

Mechanisms of Switchgrass cv. Alamo Growth Promotion by the Beneficial Bacterial Endophyte *Burkholderia phytofirmans* Strain PsJN

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



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 land
- Requires much less fertilizers and pesticides
- Can be harvested for 10 years or more once established with no or little fertilizers
- Sustainable and renewable crop
- Estimated output/input ratio about 5 :1



Endophytes

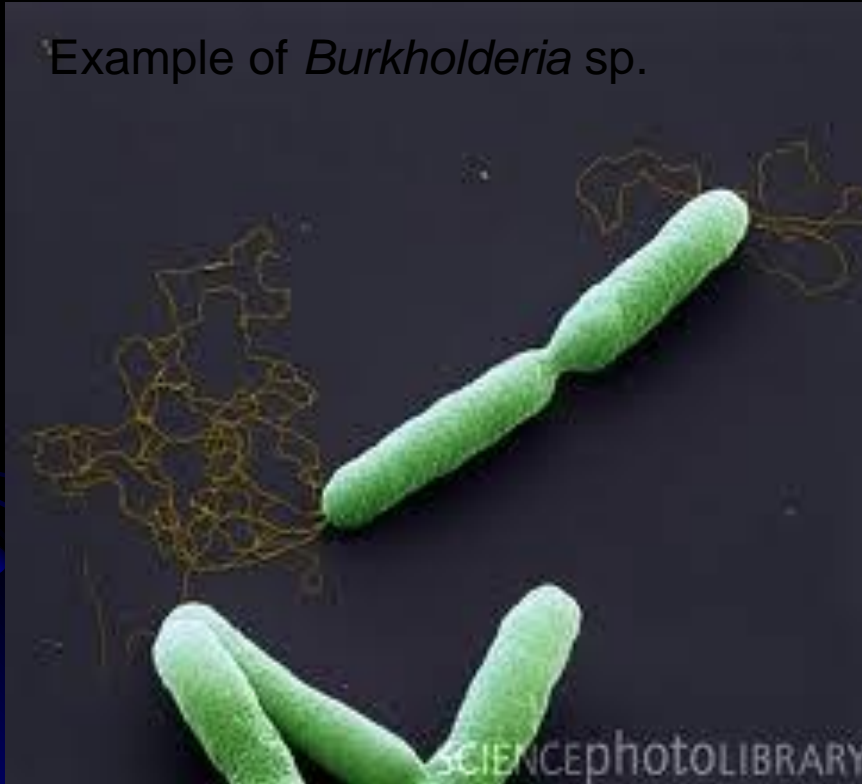
- An endophyte is an endosymbiont , often a bacterium or fungus, that lives within a plant for at least part of its life without causing apparent disease.
- Usually an endophyte benefits the plant host by
 - promoting plant growth, increasing nutrient acquisition, stress tolerance, and pathogen resistance.
- Also the endophyte is a natural existing microorganism.

Endophytes

- Endophytes have been reported in many species, such as poplar and sugar cane (Mei and Flinn, 2010).
- Fungal endophytes was reported on switchgrass (Ghimire et al. Bioenergy Research 2009 2:51-58).
- We are focusing on effects of bacterial endophytes on switchgrass growth.

Burkholderia phytofirmans strain PsJN

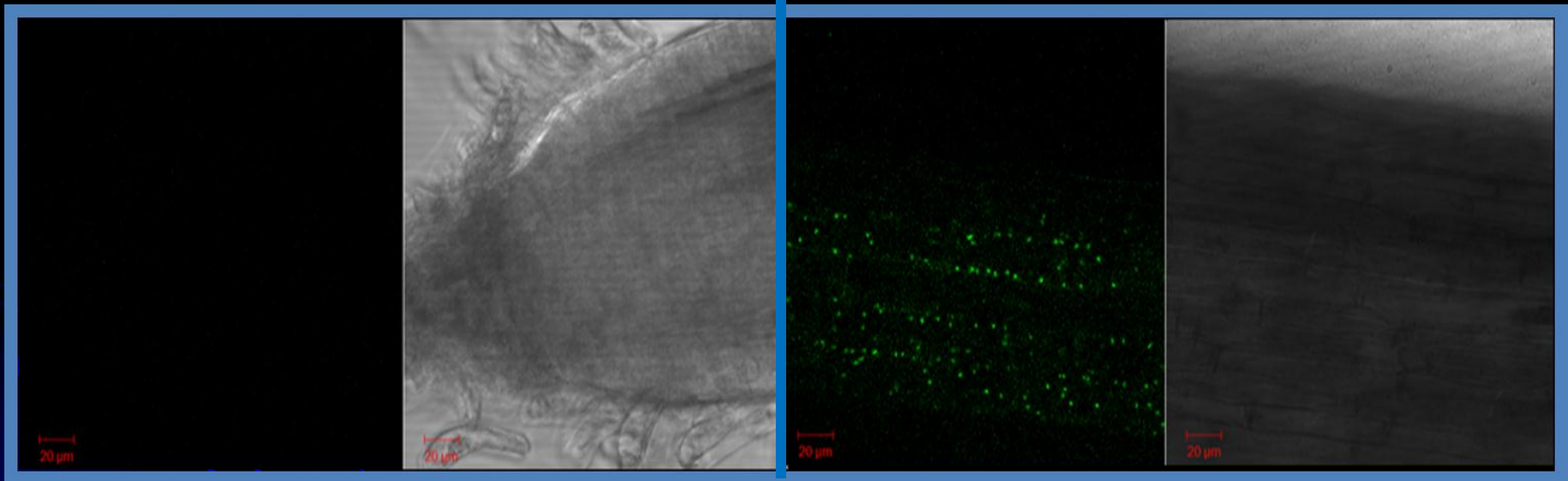
Example of *Burkholderia* sp.



- Gram negative beneficial bacterial endophyte isolated from onion roots in 1987 by Jerzy Nowak
- Plant growth promotion has been found in many species.
- Colonizes:
 - Rhizosphere
 - Root and above ground internal plant organs and tissues
- Complete genome has been sequenced*

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

Confocal Microscope



Non-infected Control

PsJN-GFP Inoculated

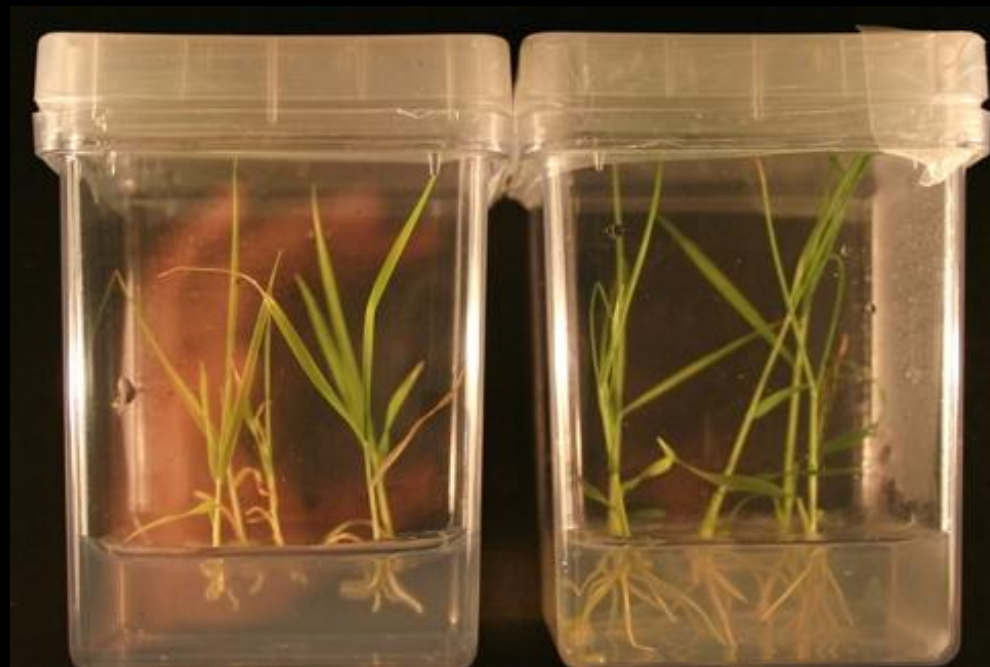
The images were taken three days after inoculation with PsJN-GFP (Appalachian State University)

