



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 economicallydepressed 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.



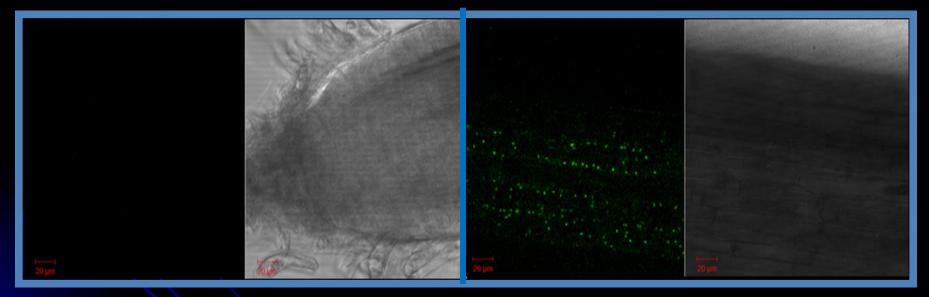
- Gram negative beneficial bacterial endophyte isolated from onion roots in 1987 by Jerzy Nowak
- Plant growth promotion has been found in many species.
- Colonizes:
 - Rhizosphere
 - Root and above ground internal plant organs and tissues
- Complete genome has been sequenced*

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





Confocal Microscope



Non-infected Control

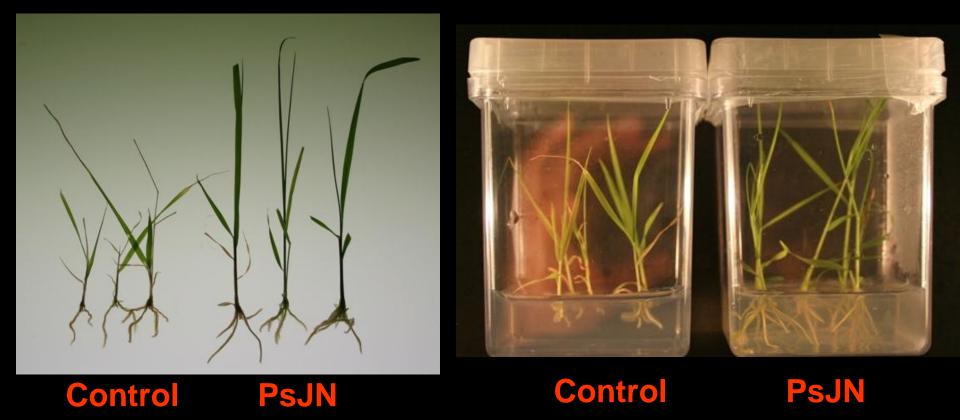
PsJN-GFP Inoculated

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





Growth Promotion by PsJN

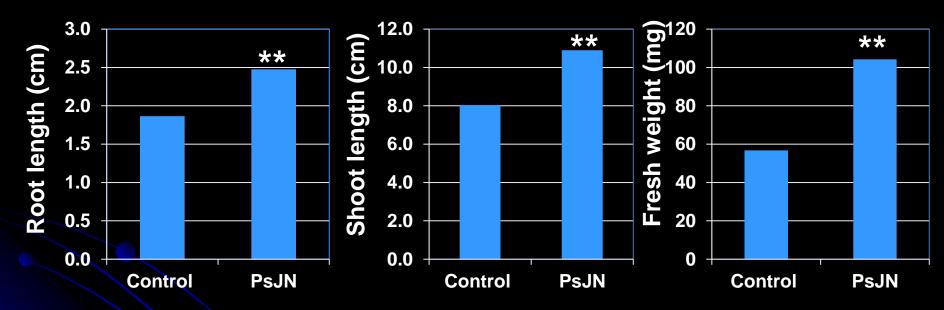


Pictures were taken one month after Alamo inoculated with PsJN.





