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David P. Horvath

USDA, Agricultural Research Service

Danny Llewellyn

Sharon A. Clay

South Dakota State University, sharon.clay@sdstate.edu

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Heterologous Hybridization of Cotton Microarrays with Velvetleaf (*Abutilon theophrasti*) Reveals Physiological Responses Due to Corn Competition

David P. Horvath, Danny Llewellyn, and Sharon A. Clay*

Microarray analysis was used to identify changes in gene expression in velvetleaf that result from competition with corn. The plants were grown in field plots under adequate N (addition of 220 kg N ha⁻¹) to minimize stress and sampled at the V6 growth stage of corn (late June). Leaf area, dry weight, and N and P concentration were similar in velvetleaf plants grown alone or with corn. Competition, however, did influence velvetleaf gene expression. Genes involved in carbon utilization, photosynthesis, red light signaling, and cell division were preferentially expressed when velvetleaf was grown in competition with corn. A less clear picture of the physiological impact of growth in monoculture was provided by the data. However, several genes involved in secondary metabolism and a gene preferentially expressed in response to phosphate availability were induced. No differences were observed in genes responsive to water stress or sequestering/transporting micronutrients.

Nomenclature: Velvetleaf, *Abutilon theophrasti* L. ABUTH.; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.

Key words: Weed competition, genomics.

Recent advances in genomics technology have opened opportunities to answer a number of fundamental questions in weed biology (Basu et al. 2004; Chao et al. 2005; Horvath et al. 2006). However, many weeds still lack the infrastructure required to allow easy access to genomic-based tools such as microarrays and deep expressed sequence tags (EST) databases. To offset this deficiency, it has been proposed that genomic tools developed for crops that are related to troublesome weed species could be used to clone and follow the expression of genes in weeds. Indeed, some very preliminary work using arabidopsis (*Arabidopsis thaliana* L.) microarrays demonstrated their usefulness in following changes in the transcriptome of weeds as diverse as wild oat (*Avena fatua* L.) and leafy spurge (*Euphorbia esula* L.) (Horvath et al. 2003a, 2003b). However, such wide cross-species hybridizations limit the number of genes that can be studied and also can confound the interpretation of the data. However, with the recent explosion of EST databases and development of microarrays from numerous and diverse crop and model species, it is now possible to obtain microarrays from species that are much more closely related to a given weed species. Indeed, recent data from cross-species hybridization of potato (*Solanum tuberosum* L.) microarrays with a battery of *Solanaceous* plants suggest that there is minimal loss of sensitivity when within-family hybridizations are attempted (Rensink et al. 2005). In interest of understanding the interactions between velvetleaf and corn, we have chosen to use whole-plant cDNA microarrays developed from cotton to study gene expression differences in velvetleaf in response to competition with corn.

Velvetleaf, like cotton, is a member of the *Malvaceae* family. It is a dicotyledonous annual weed that infests row crops in many regions of the world (Warwick and Black 1988). A native plant of China and India, velvetleaf was originally introduced to the United States before the 1700s as a possible fiber crop to be used for rope and fabric production (Mitich 1991; Spencer 1984; Warwick and Black 1988).

Velvetleaf-corn interactions have been extensively studied at the whole-plant and population levels, and the interaction between these two species is often used as a model for crop-weed interactions (Lindquist 2001; McDonald et al. 2004; Sattin et al. 1992; Teasdale 1995). Several studies have indicated that a critical period of crop-weed interaction occurs between the V3–V8 stage of corn development, and that weed removal after this period has little effect on crop growth and yield (Bryson 1990; Hall et al. 1992; Norsworthy and Oliviera 2004; Van Acker et al. 1993). The mechanisms controlling this irreversible physiological response of corn to weeds is unknown. However, work by Rajcan et al. (2004) suggests that one mechanism might be a classic shade-avoidance response due to differential reflection of red and far-red light that allows the corn to perceive and respond to the presence of weeds. The ability of the plant to “remember” the presence of weeds early in development has been hypothesized to involve epigenetic changes in gene expression due to chromatin remodeling (Horvath et al. 2006). Chromatin remodeling involves the process of modifying the histone proteins around which the DNA is wrapped in such a way as to modify the expression of a subset of affected genes (Reyes et al. 2002). Work by Horvath et al. (2006) indicated that genes involved in photosynthesis, auxin signaling, cell division, and protein degradation were preferentially expressed in the upper leaves of corn grown in velvetleaf-free plots relative to similar tissue from corn competing with velvetleaf. These changes in gene expression were observed well after the tissue in question had grown much higher than the infesting velvetleaf and thus may have been permanently altered by early exposure to velvetleaf competition.