Growth Promotion by PsJN



Control

PsJN

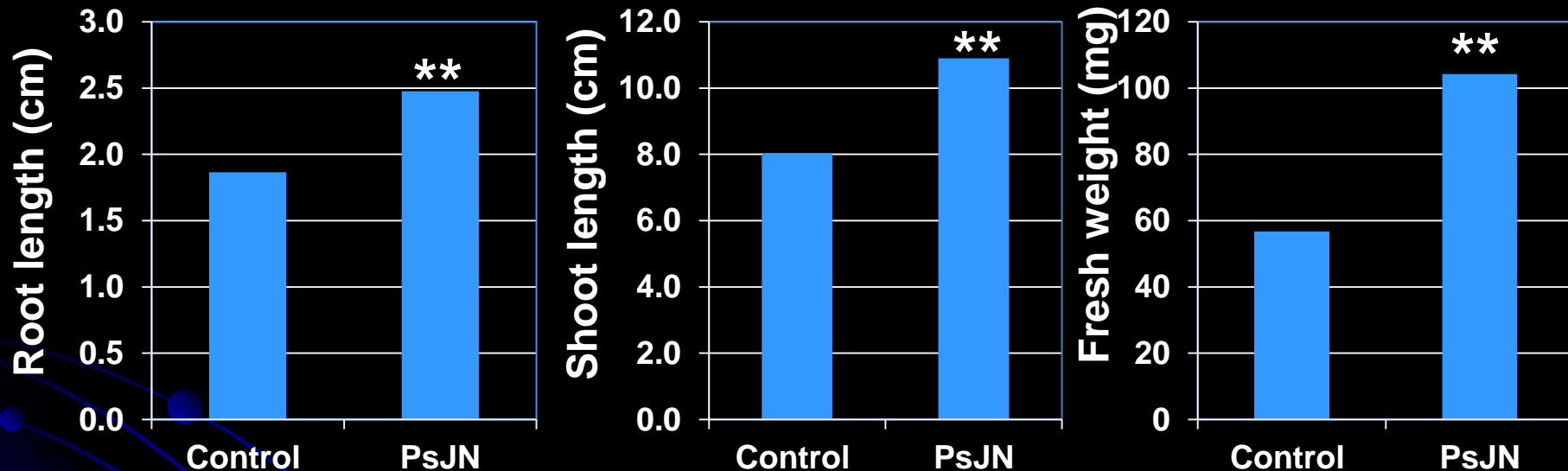


Control

PsJN

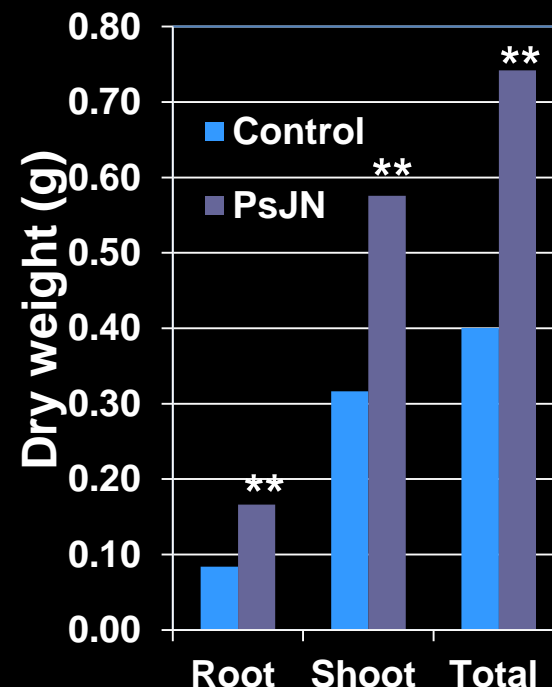
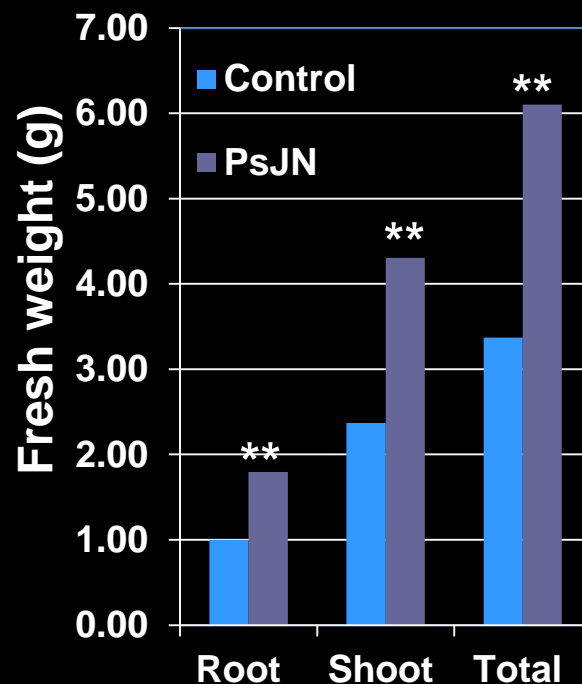
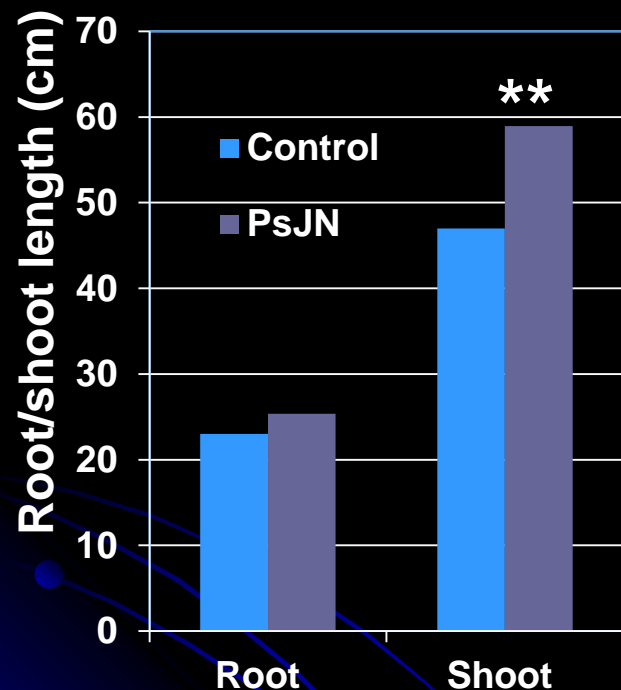
Pictures were taken one month after Alamo inoculated with PsJN.

In Vitro Experiment



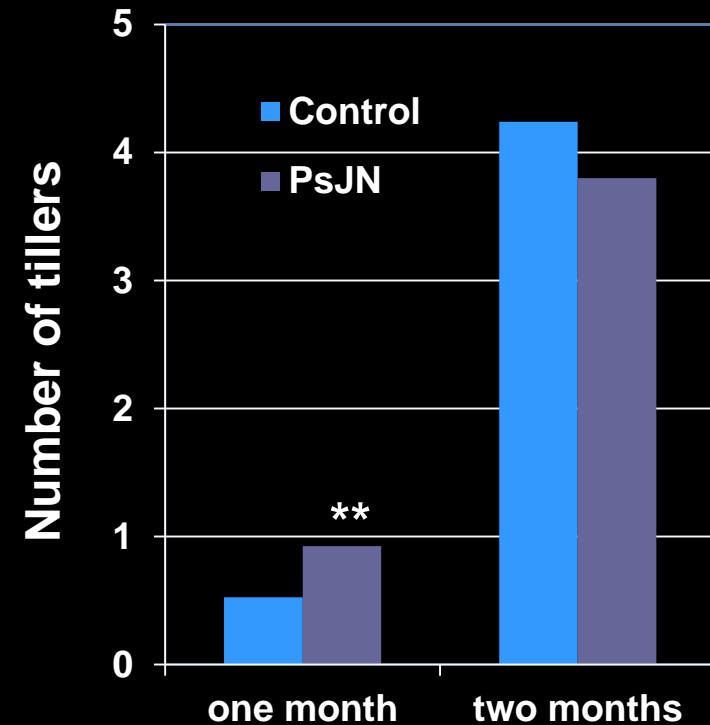
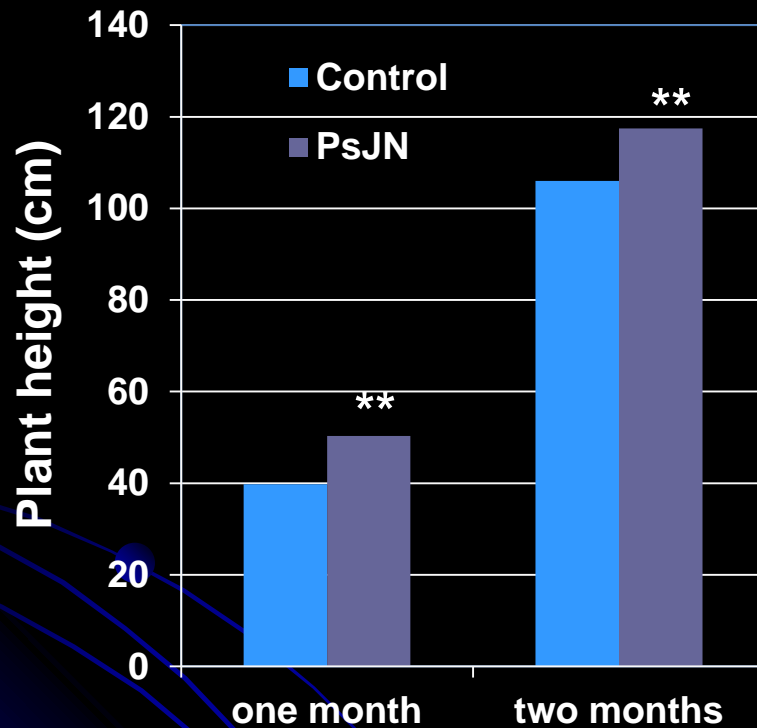
Data were from control and endophyte-inoculated plants grown in vitro for one month. Sample number was 36 for control and PsJN plants. All data from endophyte-inoculated plants were very significantly difference from control plants. Total fresh weight in endophyte-inoculated plants was **85%** higher than that of control plants.

