Global Gene Expression Profiling of Switchgrass Following Inoculation with *Burkholderia phytofirmans* Strain PsJN

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Project Goals: 1) Global gene expression profiling induced by switchgrass bacterization with the beneficial bacterial endophyte *Burkholderia phytofirmans* strain PsJN and 2) Identification of key genes from global gene expression profiling and a study of their functions

Switchgrass is one of the promising bioenergy crop candidates for the U.S. It gives relatively high biomass yields and can grow on marginal lands. However, the biomass yield varies from year to year and from location to location. The overall goal of the project is to develop a low input and sustainable switchgrass feedstock production system utilizing beneficial bacteria endophytes. Previous results on the inoculation of switchgrass lowland cultivar Alamo with *Burkholderia phytofirmans* strain PsJN indicated a significant increase of growth under *in vitro*, growth chamber and greenhouse conditions. However, no beneficial responses were recorded with the upland cultivar Cave-in-Rock. In order to explore this genotype effect further, a comparative global gene expression profiling was conducted both cultivars following PsJN inoculation using the DOE-funded switchgrass cDNA microarrays. Ten-day old seedlings were inoculated with PsJN (0.5 at OD_{600}) and tissue samples collected at 0 (prior to inoculation), 0.5, 2, 4 and 8 days after inoculation, with three biological replicates. Non-inoculated controls were treated with PBS buffer and sampled as above.

Based on the microarray data analyses, 50 candidate genes that exhibited significant differences in the expression levels between PsJN-inoculated Alamo and Cave-in-Rock were chosen for further study. These genes are being subjected to secondary verification using qPCR. So far, 20 out of the 50 have been verified. From these 20 genes, five key genes representing glutathione S-transferase, calmodulin-related calcium sensor protein, an EF-hand transcription factor, histidine-containing phosphotransfer protein and a zinc-finger protein have been chosen for further functional studies using overexpression and RNAi knockout/knockdown techniques. Overexpression constructs for glutathione S-transferase and calmodulin-related calcium sensor genes were also introduced into switchgrass embryogenic callus, and plants will be regenerated and tested for endophyte PsJN responses.

Further analysis of the microarray data focused on groups of genes that are up-regulated in Alamo and down-regulated in Cave-in-Rock at each sampling point. We identified approximately 1947, 877, 402 and 1140 genes that displayed this pattern at 0.5, 2, 4 and 8 days after inoculation,

respectively. The majority of genes showing differences are annotated as “expressed proteins” and “unknown proteins”. We are currently focused on transcription factor genes, such as AP2 domain, MYB family, F-box domain, and zinc finger protein. Further studies of these transcription factor gene functions are listed in Table 1.

Table1. 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.

		Alamo				Cave-in-Rock			
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	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

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