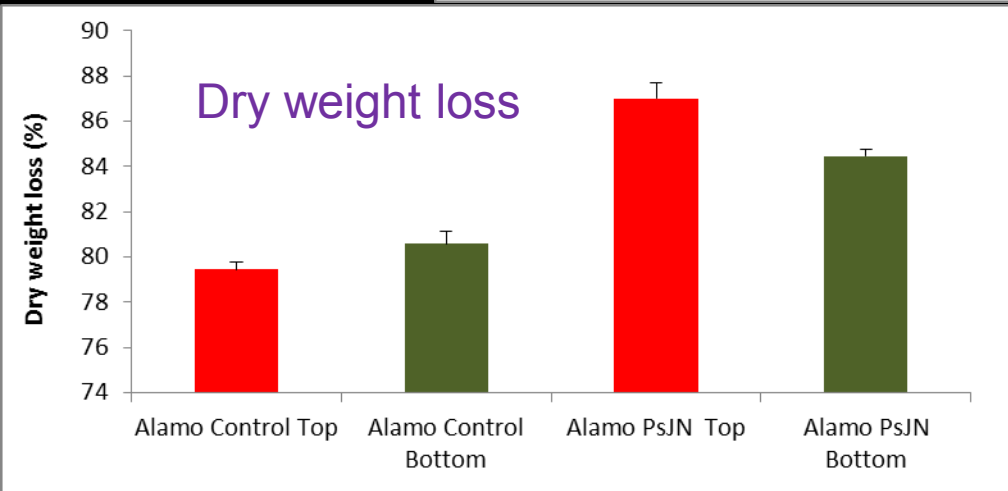
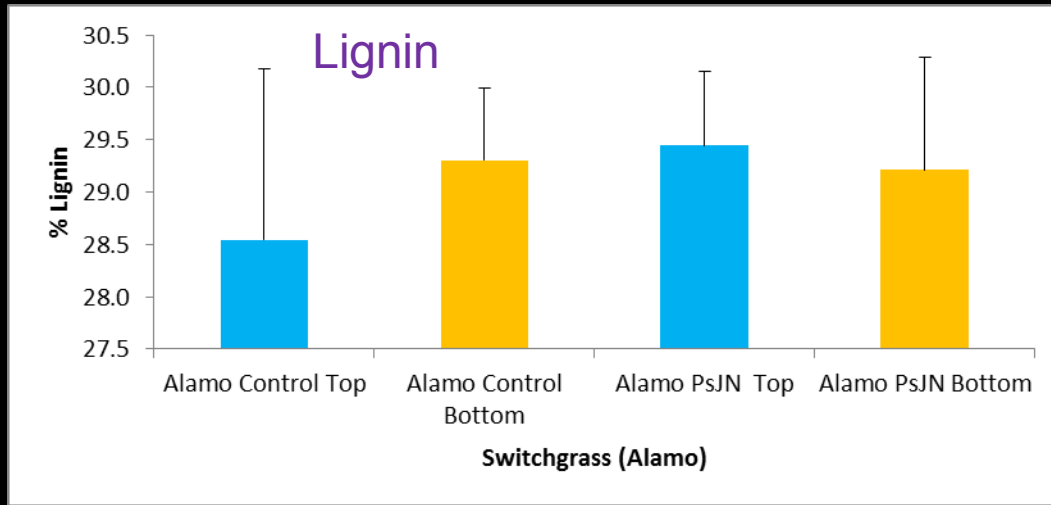
Comparison of photosynthesis rate of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Leaf area