Growth Chamber Experiment



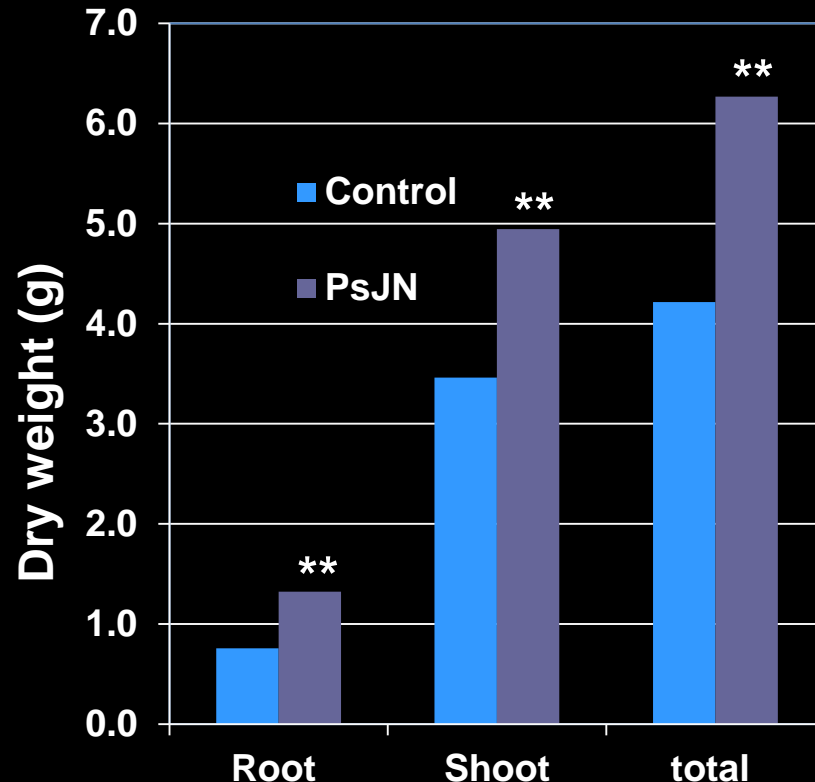
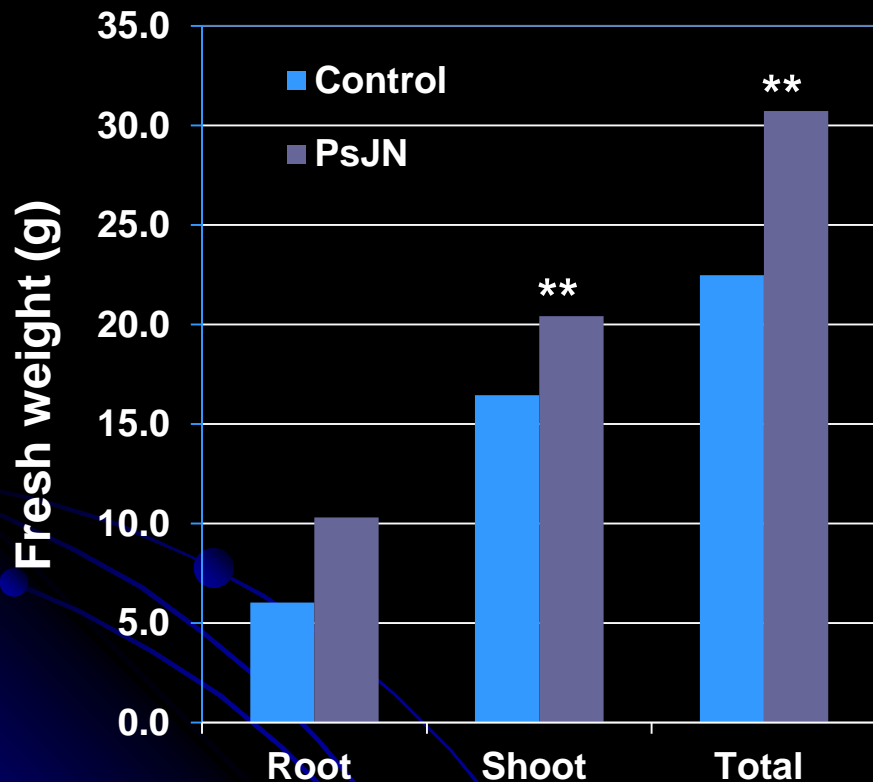
Effects of endophyte PsJN inoculation on switchgrass cv. Alamo growth in growth chamber. ** means significant difference at 0.01 level between PsJN and control using student T-test.

Greenhouse Experiment



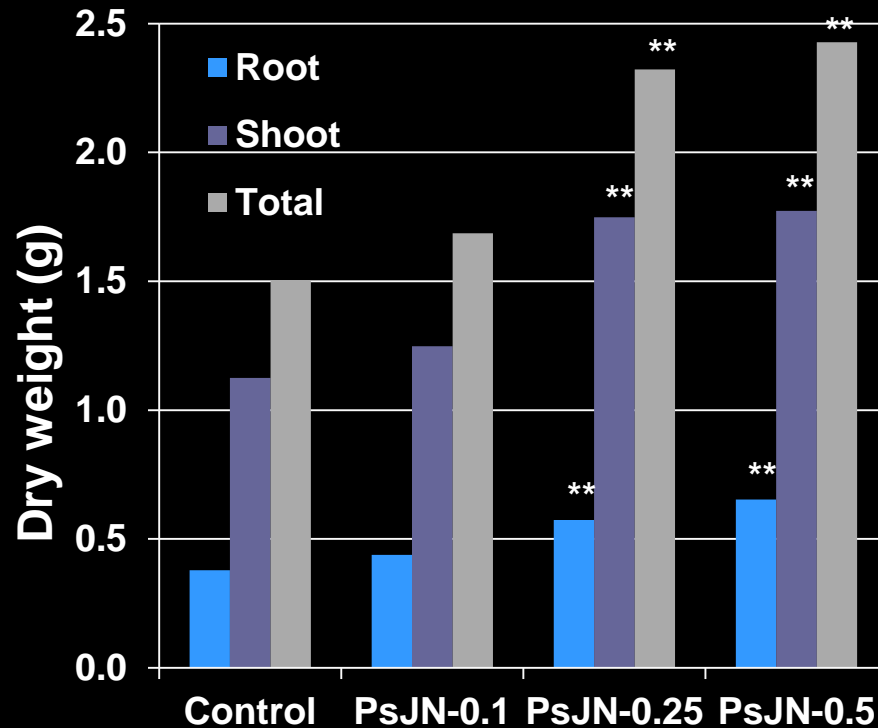
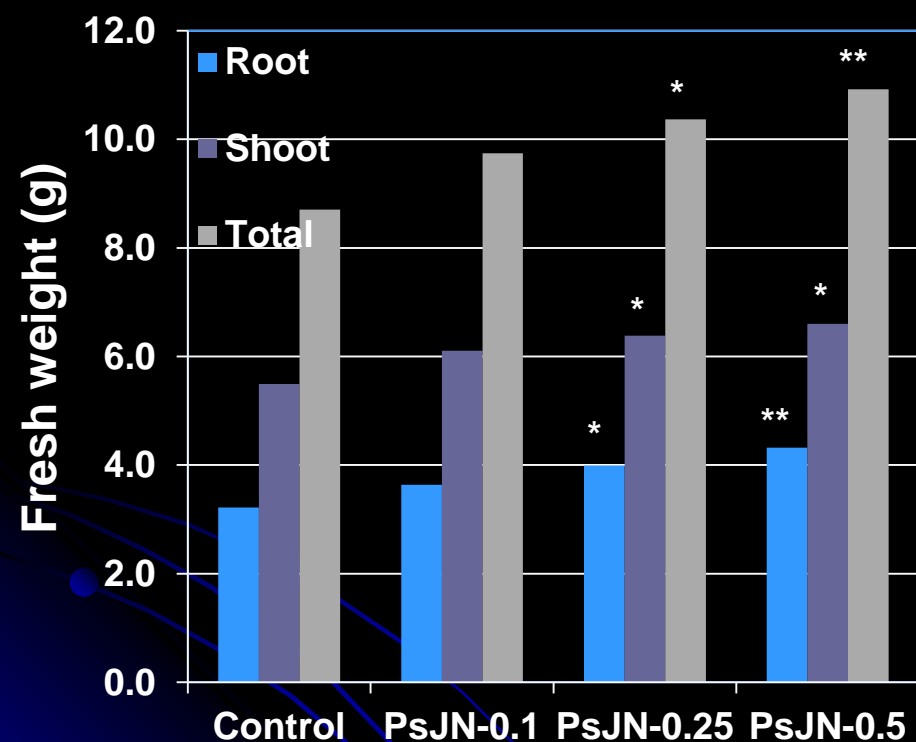
Plant height and tiller number changes after control and PsJN inoculated plants were transferred to 4-gallon pots and grown in greenhouse.

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.

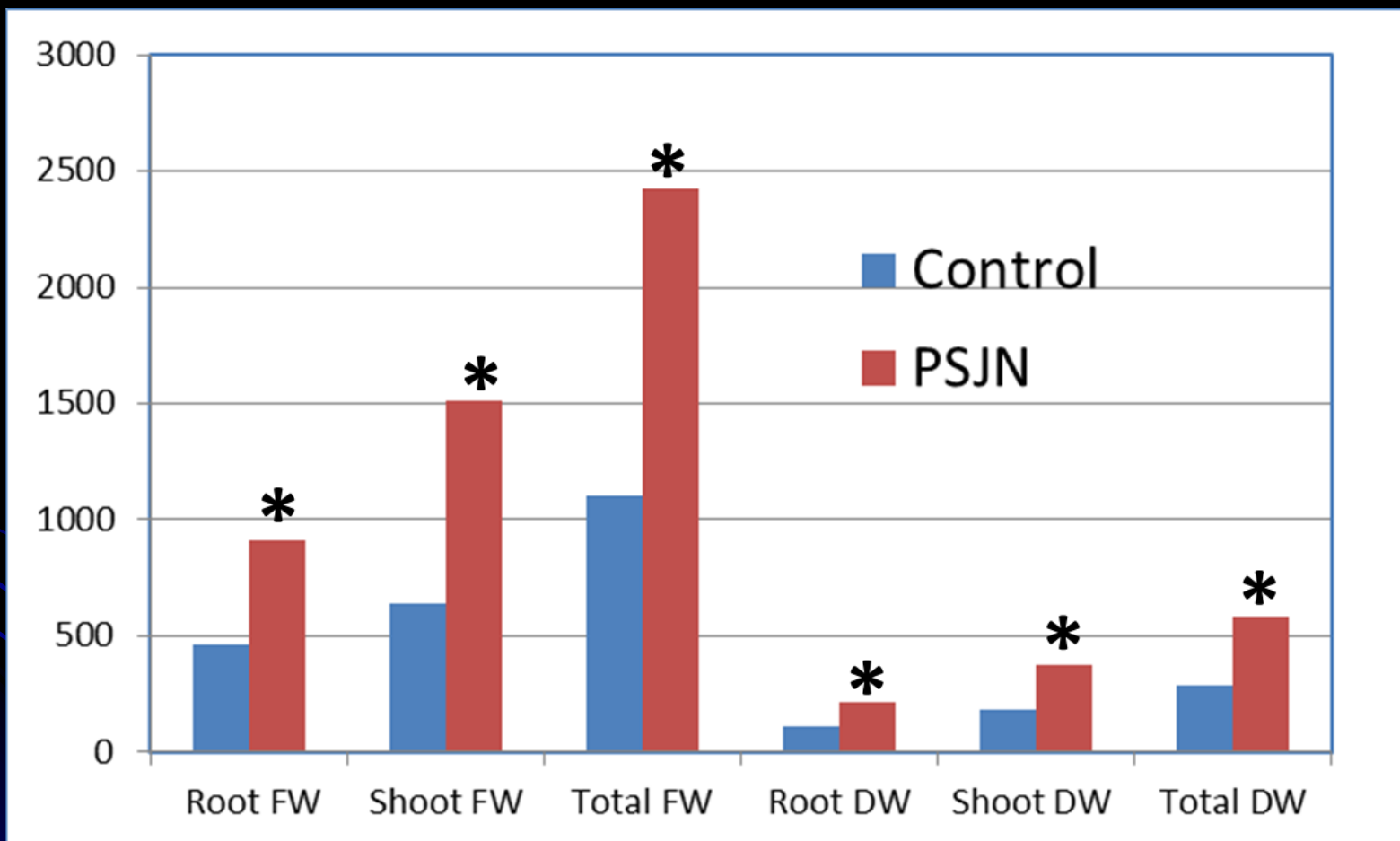
Different Concentration of PsJN



Effects of different concentrations of PsJN on switchgrass cv. Alamo growth with direct seed inoculation. * and ** mean significant difference at 0.05 and 0.01 levels respectively between PsJN and control using student T-test.