In Vitro Experiment

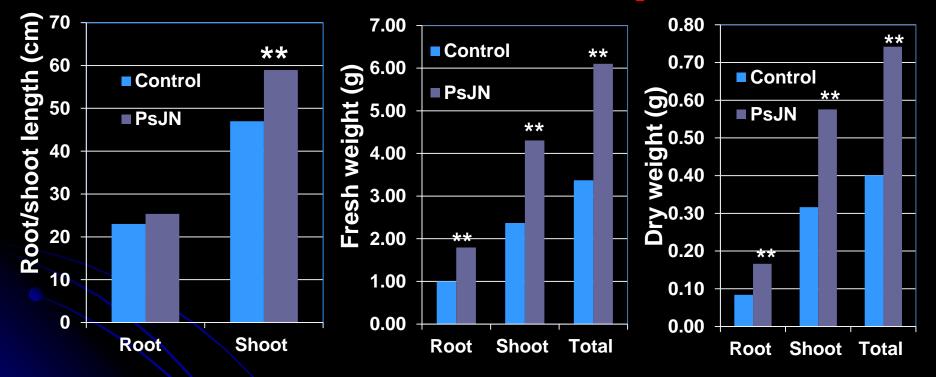


Data were from control and endophyte-inoculated plants grown in vitro for one month. Sample number was 36 for control and PsJN plants. All data from endophyte-inoculated plants were very significantly difference from control plants. Total fresh weight in endophyte-inoculated plants was 85% higher than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





Growth Chamber Experiment

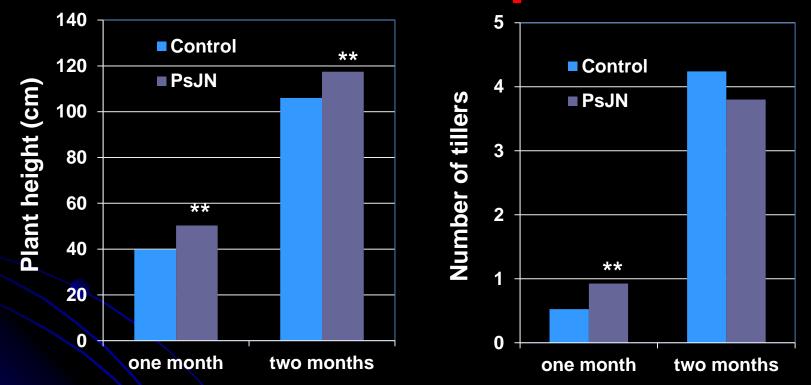


Effects of endophyte PsJN inoculation on switchgrass cv. Alamo growth in growth chamber. ** means significant difference at 0.01 level between PsJN and control using student T-test.





Greenhouse Experiment

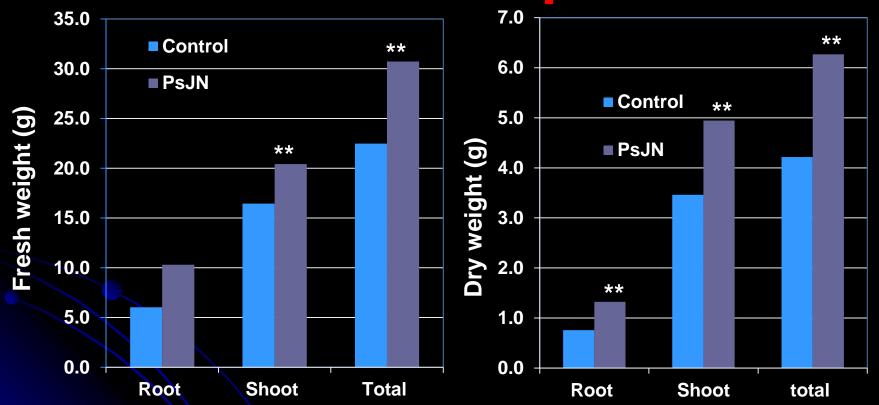


Plant height and tiller number changes after control and PsJN inoculated plants were transferred to 4-gallon pots and grown in greenhouse.