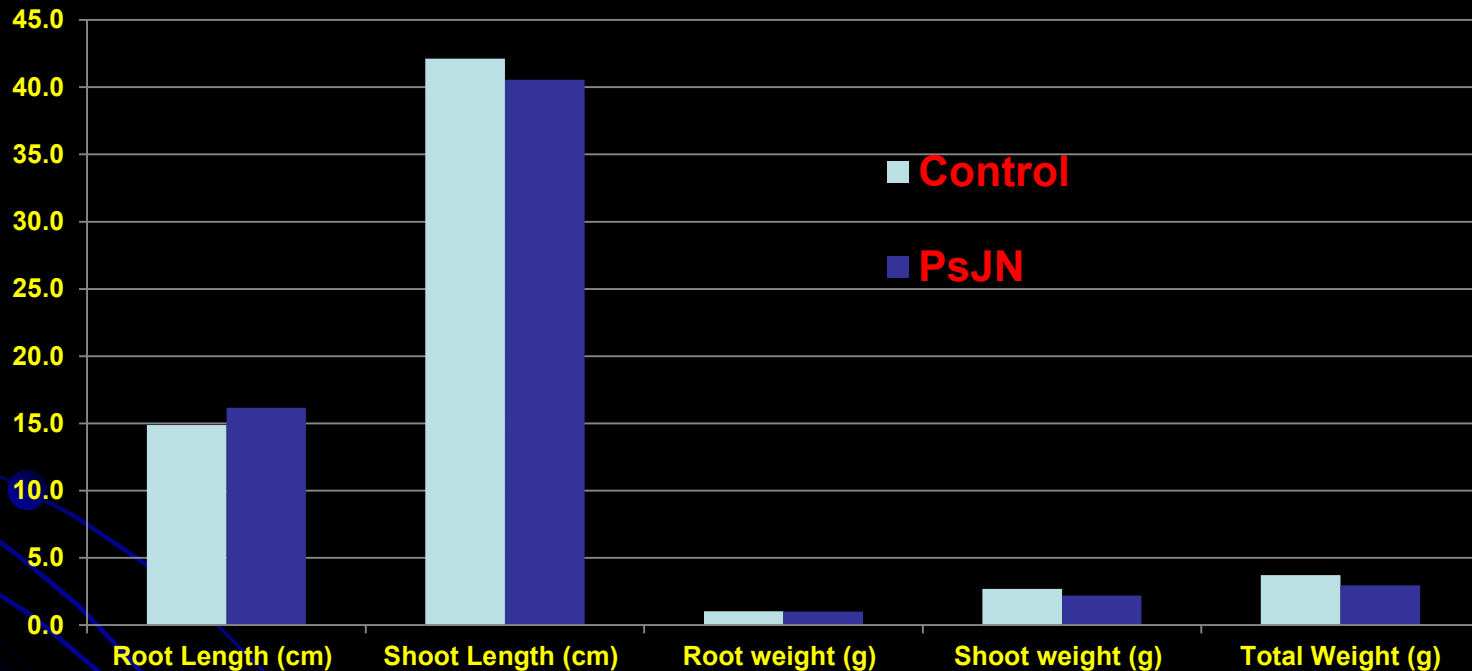
Leaf area was recorded every week from 09/27/2011 to 01/09/2012 with Photoshop CS5 (Adobe Systems Inc.).