Although a picture is emerging concerning how corn responds to velvetleaf competition, significantly less is known about what effect the corn has on the velvetleaf. Roggenkamp et al. (2000) noted a small but detectable negative effect of tall corn hybrids on fecundity of competing velvetleaf. Additionally, Nurse and DiTommaso (2005) demonstrated that corn competition altered the size and dormancy of velvetleaf seed. Velvetleaf biomass is also reduced and plant architecture altered due to competition with corn (Steinmaus and Norris 2001; Sultan et al. 2001). Corn competition has also been hypothesized to favor velvetleaf varieties that grow

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* Bioscience Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Fargo, ND 58105-5674; CSIRO Plant Industry, Canberra City ACT 2601, Australia; Department of Plant Science, South Dakota State University, Brookings SD 57007. Corresponding author's E-mail: horvathd@fargo.ars.usda.gov



Figure 1. Photo of velvetleaf growing with and without corn at time of tissue collection.

faster early in development but that respond relatively less dramatically to competition later in the season (Weinig 2000). However, no molecular studies have yet been done to identify the mechanisms that might bring about these changes in growth and development. In this report, we compare the transcriptome of velvetleaf grown in monoculture to the transcriptome of velvetleaf in direct competition with corn with the intent of identifying differential expression of numerous well-characterized genes to determine which signal transduction and physiological pathways are altered by competition with corn.

Materials and Methods

Plant Material. Velvetleaf was sown on May 8, 2006, alone or with corn (DKC46-22) at Aurora, SD, on a Brandt silty clay loam soil (fine-silty, mixed superactive, frigid Calcic Hapludoll). The sand, silt, and clay contents were 390, 383, and 226 g kg⁻¹, respectively, with a soil pH of 6.0 and organic matter content of 35 g kg⁻¹. Corn was planted at a population of about 24,000 plants ha⁻¹. Corn plots remained weed-free or were planted with velvetleaf seed. Velvetleaf was planted about 1.5 cm deep using a seed drill and was either seeded alone or in the interrow area of corn about 20 cm from the crop row. Granular urea was broadcast at 224 kg N ha⁻¹ after planting. Weed management for species other than velvetleaf consisted of a glyphosate application as a burn-down treatment prior to plant emergence and hand removal for the rest of the season. There was some variability in the density of velvetleaf between plots because of uneven germination; however, in general, velvetleaf density was about 6 plants m⁻² (ranging from 4 to 8 plants m⁻²) after germination. Treatments were placed in a randomized complete block design with three replicates. Plots were 6 m long by four rows wide with a row spacing of 76 cm and three replications per treatment.

Four individual velvetleaf plants within each treatment plot were collected on June 22 between 1:00 and 2:00 P.M. Conditions were partly cloudy and breezy with a temperatures between 25 and 27 C. The apical meristem and all of the young leaves (up to 6 cm in diameter) were collected from

each sampled plant. The samples from within each plot were pooled and immediately frozen in liquid N₂. Two representative velvetleaf and corn plants were harvested from each plot to determine leaf area, dry weight, and N and P concentrations. At physiological maturity of the corn, grain yield was determined based on hand-harvest of 10 m² area per plot.