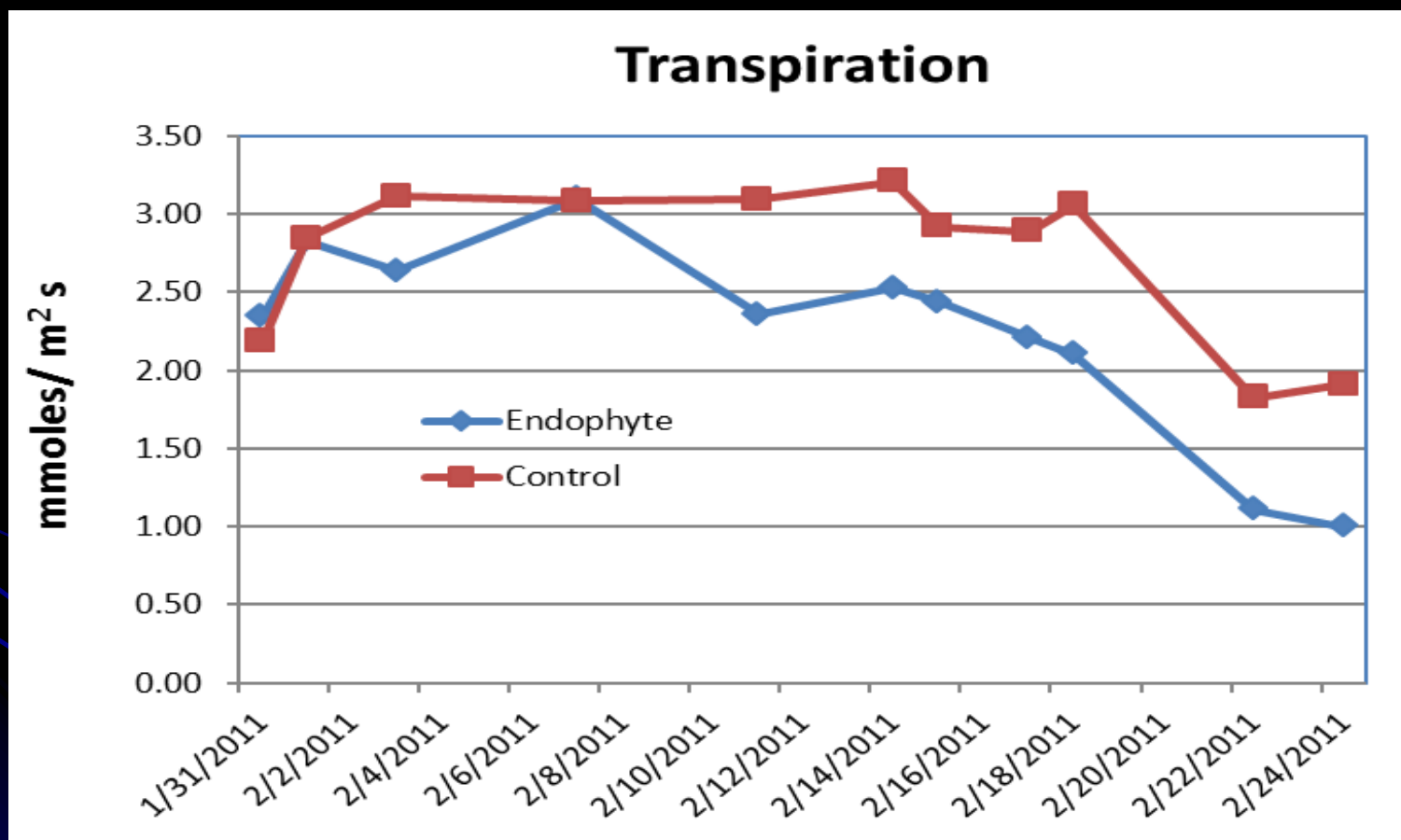
Glasshouse with Ambient Conditions

Weight (mg/plant)



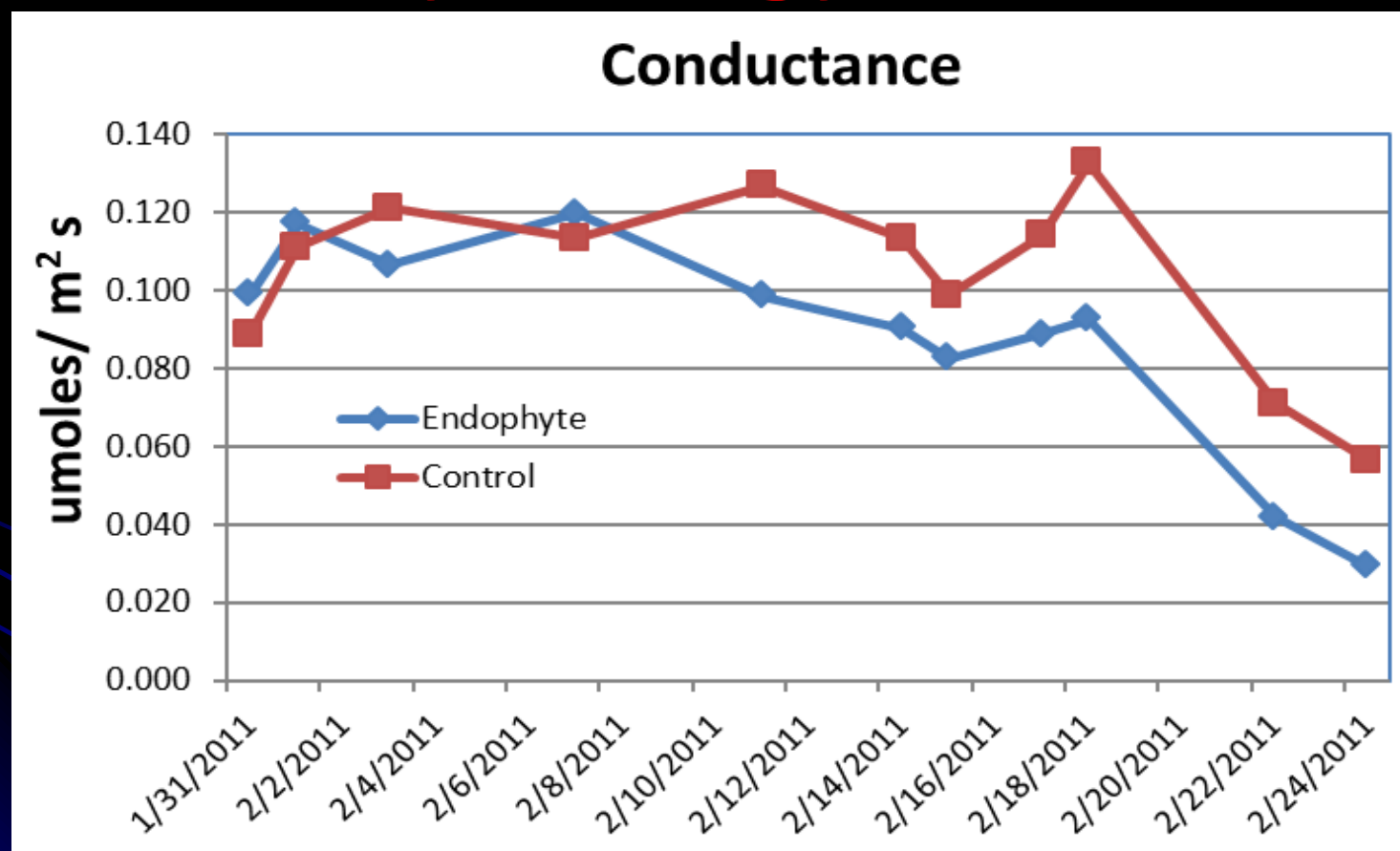
Plants were grown in field soil without fertilization in a greenhouse for 2.5 months in Fall 2010 under sub-optimal temperature. * Indicates a p-value of less than 0.005.