Greenhouse Experiment

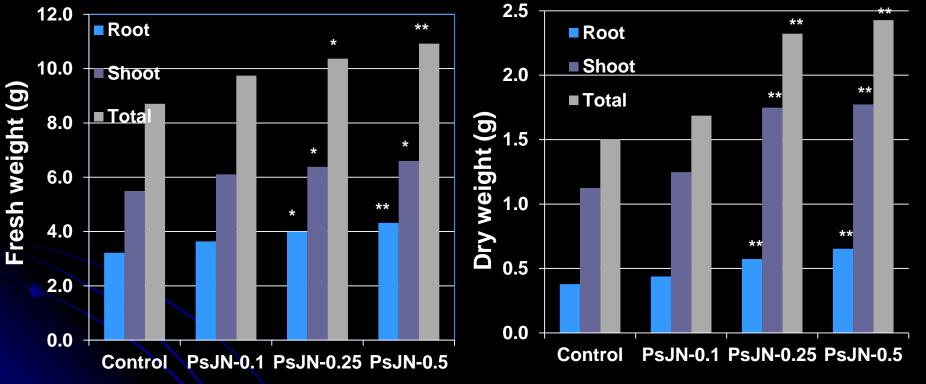


Growth promotion persistence of Alamo in greenhouse by PsJN inoculation. ** means significant difference at 0.01 level between **PsJN and control using student T-test.**





Different Concentration of PsJN

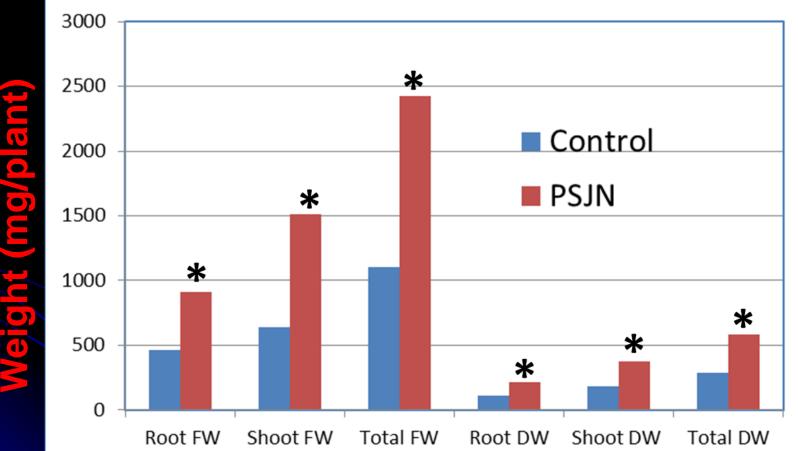


Effects of different concentrations of PsJN on swtchgrass cv. Alamo growth with direct seed inoculation. * and ** mean significant difference at 0.05 and 0.01 levels respectively between PsJN and control using student T-test.





Glasshouse with Ambient Conditions



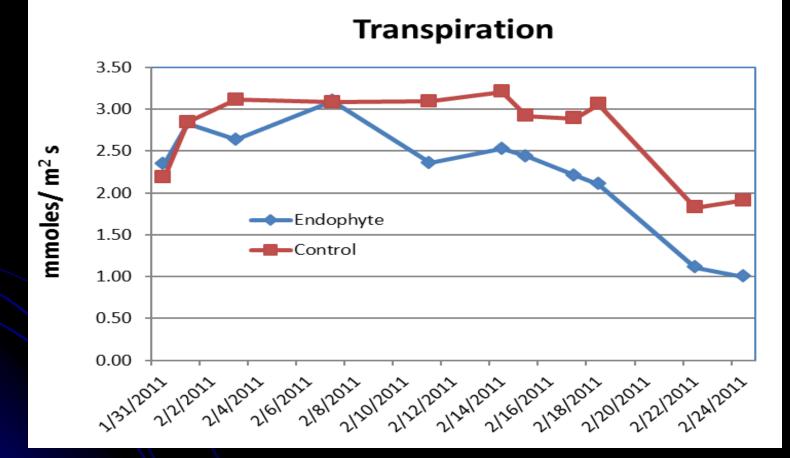
Plants were grown in field soil without fertilization in a greenhouse for 2.5 months in Fall 2010 under sub-optimal temperature. * Indicates a p-value of less than 0.005.



rass cv. Alamo inoculated with PsJN to the control plants grow: hgrass cv. Alamo inoculated with PsJN to the control plants gro



