16. Oligo microarray analysis of transcription in γ-aminobutyric acid-overaccumulating rice calli

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γ-aminobutyric acid (GABA) is a four-carbon non-protein amino acid that is produced from glutamate by glutamate decarboxylase (GAD) and is ubiquitously present in organisms ranging from bacteria to plants and vertebrates (Bouché and Fromm 2004). GABA is known to function as an inhibitory neurotransmitter in animals, but its role in plants still remains unclear. Interestingly, only plant GADs have a C-terminal extension that acts as a calmodulin binding domain (CaMBD) (Baum et al. 1993). Therefore, it is thought that transient accumulation of intracellular calcium in response to various stresses may activate GAD via interaction of the CaMBD with a Ca^{2+}/CaM complex. We recently identified a cDNA encoding a novel GAD isoform in rice (*OsGAD2*) (Akama et al. 2001). Like the GADs from dicotyledonous plants, OsGAD2 has a C-terminal extension, but it has no ability to bind to Ca^{2+}/CaM. In transgenic rice cells that overexpressed *OsGAD2* lacking the coding region for the C-terminal 30 amino acids (*OsGAD2△C*), extremely high levels of GABA were accumulated (300 pmol/mg FW and 30,000 pmol/mg FW in wild-type and *OsGAD2ΔC* calli, respectively), whereas the remaining free amino acids analyzed were present at almost the same levels as in wild-type cells, except for a several-fold increase in Gln and a several-fold decrease in Glu, Asp and Asn. Given that GABA production is induced by stress (Bouché and Fromm 2004), and that GABA functions to guide the pollen tube in *Arabidopsis* (Palanivelu et al. 2003), it is probable that GABA plays a key role in physiological regulation, for example by acting as a phytohormone, in plant cells. In order to investigate this possibility, we compared the transcription patterns of wild-type and GABA-accumulating calli using oligo microarray analysis.

Plant binary vector pCAMBIA1302 (Cambia) or pCAMBIA1302 carrying CaMV35S::OsGAD2ΔC were introduced into fresh rice calli (*Oryza sativa* L. cv. Kitaake) that had been maintained in 2N6 solid media. The cells were then transferred to 100 ml flasks containing 20 ml of liquid 2N6 media to initiate a suspension culture. The flasks were placed on a shaker 120 rpm at 28 °C in the dark. To maintain uniformity of cell size in the rice suspension cultures, cells were passed through mesh (pore size: 1 mm) and were transferred to fresh medium every 7 days to repeat for one month. Then, two days after transfer of the cells to the same fresh medium, total RNAs were extracted from

cultured cells from these two different lines. For expression profiling by microarray analysis, we used 800 ng of total RNA per microarray slide (Rice Oligo Microarray Kit, 22K; Agilent). To eliminate false-positive results, we performed a dye-swap-experiment using two arrays. The procedure used for the microarray analysis was essentially as described by Yazaki et al. (2000). After hybridization and washing, the arrays were scanned using an Agilent Microarray Scanner. For spots that showed more than 5.55-fold higher signal intensity (see below) after normalization in samples corresponding to the GABA-accumulating cells, a BLAST search was performed. cDNAs from a total of 60 spots were selected and Table 1 lists these putative genes with elevated expression according to function. First, *OsGAD2* expression was up-regulated 5.55-fold. Endogenous and introduced *GAD2* genes were both monitored in the present system, and the observed up-regulation was considered to be due to overexpression of the truncated form of *OsGAD2*. The observed up-regulation of ACC oxidase (AK058296), a key enzyme in the synthesis of ethylene, is to be expected, given that GABA induces accumulation of *ACC oxidase* mRNA (Kathiresan et al. 1998). These results suggest that expression patterns of the GABA-inducible genes could be faithfully monitored in this experiment. Of the functional classes of proteins shown in Table 1, the lipid and cell wall-related proteins are the most numerous, with ten different genes found to be up-regulated. Because previous reports have indicated that GABA controls the elongation of stems and pollen tubes (Kathiresan et al. 1998, Palanivelu et al. 2003), it is tempting to conclude that the GABA-triggered expression of genes related to cell wall structure is involved in this dynamic growth. The transcription factor, transporter and stress-response-related classes of proteins each comprised three or four genes that were up-regulated. In particular, expression of *YABBY* ortholog (AK070205), which is known to play an important role in leaf and flower formation in plants (Bowman et al. 2002), was up-regulated about 20-fold in the GABA-accumulating cells. To date, little is known about the relationship between GABA and regulation of gene expression, so in future studies it would be worth investigating the possibility that GABA functions as a signal molecule that induces expression of transcription factors such as YABBY.