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

31 samplesRMA -update_June14Sample - Microsoft Excel

Home Insert Page Layout Formulas Data Review View

Normal Page Layout Full Screen Workbook Views

Ruler Formula Bar Gridlines Headings Message Bar Show/Hide

Zoom 100% Zoom to Selection

New Window Split Arrange All Hide Freeze Panes Unhide Window

Save Switch Workspace Windows

Macros

E36

	A	B	C	D	E	F	EU	EV	EW	EX	EY	EZ	FA	FB	FC	FD	FE	FF	FG	GH
	Probe Set ID	Probe Set ID	PviUT sequence	CDD annotat	Panther anno	Arabidopsi: Rice	SD AIM_8 D	Ave CR_8 D	SD CR_8 D	Pts	Pts	CR_8 D/ALM_8D	AIM_8D:CR_8	Ave AIM_0 D	Ave AIM_0 5D	Ave ALM_2D	Ave ALM_4D	Ave ALM_8D		
1																				
2																				
3																				
4	AlamoCTG10276	AlamoCTG10276_x_at					2.9408	82.901	25.15	0.0095	0	5.6461	1	161.66	14.84	15.50	16.83	14.68		
5	AlamoCTG12624	AlamoCTG12624	AlamCTG12624			###	5.6934	375.74	37.239	0.0001	0	6.5275	1	116.24	35.08	36.33	30.37	57.56		
6	AP13CTG07854	AP13CTG07854	AP13CTG07854	pfam01214/Cas	FAMILY NOT N	AT2G44680/C	230.22	8.1624	0.7962	0.001	0	0.0071	2	2656.51	1193.22	1212.68	1168.28	1152.51		
7	AP13CTG10826	AP13CTG10826	AP13CTG10826		OS05G0506000		34.843	83.569	5.9558	0.0001	0	0.2202	2	71.41	163.40	230.07	318.66	379.55		
8	AP13CTG14907	AP13CTG14907	AP13CTG14907	pfam00083/Sug	SUBFAMILY NI		212.05	187.6	10.167	0.0011	0	2.8568	1	1519.84	586.83	463.63	294.34	555.74		
9	AP13CTG30810	AP13CTG30810	AP13CTG30810	PLN02160/thio	FAMILY NOT N		356.87	41.668	11.056	0.0046	0	0.034	2	3329.85	1210.87	570.98	471.46	1224.17		
10	AP13ITG7120	AP13ITG7120	AP13ITG7120	pfam03018/Diric	DISEASE RESI		631.13	676.13	127.38	0.0077	3E-138	0.2686	2	574.00	1457.16	2313.48	2503.42	2517.12		
11	AP13ITG52388	AP13ITG52388	AP13ITG52388			###	18.091	290	3.939	2E-05	0	11.886	1	133.78	46.57	50.14	38.55	24.40		
12	AP13ITG53099	AP13ITG53099	AP13ITG53099	pfam02956/TT			109.42	756.78	147.73	0.0233	2E-09	2.0059	1	818.87	262.97	229.88	188.30	377.27		
13	AP13ITG57727	AP13ITG57727	AP13ITG57727			###	205.29	49.464	14.522	0.044	0	0.1254	2	1092.79	283.42	209.16	278.26	394.32		
14	AP13ITG58847	AP13ITG58847	AP13ITG58847	COG0484/Dna	SUBFAMILY NI		62.843	98.093	8.4063	0.0086	2E-288	0.3577	2	708.95	161.99	241.30	269.14	274.26		
15	AP13ITG61224	AP13ITG61224	AP13ITG61224	PLN03023/expa			73.791	71.005	25.05	0.0027	2E-93	0.1932	2	68.80	298.90	709.08	690.08	367.56		
16	AP13ITG73638	AP13ITG73638	AP13ITG73638		SUBFAMILY NI	AT1G75280/P	716.79	1017.1	30.675	0.0014	0	0.2377	2	845.94	2781.09	5466.63	7858.32	4279.23		
17	AP13ITG73736	AP13ITG73736	AP13ITG73736	pfam02956/TT			21.565	28.369	8.9911	0.001	0	0.1954	2	331.15	163.47	98.79	91.58	145.19		
18	KanlowCTG12612	KanlowCTG12612	KanlowCTG12612	PLN02892/soc	ISOCITRATE L	AT3G21720/G	29.732	480.65	182.61	0.0166	3E-134	8.3768	1	724.03	201.28	100.44	24.11	57.38		