RNA Extraction and Microarray Analysis. Frozen plant material was ground to a fine powder in liquid nitrogen using a mortar and pestle. RNA was extracted from the resulting powder using the pine tree extraction method (Chang et al. 1993). Labeled cDNA was prepared from 30 µg of total RNA using the Alexa Fluor cDNA labeling kit¹ according to manufacture's protocols. Labeled cDNA was hybridized to 24K element cotton microarrays produced by Dr. Llewellyn and colleagues, CSIRO Plant Industry, Australia, from cotton cDNA libraries according to previously published protocols (Horvath et al. 2006). The array contained clones predominantly from young ovules and elongating fibers, but also included leaves, roots, and immature embryos. A full list of the clones and the layout of the microarrays can be obtained from the Gene Expression Omnibus (GEO), accession number GPL4043. A rolling-circle dye-swap hybridization scheme with two cross-circle comparisons (Churchill 2002) was used to compare gene expression between velvetleaf monocultures and velvetleaf grown in competition with corn from three biological replicates of each treatment. Eight different two-dye hybridizations were performed that compared velvetleaf alone vs. velvetleaf with corn treatments. Chips were hybridized and washed following previously published protocols (Horvath et al. 2006). Hybridization intensities (based on fluorescence) for each probe (spotted clone) were visualized and quantified using an Affy 428 scanner² and Jaguar software².

Northern Hybridization. Twenty micrograms of total RNA from each of the three biological replicates from each treatment were separated on 1% denaturing agarose gels. Gels were blotted and probed with radiolabeled cDNA from selected cotton probes that were used in preparation of the cotton microarrays. Blots were washed at least one time at

Table 1. Expression data from all genes preferentially expressed in velvetleaf grown in monoculture (P values less than 0.05 and/or q values of less than 20%). Bold type indicates significant P or q values.