Plant Physiology Parameters

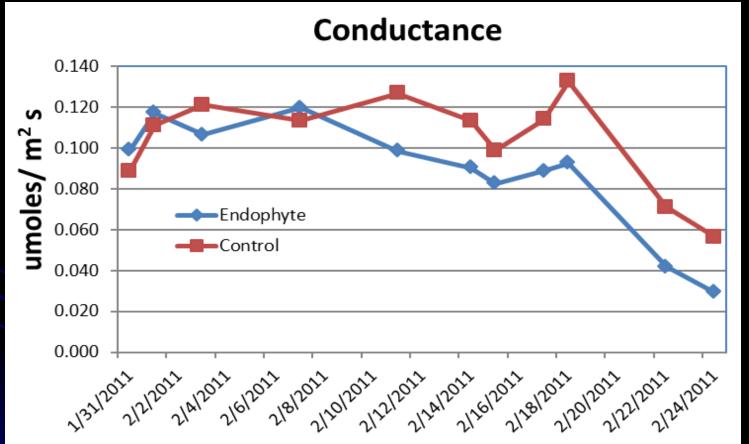


Comparison of transpiration of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.





Plant Physiology Parameters

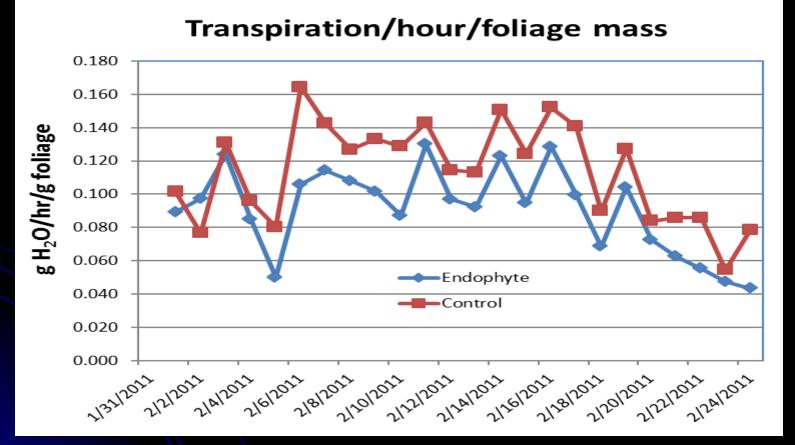


Comparison of conductance of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.





Plant Physiology Parameters

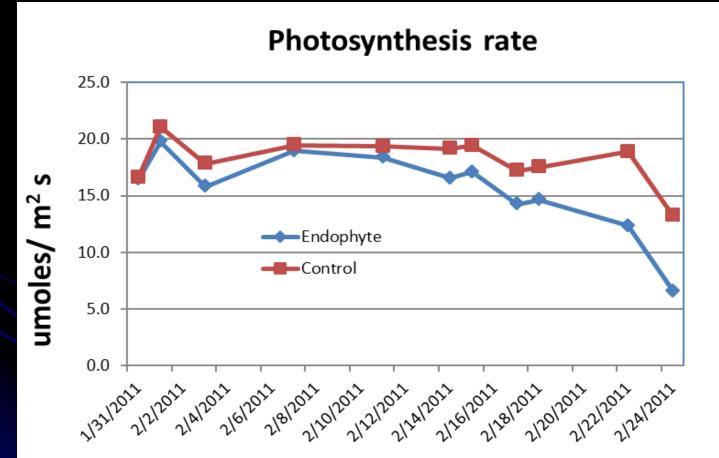


Comparison of water use efficiency of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.





Plant Physiology Parameters

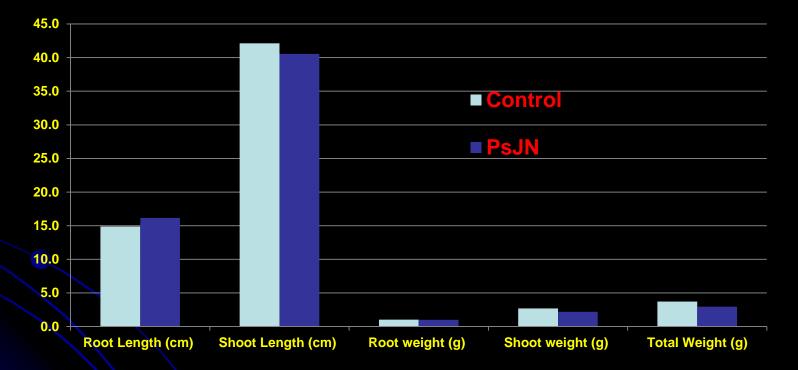


Comparison of photosynthesis rate of switchgrass cv. Alamo inoculated with PsJN to the control plants grown in greenhouse conditions.