19	KanlowCTG13620	KanlowCTG13620	KanlowCTG13620	PLN02892/soc	ISOCITRATE L	AT3G21720/G	43.082	258.7	42.645	0.0113	3E-10	2.5065	1	511.26	164.36	68.75	40.27	103.21		
20	KanlowCTG18939	KanlowCTG18939	KanlowCTG18939	pfam00891/O-m	FAMILY NOT N	AT5G54160/F	70.673	1139.2	180.43	0.0007	0	15.174	1	17.96	42.88	231.47	67.24	75.08		
21	KanlowCTG34558	KanlowCTG34558	KanlowCTG34558			###	235.14	2369.2	658.49	0.0123	4E-38	3.8363	1	2836.15	190.93	379.23	485.40	617.57		
22	KanlowCTG37633	KanlowCTG37633	KanlowCTG37633			###	144.66	986.55	76.33	0.0029	0	2.6556	1	1145.57	555.14	411.57	353.38	371.50		
23	KanlowCTG40578	KanlowCTG40578	KanlowCTG40578		SUBFAMILY NI		0.3746	105.5	12.945	0.0003	0	5.9142	1	86.15	33.18	30.28	27.15	17.84		
24	VS16ITG01008	VS16ITG01008	VS16ITG01008	cd01910/This do	FAMILY NOT N		603.39	491.6	166.96	0.0094	5E-69	0.225	2	5976.67	1035.43	1043.59	1216.23	2184.56		
25	AlamoCTG03112	AlamoCTG03112	AlamoCTG03112		SUBFAMILY NI	AT5G06580/F	131.19	1355.6	91.487	0.0004	0	3.6812	1	1094.16	505.14	317.65	245.59	368.24		
26	AlamoCTG14573	AlamoCTG14573	AlamoCTG14573			###	19.455	200.04	44.000	0.0007	0	0.2007	1	123.00	55.10	44.20	34.00	34.00		
27	VS16ITG26521	VS16ITG26521	VS16ITG26521			###	74	340.81	28.331	0.3502	0.0031	0.8758	0	256.40	499.46	407.18	444.02	389.15		
28	VS16ITG26801	VS16ITG26801	VS16ITG26801			###	0.2325	14.592	9.3207	0.2683	0	1.9007	0	7.04	7.54	7.46	7.48	7.68		
29	VS16ITG26971	VS16ITG26971	VS16ITG26971			###	24.07	13.451	2.5556	0.1678	4E-57	0.3639	0	19.22	18.47	22.29	50.39	36.96		
30	VS16ITG27139	VS16ITG27139	VS16ITG27139			###	4.2936	120.62	7.5374	0.004	4E-33	1.3268	0	87.63	50.60	69.96	94.51	90.91		
31	VS16ITG27178	VS16ITG27178	VS16ITG27178		OS11G0524800		5.5347	6.4299	0.8504	0.0221	2E-126	0.3538	2	8.25	6.16	13.03	15.42	18.17		
32	VS16ITG27721	VS16ITG27721	VS16ITG27721			###	3.7526	10.848	1.7019	0.0402	4E-13	0.6036	0	17.21	13.58	22.67	23.39	17.97		
33	VS16ITG27876	VS16ITG27876	VS16ITG27876			###	5.0219	11.102	1.4235	0.0118	3E-58	0.4564	2	17.77	13.77	19.40	28.20	24.32		
34	VS16ITG27891	VS16ITG27891	VS16ITG27891			###	14.412	8.2813	0.9407	0.2051	4E-119	0.3965	0	11.19	8.90	13.01	25.79	20.89		
35	VS16ITG27979	VS16ITG27979	VS16ITG27979			###	18.325	7.0965	1.2222	0.1903	7E-124	0.2982	2	8.09	6.99	8.80	19.44	23.80		
36	VS16ITG28003	VS16ITG28003	VS16ITG28003			###	12.235	11.749	2.0001	0.1145	1E-35	0.4493	0	22.37	18.28	17.86	46.80	26.15		
37	VS16ITG28088	VS16ITG28088	VS16ITG28088			###	8.3246	39.286	12.012	0.2413	0.0158	1.4188	0	20.14	19.09	25.15	17.06	27.69		
38	VS16ITG28328	VS16ITG28328	VS16ITG28328			###	7.3519	14.248	1.7803	0.0222	1E-53	0.4734	2	20.91	17.37	20.95	18.70	30.09		
39	VS16ITG30113	VS16ITG30113	VS16ITG30113			###	0.8038	10.861	1.4895	0.0949	5E-06	1.2438	0	7.08	8.14	7.40	9.18	8.73		
40	VS16ITG30180	VS16ITG30180	VS16ITG30180			###	21.582	7.7726	0.6397	0.2529	0	0.3184	2	8.37	7.40	12.30	22.71	24.41		