GEO ID	Log ₂ ratio	Ave A value	q value %	P value	GenBank accession no.	BLAST hit	Functional category
2875	-0.38	10.25	43.59	0.04	BG446836	Acetylglutamate kinase	Amino acid metabolism
926	-0.5	11.65	43.59	0.03	CD486399	AT1G05210	Carbon metabolism
14502	-0.24	11.81	43.59	0.04	DT458137	AT1G05170	Carbon metabolism
194	-0.49	9.75	43.59	0.02	DV850171	Probable pantothenate kinase 1	Carbon utilization
8947	-0.43	11.36	43.59	0.05	BG446732	Plastidic glucose-6-phosphate dehydrogenase	Carbon utilization
8999	-0.44	11.17	43.59	0.04	BQ403038	Acetyl coA acetyltransferase	Carbon utilization
11136	-0.48	10.41	43.59	0.01	BG440091	NADH-dependent glutamate synthase	Carbon utilization
11552	-0.39	11.94	43.59	0.03	CD486505	acetyl-coA C-acyltransferase	Carbon utilization
14365	-0.35	12.08	43.59	0	DV849957	Carbohydrate kinase-like protein	Carbon utilization
15864	-0.31	10.97	43.59	0.04	BF269993	3,5-epimerase/4-reductase	Carbon utilization
16335	-0.39	10.72	43.59	0.05	BG445737	Pyruvate dehydrogenase e1 beta subunit isoform 1	Carbon utilization
16337	-0.33	10.78	43.59	0.04	BG445442	UDP-glucose dehydrogenase 2	Carbon utilization
16596	-0.34	10.11	43.59	0.02	N/A	Pyruvate kinase, cytosolic isozyme	Carbon utilization
17128	-0.33	10.94	43.59	0.01	BQ404445	ATP citrate lyase B-subunit	Carbon utilization
23385	-0.66	10.26	43.59	0.01	BG442925	Glutamine-fructose-6-phosphate transaminase 2	Carbon utilization
1663	-0.57	9.63	43.59	0.03	BG445392	Histone H4	Cell division
2892	-0.37	10.57	43.59	0.02	DT459013	Translationally controlled tumor protein homolog	Cell division
3391	-0.3	10.83	43.59	0.05	BQ409394	Tubulin beta-1	Cell division
13261	-0.35	10.63	43.59	0.01	BG443267	Pescadillo-like protein	Cell division
18659	-0.45	10.6	43.59	0.02	BQ405860	Male sterility MS5 family protein	Cell division
19086	-0.38	12.12	43.59	0.01	DT458835	Meiotic serine proteinase TMP	Cell division
21238	-0.54	9.71	43.59	0.04	BF274181	Tubulin folding cofactor A	Cell division
23101	-0.34	12.89	43.59	0.01	DT458514	MOB1/phoccein family protein	Cell division
4412	-0.3	11.53	43.59	0.03	DT458789	Porin	Cellular communication
8433	-0.39	11.47	43.59	0.02	DT457819	Porin	Cellular communication
23132	-0.4	12.89	43.59	0	DT459107	Mitochondrial import receptor subunit TOM6 homolog	Cellular communication
4440	-0.3	14.35	43.59	0.03	DT459696	Ovate protein	Development and growth
10990	-0.29	10.64	43.59	0.03	DT458803	Crumpled leaf	Development and growth
18870	-0.26	9.83	43.59	0.03	BG445320	Vacuolar ATP synthase subunit B isoform 2	Energy production
18684	-0.29	9.57	43.59	0.01	BQ406947	Dihydroflavonol reductase	Flavone biosynthesis
19282	-0.25	13.63	43.59	0.03	BG441541	Reduced vernalization response 1	Flowering
21661	-0.44	9.59	43.59	0.03	BQ402258	Flowering time control protein	Flowering
7450	-0.4	9.93	43.59	0.03	DT458547	Dolichyl pyrophosphate alpha-1,3-glucosyltransferase	Glycosylation
2118	-0.33	10.1	43.59	0.05	BG443772	Xyloglucan endotransglycosylase	Growth