Genotype Specificity of PsJN



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



Genomic Science Program

Systems Biology for Energy and Environment

ABOUT	RESEAR	ксн	TECHNO	LOGIES	MISSIONS	COMPUT	NG EDUC	ATION	BIOFUELS	BIOENERGY	RESEARCH CENTERS
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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

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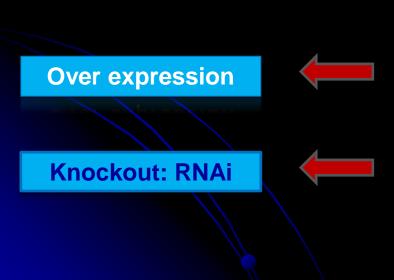
Overall Procedure

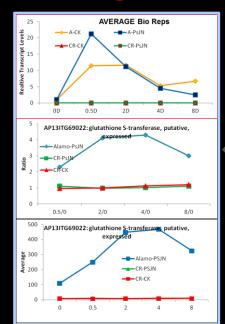
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16	AP13TG57727-R	AP13/TG57727-R0	C_at				-	205.29			0.044			2	1092.7					
		AP13/T058847_a				1	LOC_0	62.043						2	708.9					
		AP13IT061224_at				1	LOC_	73.791						2	68.8					
		AP13/TG73638_s AP13/TG73736_a			SUBFAMILYN	AT 15/52500	LOC	716.79				- 1		2	845.9			91.5		
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		VS16ITG26971_at			1	1	ш	24.07	13.45					0	19.2					
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		VS101TG27891-FIC						14.412	8,285		0.2051			0	11.1					
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		VS16ITG28088-PIC						0.3246						0	20.5					
		VS16ITG28328-RC						7.3519						2	20.5					
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Ready															- CE CI A	B 60%	(-)			

Microarray data

Parks Cat ID	A un shatter
Probe Set ID	Annotation
AP13CTG03773_s_at	1,4-alpha-glucan-branching enzyme, chloroplast precursor, putative, expressed
AP13CTG00783_s_at	4-alpha-glucanotransferase, 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_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	BTBN10 - 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 depedent 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
AP13ITG73149_at	decarboxylase, putative, expressed
AP13ITG41174_at	DUF581 domain-containing protein
AP13ITG73622RC_at	EF hand family protein
AP13CTG25046_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 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





Key genes selected

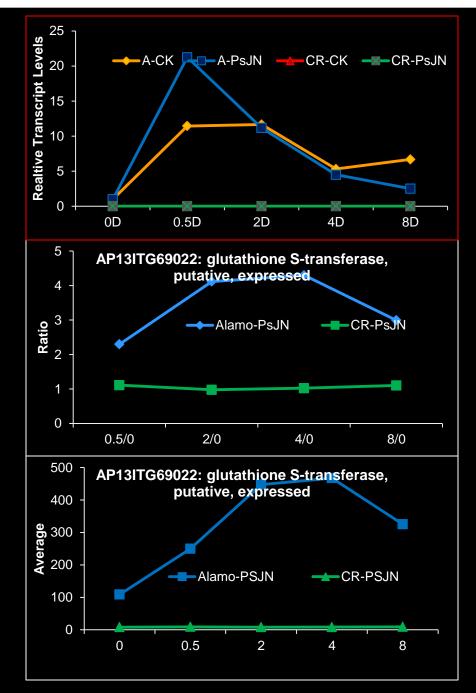


Real-time PCR

G1: AP13ITG69022: glutathione Stransferase, 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.