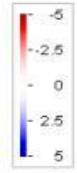
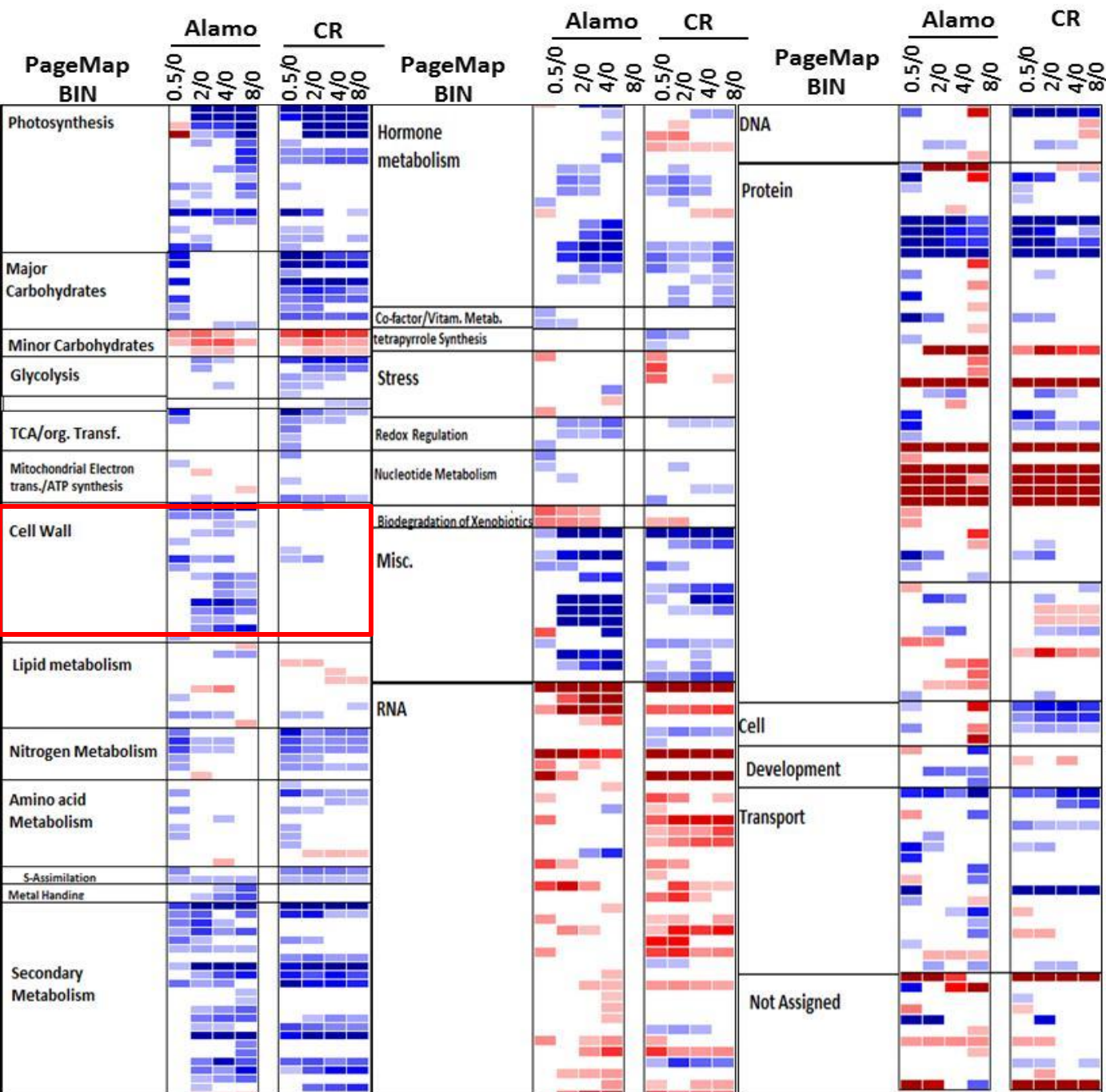
Sheet1 selections Up_5A Up_2A Up_4A Up_8A D1