4744	-0.38	10.66	43.59	0.02	BF268379	Endochitinase 1 precursor	Growth
13033	-0.38	10.12	43.59	0.02	BQ410959	Actin-related complex protein	Growth
15570	-0.22	11.73	43.59	0.05	DT459961	Endomembrane protein EMP70 precursor isolog	Growth
16538	-0.38	11.08	43.59	0.01	BG446894	Extensin	Growth
22854	-0.37	13.3	43.59	0.04	DT527466	Beta-1,3-glucanase	Growth
22894	-0.64	9.85	43.59	0.02	DV850326	Brassinosteroid-regulated protein BRU1	Growth
15127	-0.42	13.4	43.59	0.04	CD486328	Dormancy-associated protein	Hormone response auxin
22641	-0.52	11.31	43.59	0.01	DT460588	Auxin-induced protein	Hormone response auxin
4637	-0.33	14.23	43.59	0.02	DT527469	Ripening regulated protein DDTFR18	Hormone response ethylene
20628	-0.47	10.72	43.59	0.04	DT460236	EIN2	Hormone response ethylene
14785	-0.45	11.27	43.59	0	DV848799	Enolase	Hormone response ethylene?
18568	-0.55	12.62	43.59	0.04	DT457054	Cinnamoyl-coA reductase	Lignin biosynthesis
5414	-0.35	10.77	43.59	0.03	BQ409890	Lipoic acid synthase LIP1 (LIP1)	Lipid metabolism
7973	-0.26	9.6	43.59	0.05	BQ410871	Lysophospholipase	Lipid metabolism
10238	-0.32	10.8	43.59	0.04	BG444883	Phospholipase D alpha 1 precursor	Lipid metabolism
11029	-0.4	12.18	43.59	0.01	N/A	Oleosin 164 KDA	Lipid metabolism
19110	-0.28	13.36	43.59	0.01	DT460220	AT1G04970	Lipid metabolism
22601	-0.51	12.52	43.59	0.03	DT457875	Phospholipase	Lipid metabolism
23358	-0.45	9.65	43.59	0	BG442184	Lipase/hydrolase	Lipid metabolism
6421	-0.21	14.07	43.59	0.01	DT457336	AT1G11060	Mitochondrial
4385	-0.35	10.43	43.59	0.02	DT457802	Leaf ubiquitous urease	Nitrogen metabolism
7110	-0.31	11.25	43.59	0.05	BG440999	Purine permease	Nitrogen metabolism
9466	-0.42	10.63	43.59	0.03	BQ408932	Cyanate lyase (CYN)	Nitrogen metabolism
17567	-0.33	10.57	43.59	0.04	DT458966	Cystathionine gamma-synthase isoform 2	Nitrogen metabolism
333	-0.31	10.55	43.59	0.02	DT458185	DNA polymerase delta catalytic subunit	Nucleic acid metabolism
2271	-0.29	9.95	43.59	0.03	BF275099	RNA helicase	Nucleic acid metabolism
2358	-0.37	12.27	43.59	0.02	DT458092	AMP deaminase	Nucleic acid metabolism
4231	-0.27	10.16	43.59	0.02	BF269375	Poly(a)-binding protein II-like	Nucleic acid metabolism
10989	-0.35	10.61	43.59	0.03	DT458922	6-4 photolyase	Nucleic acid metabolism
12696	-0.34	13.04	43.59	0.01	DT527154	Poly(a) polymerase	Nucleic acid metabolism
14558	-0.39	11.63	43.59	0.04	DT459972	Oligouridylylate binding protein, putative	Nucleic acid metabolism
16249	-0.22	13.42	43.59	0.04	DT526980	Small nuclear ribonucleoprotein U2B	Nucleic acid metabolism
17054	-0.31	11.16	43.59	0.02	BQ409501	RNA helicase	Nucleic acid metabolism
18302	-0.22	13.64	43.59	0.05	N/A	Similarity to RNA-binding protein	Nucleic acid metabolism
19646	-0.38	9.77	43.59	0.04	DT460942	AT1G05470	Nucleic acid metabolism
21049	-0.53	10.22	43.59	0.01	BF276643	Polyadenylate-binding protein	Nucleic acid metabolism