Plant Physiology Parameters



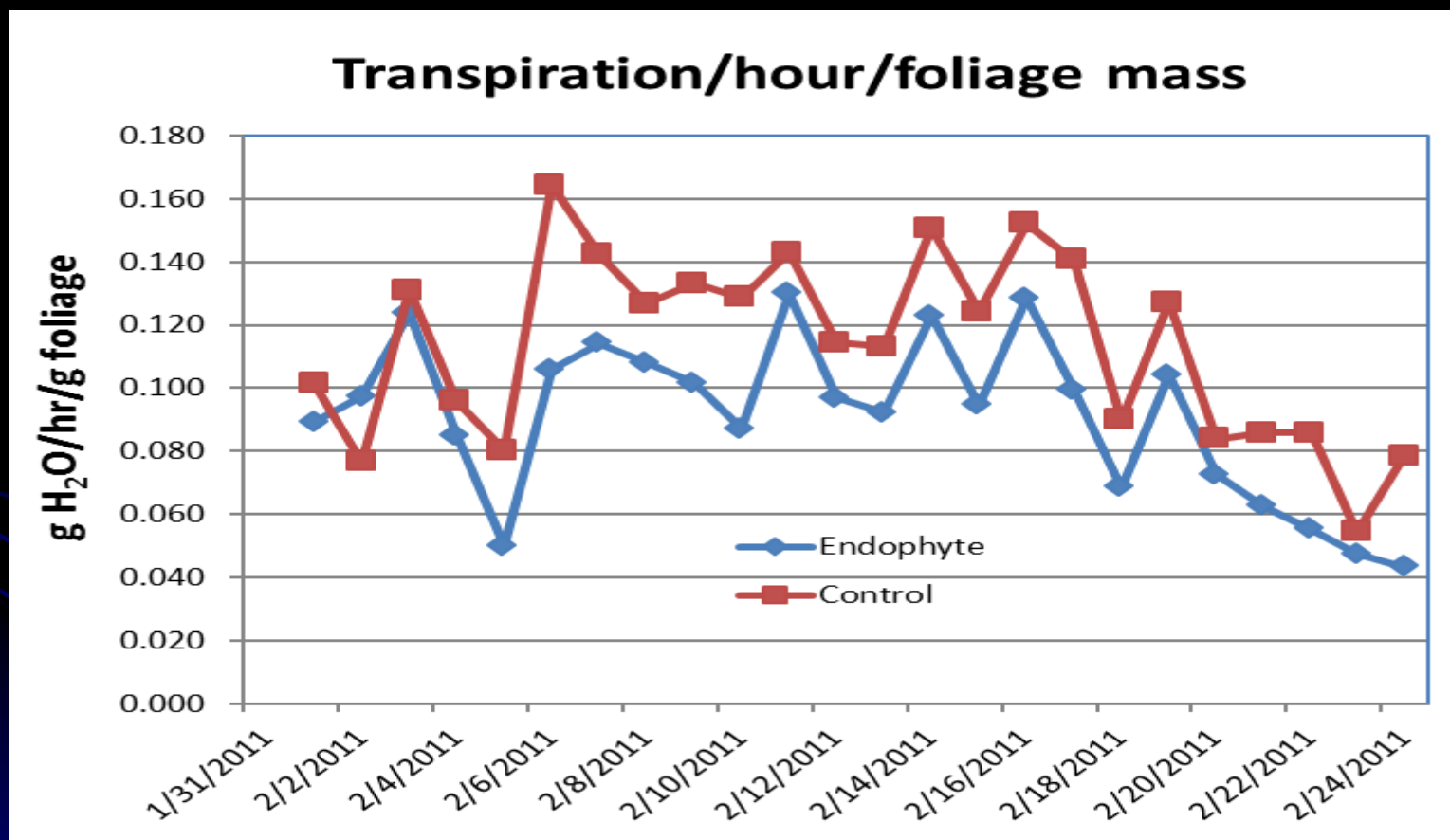
Comparison of transpiration of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



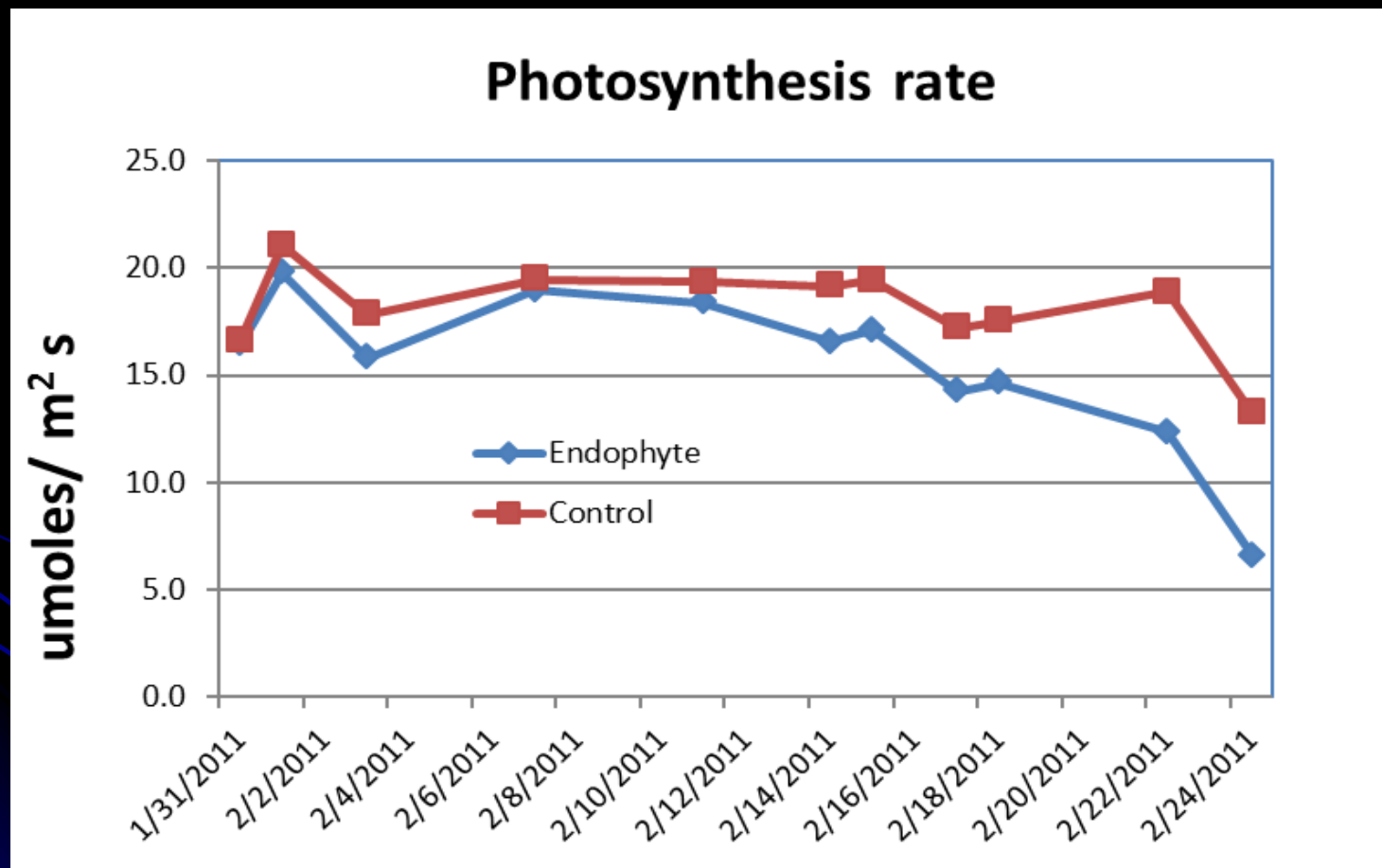
Comparison of conductance of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



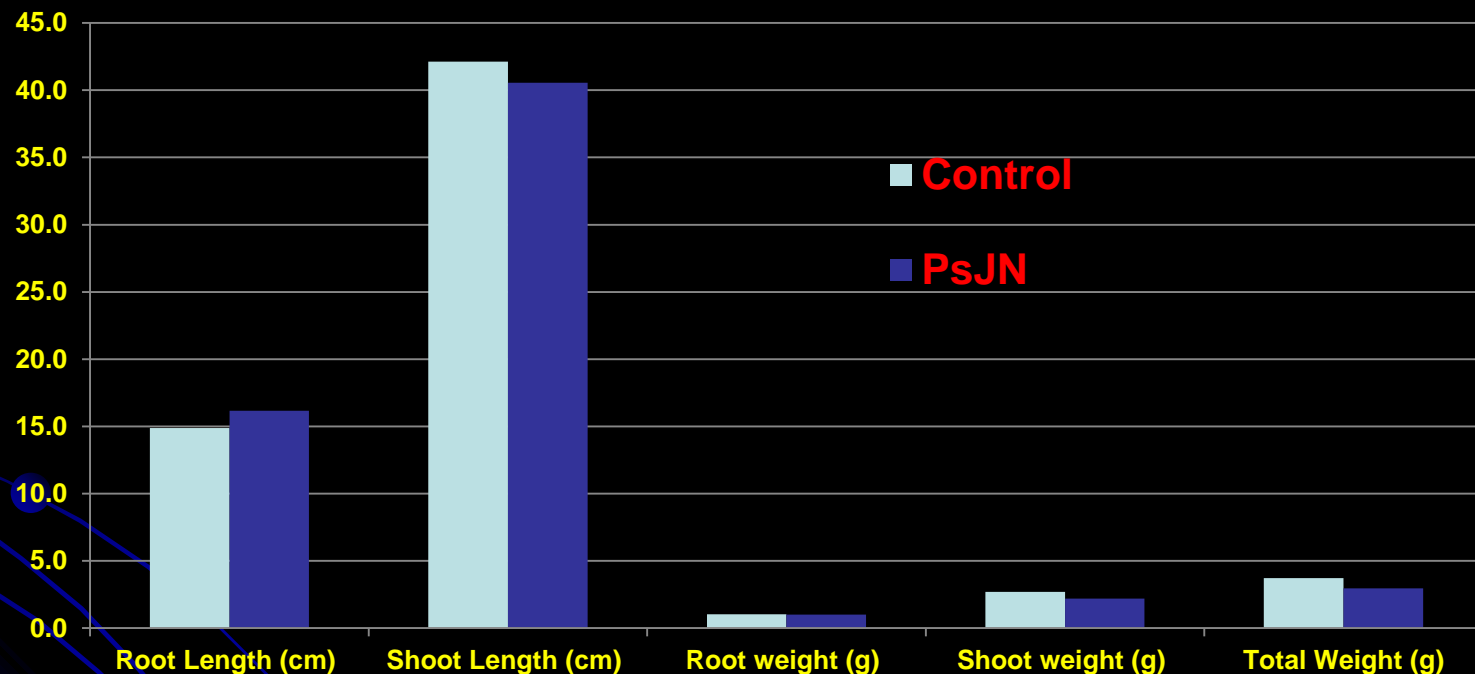
Comparison of water use efficiency of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.