Ready 60%

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

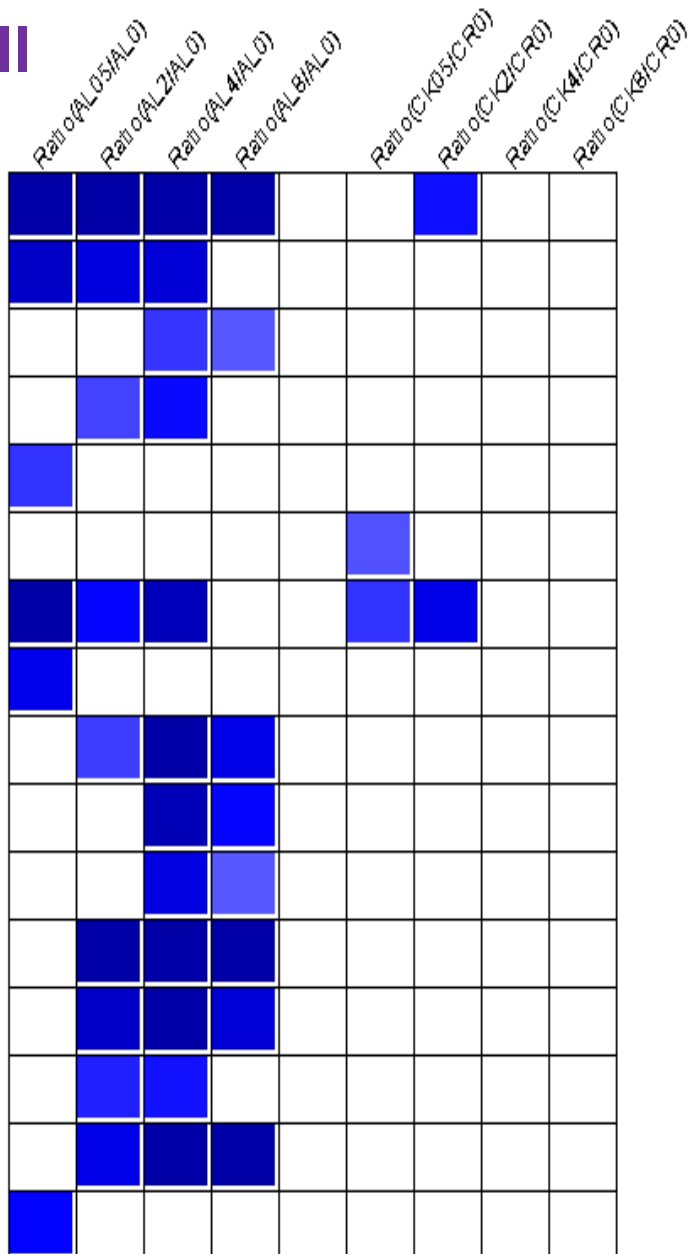
Expression level changes of transcription factor genes of interest in Alamo and Cave-in-Rock at 0.5, 2, 4 and 8 days following inoculation with PsJN, compared with expression level at 0 day, respectively.