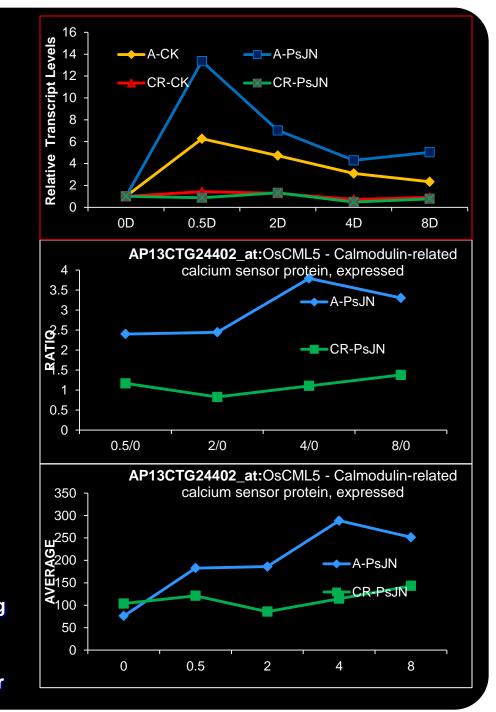
A newly discovered plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress, and heavymetal toxicity.

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.



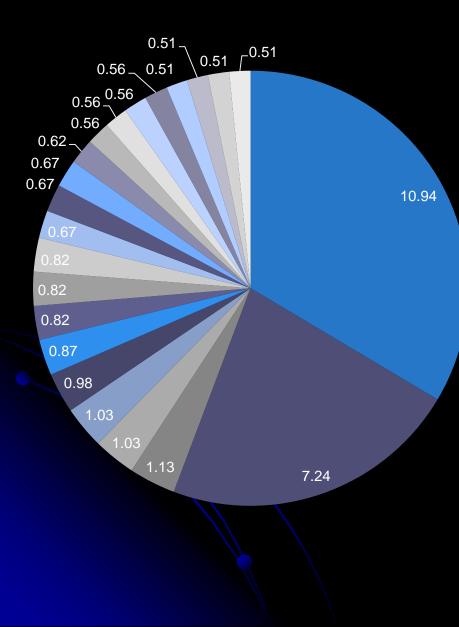
G4: AP13CTG24402_at:OsCML5 - Calmodulinrelated calcium sensor protein, expressed The calmodulin (CaM) family is a major class of calcium sensor proteins which collectively play a crucial role in cellular signaling cascades through the regulation of numerous target proteins.

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



avalatas

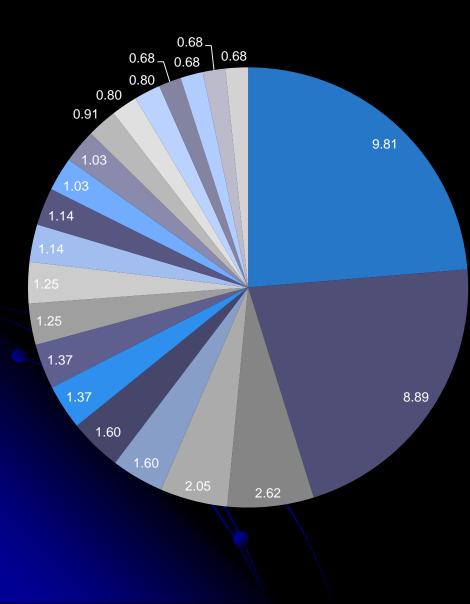
Up Alamo_Down CR: 0.5 days post PsJN expressed protein 1947 genes 0.5 days post PsJN 0.5



10.94%

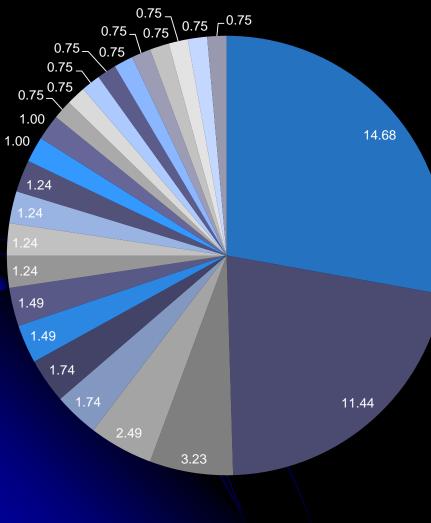
- **7.24%** ↑
- ribosomal protein 1.13%
- cytochrome P450 protein 1.03% ↑
- pentatricopeptide repeat domain containing protein
- **0.98%** ↑ DUF domain containing protein
- PPR repeat containing protein
- glycosyltransferase protein
- retrotransposon protein
- transposon protein
- peroxidase precursor 0.67% 1
- 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