Plant Physiology Parameters



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

Genotype Specificity of PsJN



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

Genomic Science Program

Systems Biology for Energy and Environment

ABOUT RESEARCH TECHNOLOGIES MISSIONS COMPUTING EDUCATION BIOFUELS BIOENERGY RESEARCH CENTERS

OVERVIEW FUNDING RESEARCH HIGHLIGHTS DOE USDA FEEDSTOCKS DATA SHARING

Plant Feedstock Genomics
for Bioenergy



2010 Awardee

Development of a Low Input and Sustainable Switchgrass Feedstock Production System Utilizing Beneficial Bacterial Endophytes

INVESTIGATORS: Mei, Chuansheng; Flinn, Barry; Seiler, John (Virginia Polytechnic Institute & State University); Nowak, Jerzy (Virginia Polytechnic Institute & State University).

INSTITUTION: Institute for Advanced Learning and Research

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

</

Overall Procedure

31 samplesRNA-update_June14sample - Microsoft Excel

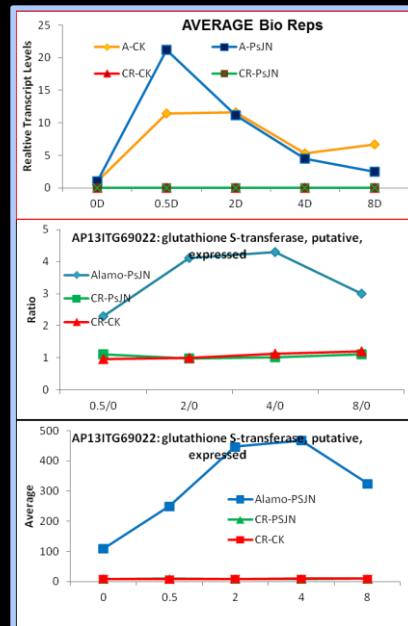
Microarray data

Probe Set ID	Annotation
AP13CTG03773_s_at	1,4-alpha-glucan-branching enzyme, chloroplast precursor, putative, expressed
AP13CTG03783_s_at	4-alpha-glucanase, putative, expressed
AP13ITG38548_at	aldehyde dehydrogenase, putative, expressed
AP13ITG43557_at	alpha-amylase precursor, putative, expressed
KanlowCTG08576_at	aminotransferase, classes I and II, domain containing protein, expressed
KanlowCTG45043_s_at	AMP-binding enzyme family protein, expressed
KanlowCTG15922_s_at	AP2 domain containing protein, expressed
AP13ITG57253_s_at	avr9/CF-9 rapidly elicited protein
AP13CTG21374_s_at	BTB10 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3
AP13ITG40096_s_at	CAMK_KIN1/SNF1/Nim1_like 30 - CAMK includes calcium/calmodulin dependent protein kinases
OTHSWCTG10190_s_at	CCT/B-box zinc finger protein
AP13CTG13208_s_at	CCT/B-box zinc finger protein, putative, expressed
OTHSWCTG12672_at	CCT/B-box zinc finger protein, putative, expressed
AP13ITG72700_at	chlorophyllase-2, chloroplast precursor, putative, expressed
AP13CTG27051_at	CSLC3 - cellulose synthase-like family C, expressed
AP13ITG31419_at	decarboxylase, putative, expressed
AP13ITG41174_at	DUF581 domain-containing protein
AP13ITG73622RC_at	EF hand family protein
AP13CTG5046_at	EF hand family protein, putative, expressed
AP13ITG75366RC_at	EF hand family protein, putative, expressed
AP13ITG73524_at	ethylene-responsive transcription factor, putative, expressed
AP13ITG75664RC_at	glutathione S-transferase, putative, expressed
AP13ITG69022_at	glutathione S-transferase, putative, expressed
KanlowCTG10254_at	glutathione S-transferase, putative, expressed
AP13ITG59013_s_at	glycosyl hydrolases family 17, putative, expressed
AP13ITG60135_s_at	glycosyl transferase 8 domain containing protein, putative, expressed
KanlowCTG47396_s_at	glycosyl transferase family 17 protein, putative, expressed
AP13ITG55791_at	glycosyltransferase protein, putative, expressed
AP13ITG41264RC_at	harpin-induced protein 1 domain containing protein, expressed
KanlowCTG27474_at	HEV3 - Hevein family protein precursor, expressed
OTHSWCTG10229_s_at	histidine-containing phosphotransfer protein, putative, expressed

50 genes have been selected

Over expression

Knockout: RNAi



Key genes selected



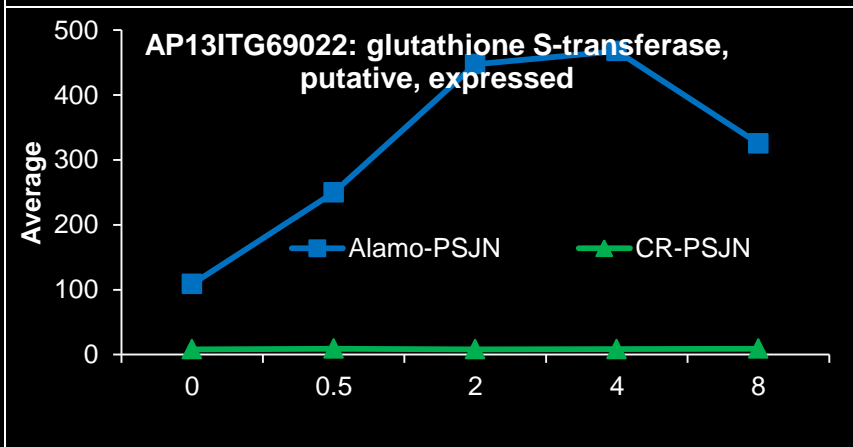
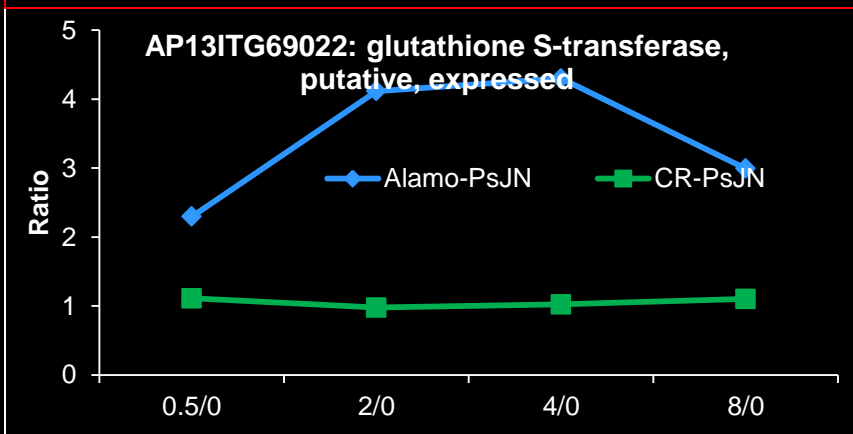
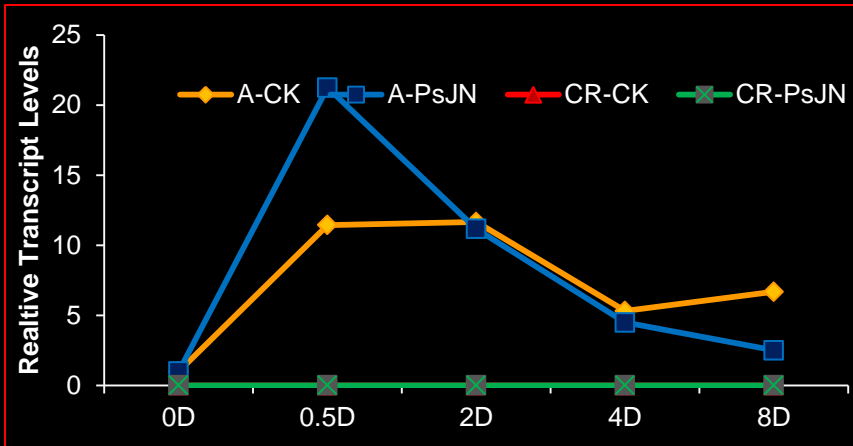
Real-time PCR

G1: AP13ITG69022: glutathione S-transferase, putative, expressed

Glutathione S-transferases (GSTs) play roles in both normal cellular metabolism as well as in the detoxification of a wide variety of xenobiotic compounds, and they have been intensively studied with regard to herbicide detoxification in plants.

A newly discovered plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress, and heavy-metal toxicity.

In addition, plant GSTs play a role in the cellular response to auxins and during the normal metabolism of plant secondary products like anthocyanins and cinnamic acid.