Table 1. Genes that were overexpressed in GABA-overaccumulating rice calli

Accession No	Putative Gene Function	P-value	Fold increase
	Transcription factor		
AK070205	yabby14 protein	1.00E-71	18.92
AK100786	transcription factor	3.00E-09	7.36
AK068359	DNA binding / transcription factor	2.00E-09	8.50
	Transporter		
AK059423	putative sorbitol transporter	0	6.26
AK060520	C4-dicarboxylate transporter/malic acid transport protein	0	10.66
AK064565	PPAK motif; Heavy metal transport/detoxification protein	8.00E-17	6.25
AK066565	tetracycline:hydrogen antiporter/ transporter	2.00E-108	7.00
	Lipid and cell wall related		
AK073016	fatty acid elongase	3.00E-65	9.97
AK104609	carboxylic ester hydrolase	2.00E-110	11.70
AK058583	Lipoxygenase, LH2	1.00E-25	8.77
AK067173	beta-1,3-glucanase-like protein	7.00E-94	8.11
AK103715	extensin-like protein	7.00E-24	7.17
AK106266	CXE carboxylesterase	6.00E-100	6.36
AK102449	beta-N-acetylhexosaminidase	0	9.55
AK058634	pectinacetylesterase precursor	5.00E-138	6.76
AK071034	UDP-D-glucuronate decarboxylase	7.00E-178	6.09
AK110615	2-alkenal reductase	5.00E-126	7.23
	Stress responsive related		
AK068340	glutamate decarboxylase OsGAD2	0	5.55
AK069815	stress responsive protein	4.00E-66	6.66
AK108464	Avr9/Cf-9 rapidly elicited protein 146	2.00E-16	6.20
AK071605	rust-resistance protein Lr21	6.00E-67	6.91
	Unclasiffied		
AK110972	oxidoreductase	3.00E-74	6.98
AK061856	oxidoreductase	2.00E-61	6.72
AK059489	putative peroxidase	4.00E-77	6.20
AK069636	GDP dissociation inhibitor protein	0.00E+00	7.58
AK061298	RNA helicase GLH-2	4.00E-10	6.66
AK111398	Transposase, IS4	1.00E-45	6.77
AK108549	putative cell division protein	5.8	6.67
AK107262	TNP1	0.37	6.66
Accession No	**Putative Gene Function**	**P-value**	**Fold increase**
(continued)			
AK105307	nutrient reservoir	5.00E-73	6.31
AK058296	1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)	0	6.27
AK108464	Avr9/Cf-9 rapidly elicited protein 146	2.00E-16	6.20
AK108504	hypothetical telomeric SfiI fragment 20 protein 3	4.00E-06	6.17
AK107430	similar to myosin, heavy polypeptide 13, skeletal muscle isoform 2	0.24	11.69
AK107210	Chalcone and stilbene synthases, N-terminal	4.00E-152	10.86
AK059149	hydrolase, hydrolyzing O-glycosyl compounds	8.00E-149	9.16
AK062203	ribulose bisphosphate carboxylase large chain	1.00E-92	7.98
AK058623	ribulose bisphosphate carboxylase large chain	1.00E-92	7.90
	UnKnown function		
AK067296	ACR4; amino acid binding	3.00E-132	6.34
AK061054	putative alliin lyase	4.00E-62	6.37
AK102039	ABA induced plasma membrane protein PM 19	5.00E-55	8.35
AK105608	putative receptor-like protein kinase	2.00E-104	7.40
AK109898	permease	4.7	5.75
AK109325	putative protein	6.00E-08	7.64
AK069283	Protein of unknown function DUF6, transmembrane	2.00E-79	12.70
AK064117	ankyrin-like protein-like protein	3.00E-75	21.58
AK063903	hypothetical protein	2.1	6.06
AK073556	CRK11 (CYSTEINE-RICH RLK11); kinase	5.00E-93	6.09
AK073710	No significant similarity found	-	14.54
AK103860	No significant similarity found	-	6.01
AK059204	No significant similarity found	-	6.11
AK060047	unknown protein	9.00E-05	6.32
AK099538	unknown protein	6.00E-91	9.42
AK069354	unknown protein	1.00E-14	9.92
AK107423	unknown protein	6.00E-39	9.57
AK073398	unknown protein	3.00E-17	10.06
AK063682	unknown protein	0.002	8.89
AK099618	unknown protein	4.00E-05	8.87
AK061581	unknown protein	8.00E-45	7.45
AK107707	unknown protein	7.00E-12	6.11
AK103494	unknown protein	1.00E-62	5.97

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