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

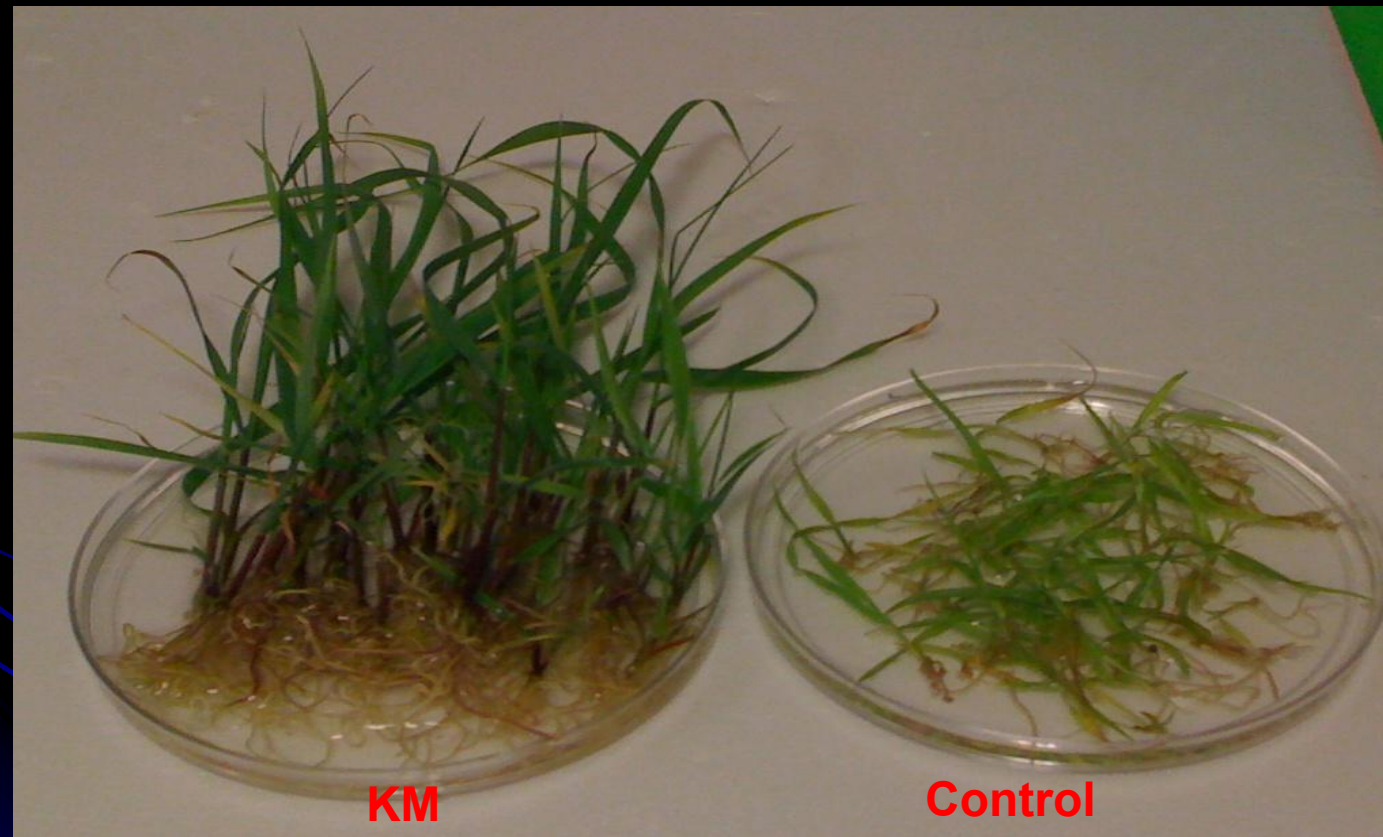
Cell wall

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



KM

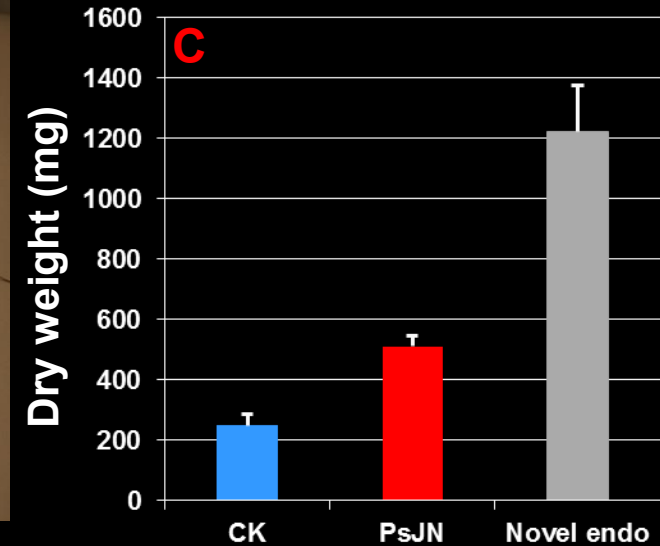
Control



CK

PsJN

KM



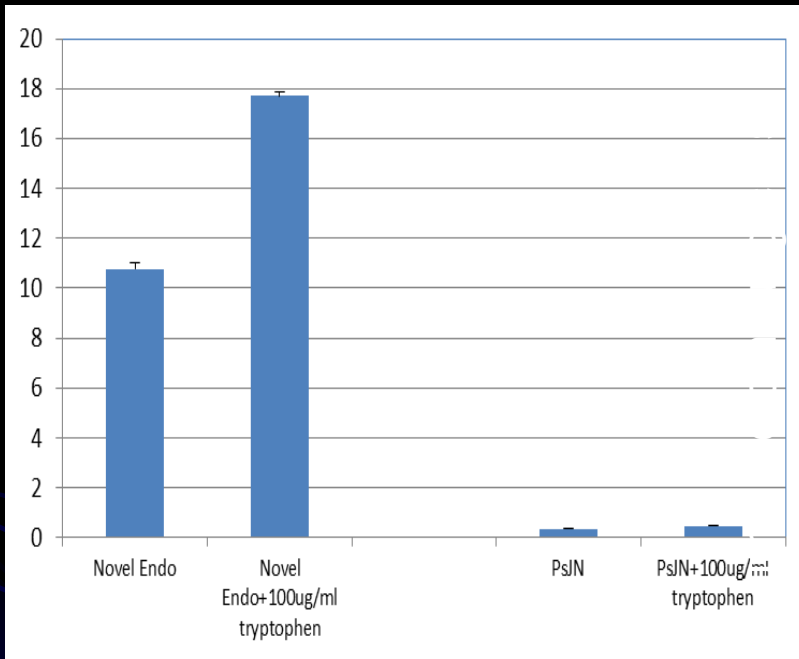
Comparison of KM with PsJN in growth promotion on Alamo under in vitro condition.