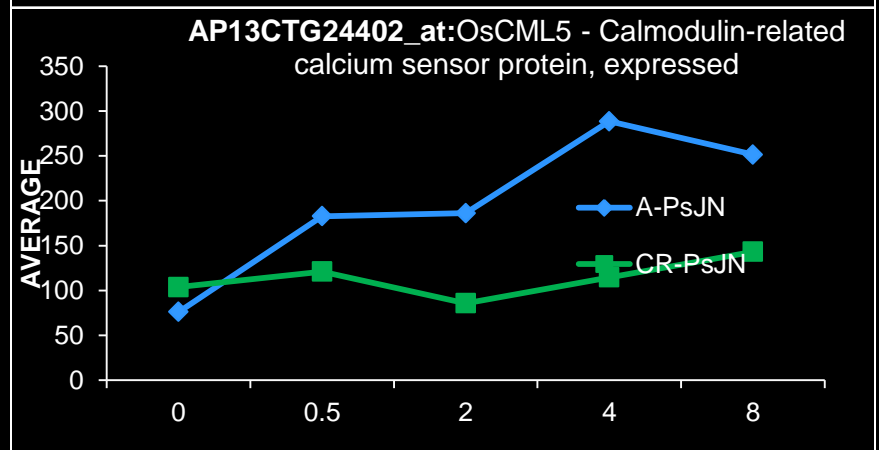
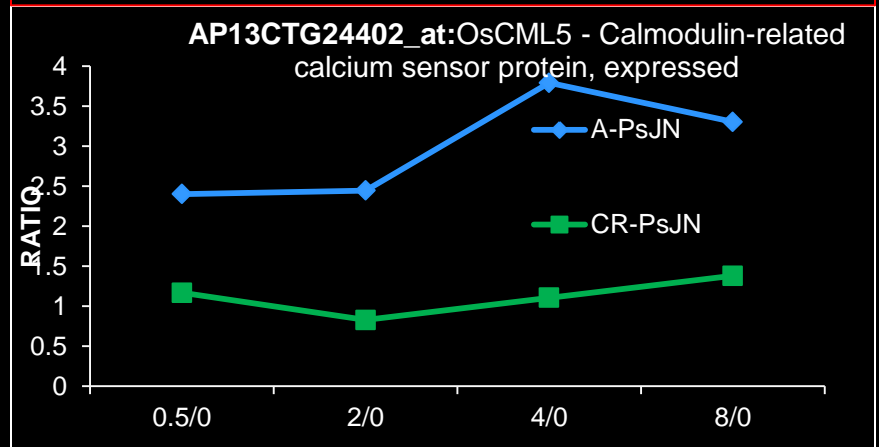
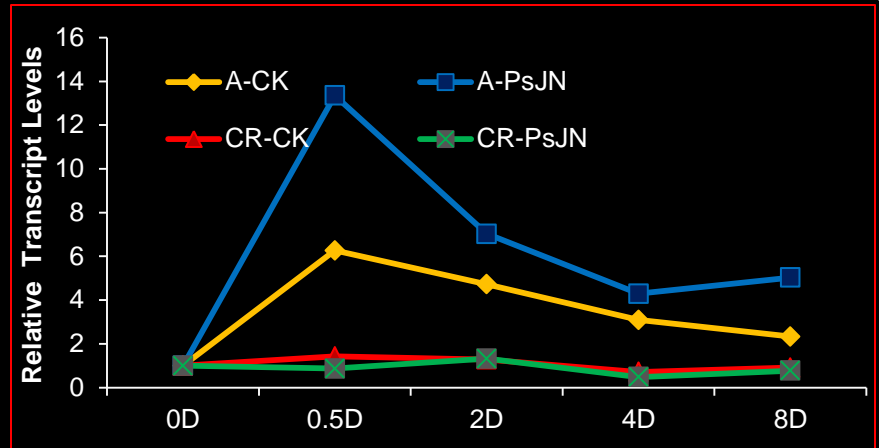


G4: AP13CTG24402_at:OsCML5 - Calmodulin-related calcium sensor protein, expressed

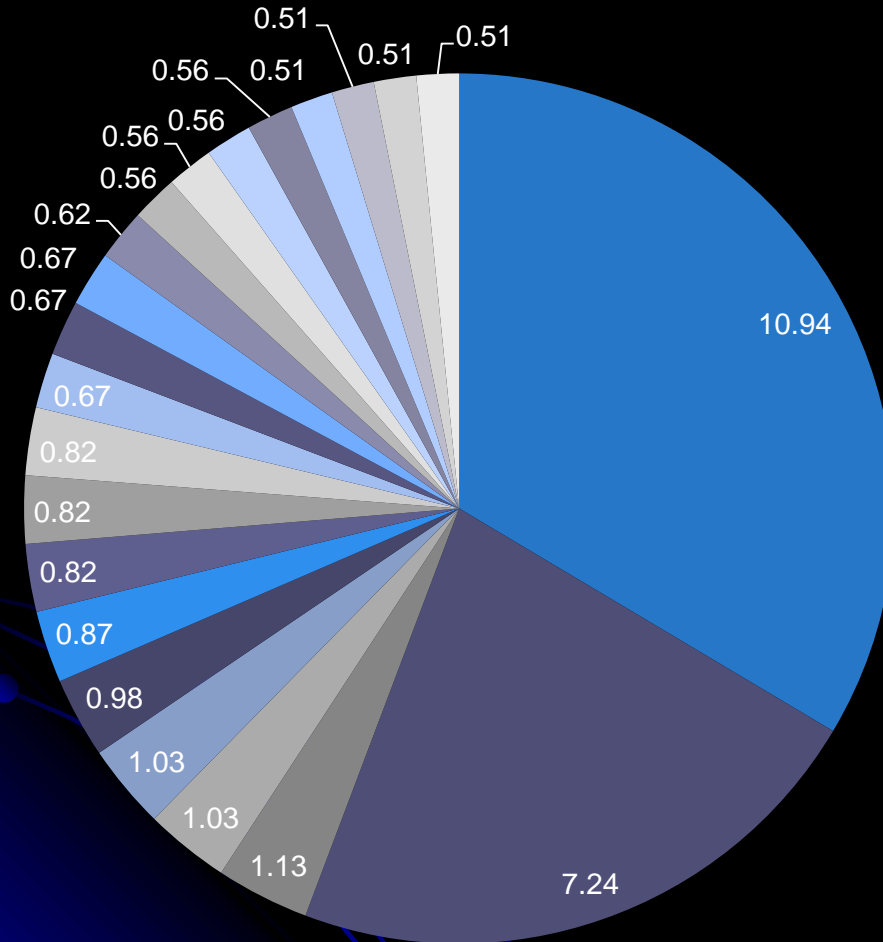
The calmodulin (CaM) family is a major class of calcium sensor proteins which collectively play a crucial role in cellular signaling cascades through the regulation of numerous target proteins.

Ca²⁺ is an essential second messenger in all eukaryotic cells in triggering physiological changes in response to external stimuli.

In plant cells, a wide range of stimuli trigger cytosolic [Ca²⁺] increases of different magnitude and specialized character [1,2], which are typically transmitted by protein sensors that preferably bind Ca²⁺. Ca²⁺ binding results in conformation changes that modulate their activity or their ability to interact with other

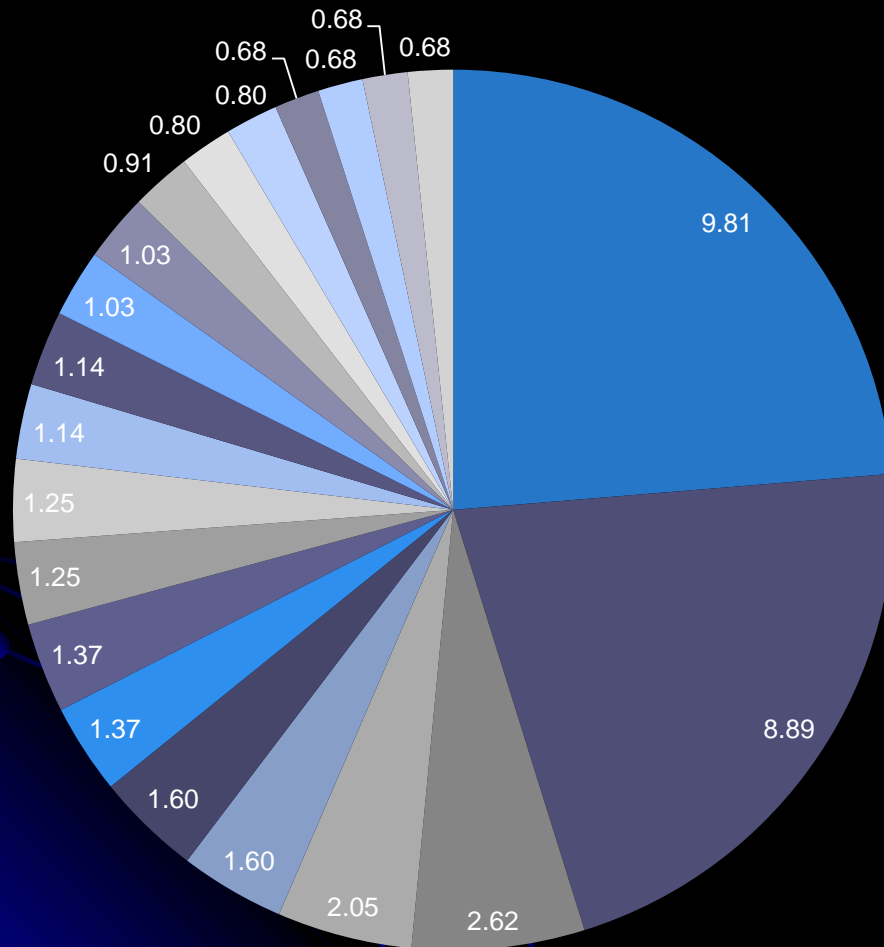


Up Alamo_Down CR: 0.5 days post PsJN 1947 genes



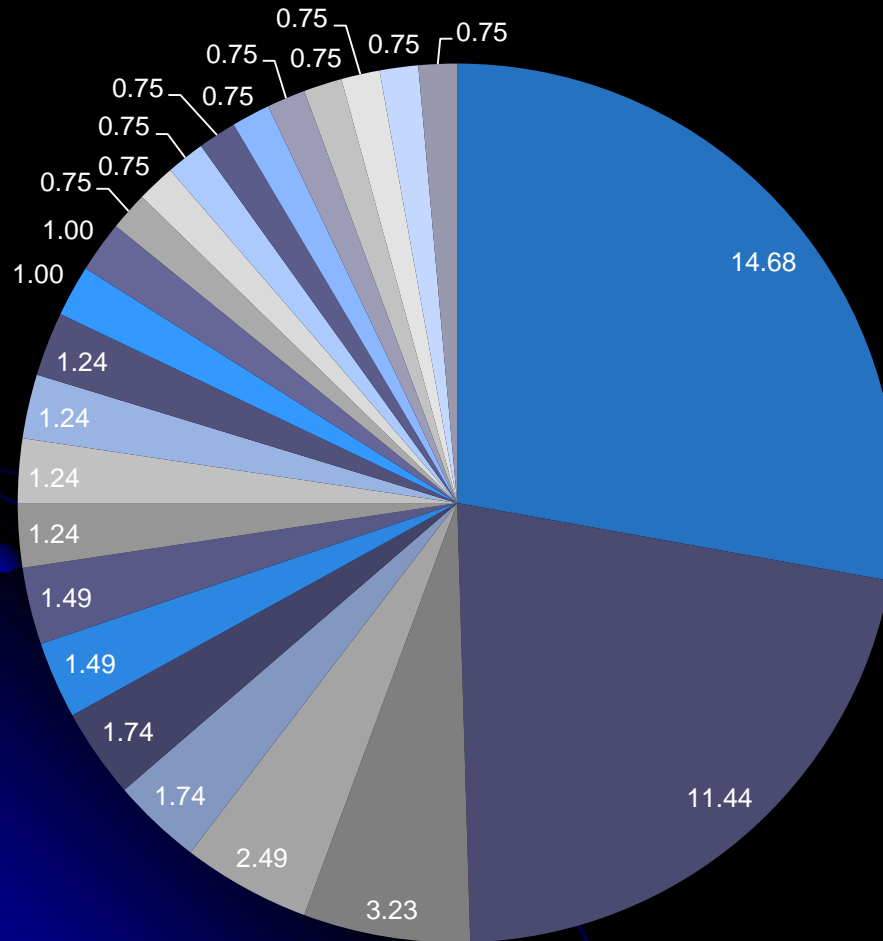
- expressed protein **10.94%**
- unknown **7.24%** ↑
- ribosomal protein **1.13%** ↓
- cytochrome P450 protein **1.03%** ↑
- pentatricopeptide repeat domain containing protein
- DUF domain containing protein **0.98%** ↑
- PPR repeat containing protein
- glycosyltransferase protein
- retrotransposon protein
- transposon protein
- peroxidase precursor **0.67%** ↑
- protein kinase domain containing protein
- transporter, putative
- hydrolase, alpha/beta fold family domain containing protein
- bifunctional protein fold
- OsFB- F-box domain containing protein
- peptidyl-prolyl isomerase
- RNA recognition motif containing protein
- ABC type transporter domain containing protein
- glycosyl hydrolase
- protein phosphatase
- WD domain, G-beta repeat domain containing protei