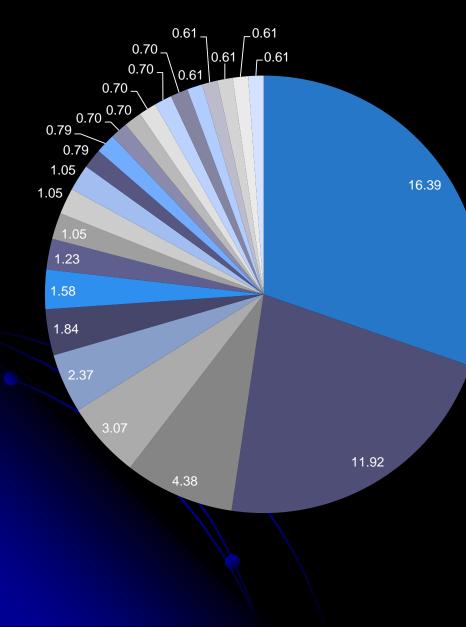
- unknown 9.81%↑
- expressed protein 8.89%
- cytochrome P450 protein 2.62%↑
- peroxidase precursor, putative 2.05%↑
- UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein
- LRP1, putative
- pentatricopeptide repeat domain containing protein
- retrotransposon protein, putative, unclassified
- 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% ↑										
N	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-glucoronosyl 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-glucoronosyl 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

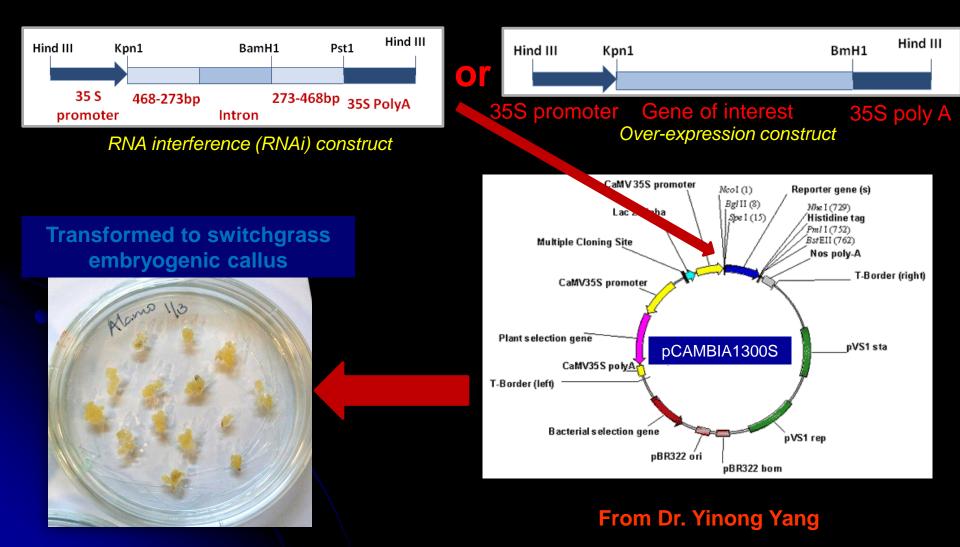
Transcription Factors

				sJN inocula	tion	Cave-in-Rock (days after PsJN inoculation)					
ID probe	Annotation	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		1.52	1.68	2.06	1.24	0.95	0.94	0.91	0.98		
KanlowCTG34263_at	MYB family	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		1.53	2.03	2.26	2.88	1.15	1.06	0.79	0.98		
KanlowCTG42852_s_at	F-box domain	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	1.56	1.76	2.07	2.28	0.74	0.62	0.67	0.71		
AlamoCTG04292_s_at	type	2.26	1.22	1.16	1.11	1.65	1.95	1.89	1.90		
AP13CTG19863_at	TFs having WRKY	3.13	1.94	1.70	1.69	0.12	0.11	0.17	0.17		
AP13CTG44559_s_at	and zinc finger domain	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,







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