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

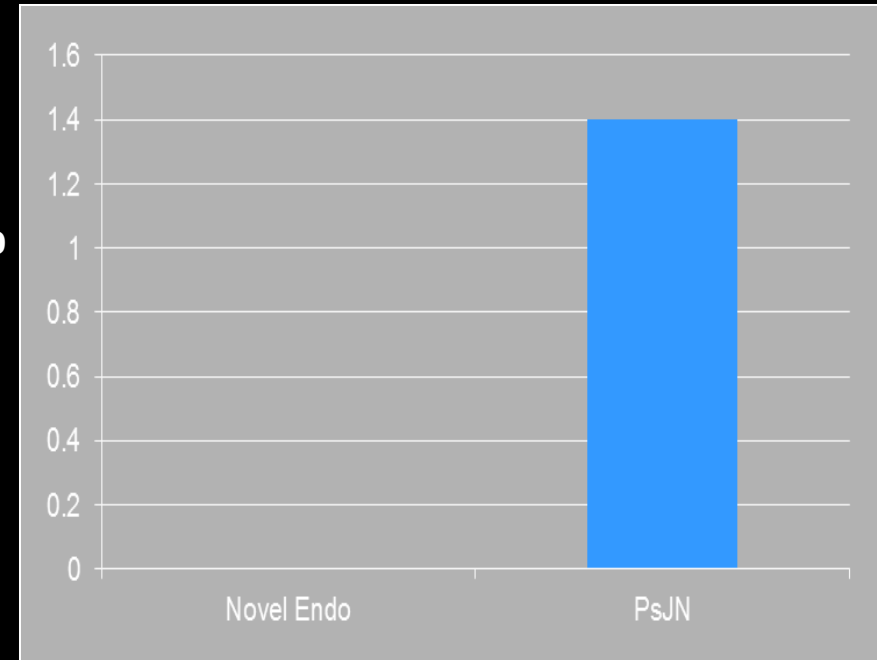
IAA and ACC Deaminase

IAA amount ($\mu\text{g}/\text{mg}$ bacterial cells)



IAA concentration

umol Keto/hour/g cells



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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