Up Alamo_Down CR: 2 days post PsJN 877 genes



- unknown 9.81%↑
- expressed protein 8.89%
- cytochrome P450 protein 2.62%↑
- peroxidase precursor, putative 2.05%↑
- UDP-glucuronosyl and UDP-glucosyl transferase domain containing protein
- LRP1, putative
- pentatricopeptide repeat domain containing protein
- retrotransposon protein, putative, unclassified
- DUF domain containing protein 1.25%↑
- expansin precursor
- glycosyltransferase protein
- glutathione S-transferase
- gibberellin receptor GID1L2
- transferase family protein
- hypothetical protein
- ribosomal protein 0.80%↓
- hydrolase, alpha/beta fold family domain containing protein
- glycosyl hydrolase, putative
- oxidoreductase, aldo/keto reductase family protein
- plastocyanin-like domain containing protein
- SCP-like extracellular protein

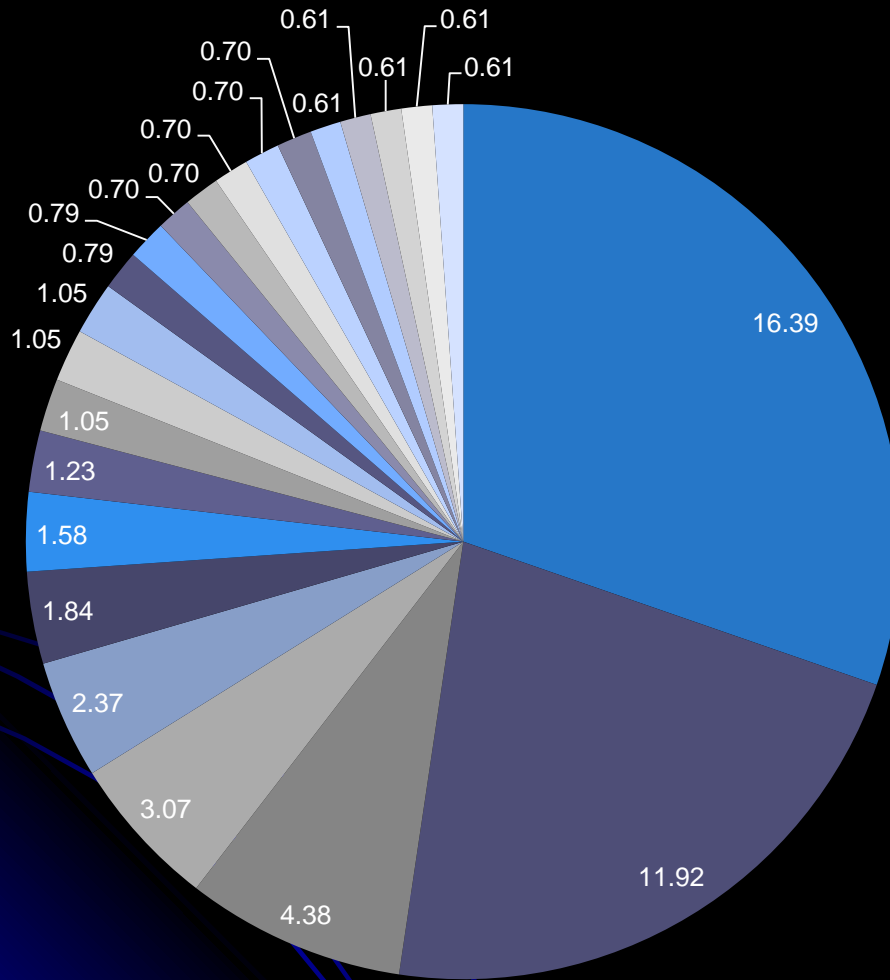
Up Alamo_Down CR: 4 days post PsJN 402 genes



- unknown 14.68%↑
- expressed protein 11.44%
- cytochrome P450 protein 3.23%↑
- DUF domain containing protein 2.49%↑
- transferase family protein
- MYB family transcription factor
- peroxidase precursor 1.49%↑
- transposon protein, putative, unclassified
- glutathione S-transferase 1.24%↓
- hypothetical protein
- harpin-induced protein 1 domain containing protein
- conserved hypothetical protein
- glycosyl hydrolase
- transporter, putative
- UDP-glucuronosyl and UDP-glucosyl transferase domain
- retrotransposon protein, putative, unclassified
- gibberellin receptor GID1L2
- zinc finger, C3HC4 type domain containing protein
- DNA binding protein, putative
- nodulin, putative
- heavy-metal-associated domain-containing protein
- zinc finger family protein, putative
- helix-loop-helix DNA-binding domain containing protein
- IQ calmodulin-binding motif family protein

Up Alamo_Down CR: 8 days post PsJN

1141 genes



- unknown 16.39%↑
- expressed protein 11.92%↑
- retrotransposon protein
- cytochrome P450 protein 3.07%↑
- transposon protein
- DUF domain containing protein
- peroxidase precursor 1.58%↑
- hypothetical protein
- transferase family protein, putative
- UDP-glucuronosyl and UDP-glucosyl transferase
- glycosyltransferase protein, putative
- gibberellin receptor GID1L2
- LTP family protein precursor, expressed
- glutathione S-transferase, putative
- EF hand family protein
- expansin precursor
- peptide transporter PTR
- pentatricopeptide repeat domain containing protein
- MYB family transcription factor
- harpin-induced protein 1 domain containing protein
- zinc finger, C3HC4 type domain containing protein
- aspartic proteinase nepenthesin precursor
- GDSL-like lipase/acylhydrolase

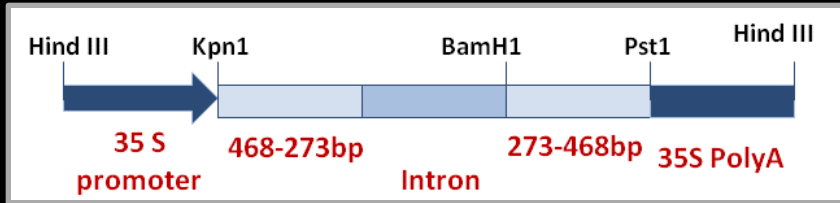
Transcription Factors

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.

Functional Studies of Key Genes

1. **G1:** AP13ITG69022: glutathione S-transferase, putative, expressed
2. **G4:** AP13CTG24402_at:OsCML5 - Calmodulin-related calcium sensor protein,



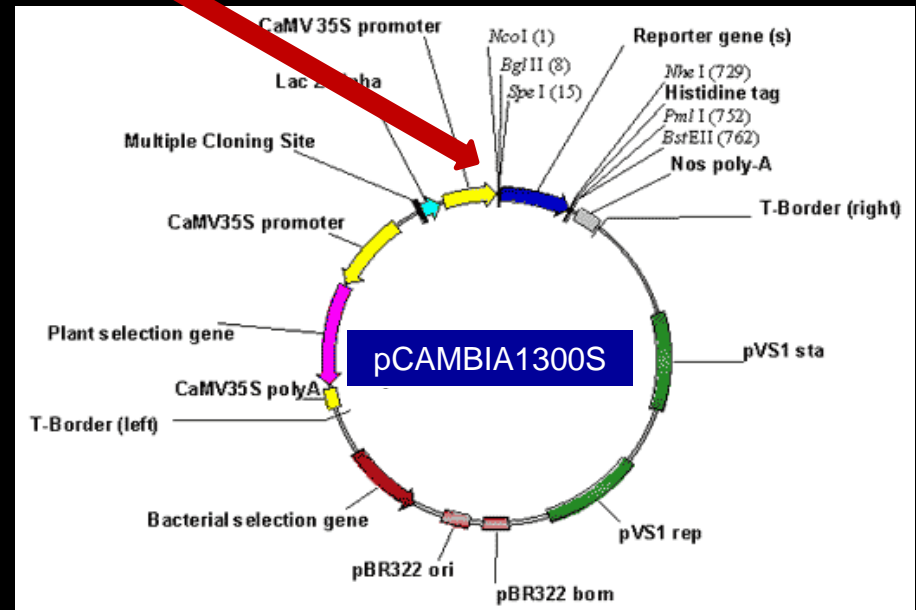
RNA interference (RNAi) construct

or



Over-expression construct

Transformed to switchgrass
embryogenic callus



From Dr. Yinong Yang

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Ms. Bingxue Wang (Ph.D. student)
Ms. Bethany Gregory (Undergraduate)

Collaborators

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State University
Dr. Bingyu Zhao, VT



United States
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Agriculture

National Institute
of Food and
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U.S. DEPARTMENT OF
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Office of
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Plant Feedstock Genomics for Bioenergy

U.S. Departments of Agriculture and Energy

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

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