Table 1. Continued

GEO ID	Log ₂ ratio	Ave A value	q value -%-	P value	GenBank accession no.	BLAST hit	Functional category
21424	-0.44	9.91	43.59	0.04	BE055620	Small nuclear ribonucleoprotein D1	Nucleic acid metabolism
22612	-0.38	13.32	43.59	0.04	DT457546	RNA helicase	Nucleic acid metabolism
5458	-0.4	9.74	43.59	0.01	BQ402979	Cytochrome c oxidase-related	Oxidative stress
17669	-0.33	13.05	43.59	0	N/A	Phosphate-induced protein cell cycle-related	Phosphate response
20436	-0.46	12.14	43.59	0.01	DV850059	Phosphate inducible protein 1	Phosphate response
4399	-0.41	12.08	43.59	0.04	DT457109	Aspartate aminotransferase	Photosynthesis
10789	-0.49	12.44	43.59	0.01	DV849803	67-KDa chloroplastic rna-binding protein	Photosynthesis
13138	-0.41	11.08	43.59	0.03	BQ407400	DAG protein, chloroplast precursor	Photosynthesis
21080	-0.38	12.13	43.59	0.04	DT458167	Oxygen-evolving enhancer protein 2-1	Photosynthesis
22886	-0.51	10.43	43.59	0	BG445014	COP9 complex subunit 3	Photosynthesis
2383	-0.39	10.79	43.59	0.05	DT459361	Subtilisin-like protease-like protein	Protein metabolism
3508	-0.34	9.89	43.59	0.03	BQ406506	Ribosomal protein s5	Protein metabolism
5194	-0.57	10.53	43.59	0.01	BG445810	Ribosome biogenesis regulatory protein homolog	Protein metabolism
6406	-0.27	11.89	43.59	0.01	DT458104	60S ribosomal protein L5	Protein metabolism
6746	-0.38	10.32	43.59	0.04	DV849545	Ribosomal protein L12	Protein metabolism
8102	-0.43	11.21	43.59	0.02	BG439951	F-box family protein	Protein metabolism
8742	-0.22	9.68	43.59	0.01	DV849184	Immunophilin	Protein metabolism
8939	-0.41	13	43.59	0.02	DT457814	60S ribosomal protein L37	Protein metabolism
10364	-0.32	9.45	43.59	0.01	BF275448	Translation factor	Protein metabolism
10499	-0.38	10.54	43.59	0.04	BQ411689	60S ribosomal protein L13A-4	Protein metabolism
11017	-0.36	12.5	43.59	0.04	DT459831	Cullin 1	Protein metabolism
11186	-0.31	12.56	43.59	0.04	BE053077	60S ribosomal protein L14	Protein metabolism
11472	-0.36	9.67	43.59	0.02	BG447322	40S ribosomal protein S27-2	Protein metabolism
12340	-0.39	12.47	43.59	0.01	DV850055	60S ribosomal protein L17	Protein metabolism
14589	-0.38	10.77	43.59	0.05	N/A	Glu-tRNA(GLN) amidotransferase subunit B	Protein metabolism
15095	-0.38	11.5	43.59	0	N/A	Proteasome subunit alpha type 5	Protein metabolism
15830	-0.3	10.07	43.59	0.05	BG445597	50S ribosomal protein L29	Protein metabolism
16389	-0.3	12.38	43.59	0.03	DV849958	Ubiquitin activating enzyme 1	Protein metabolism
17033	-0.33	10.08	43.59	0.05	DT458039	Translational inhibitor protein	Protein metabolism
17064	-0.25	11.56	43.59	0	DT458608	40S ribosomal protein S24	Protein metabolism
18390	-0.44	11.06	43.59	0.02	BE054519	Ubiquitin-conjugating enzyme 8	Protein metabolism
18609	-0.36	12.9	43.59	0.01	DT459642	Ubiquitin-conjugating enzyme	Protein metabolism
21079	-0.36	10.69	43.59	0.04	DT458265	40S ribosomal protein S15	Protein metabolism
21390	-0.37	13.6	43.59	0.03	DV849252	Protein translation factor SUI1	Protein metabolism
22244	-0.49	12.73	43.59	0	DV850318	60S ribosomal protein L6 (YL16-LIKE)	Protein metabolism
22597	-0.52	10.2	43.59	0.04	DT458273	F-box protein family, ATFB16	Protein metabolism
2917	-0.43	10.15	43.59	0.03	DT460254	Non-LTR retroelement reverse transcriptase	Retrovirus
2970	-0.38	9.62	43.59	0.03	BQ403289	Reverse transcriptase	Retrovirus
3260	-0.4	9.82	43.59	0.03	DT464634	Polyprotein	Retrovirus
14897	-0.27	11.82	43.59	0.05	DT464774	Gag-Pol	Retrovirus
16523	-0.23	12.58	43.59	0.01	DT458494	Transposase protein	Retrovirus
16547	-0.32	9.58	43.59	0.02	BQ409625	<i>cis</i> -Prenyltransferase	Secondary metabolism
17353	-0.34	10.7	43.59	0.02	BG445132	Hydroxymethylglutaryl-coA lyase	Secondary metabolism
19619	-0.3	12.76	43.59	0.02	DT459862	Mevalonate kinase	Secondary metabolism
1973	-0.21	9.54	43.59	0.03	BQ405267	Signal recognition particle 19-KDa protein	Signal transduction
4383	-0.4	12.41	43.59	0.03	DT457997	Zinc finger protein-like; Ser/Thr protein kinase	Signal transduction
4392	-0.41	11.7	43.59	0.04	BG446905	Clathrin coat assembly protein	Signal transduction
6962	-0.42	10.05	43.59	0.01	BQ410930	MAP kinase kinase	Signal transduction
7291	-0.54	9.53	43.59	0.01	BF270995	RAB7A	Signal transduction
11266	-0.32	11.46	43.59	0.02	BG445837	Cleft lip and palate transmembrane protein	Signal transduction
14514	-0.32	12.02	43.59	0.03	BG446753	Ser-Thr protein kinase	Signal transduction
15603	-0.51	10.17	43.59	0	N/A	RAB11G	Signal transduction
16567	-0.33	12.12	43.59	0.03	DT460835	Calmodulin-binding family protein	Signal transduction
16581	-0.22	12.76	43.59	0.01	DT460105	Leucine-rich repeat transmembrane protein kinase	Signal transduction
17504	-0.37	10.37	43.59	0.01	BF277629	Mitogen-activated protein kinase homologue	Signal transduction
18010	-0.5	10.38	43.59	0.04	BF276827	Potassium channel 5 outward-rectifying	Signal transduction
19015	-0.29	12.5	43.59	0	DT468110	Receptor kinase	Signal transduction
19083	-0.4	11.14	43.59	0.02	DT459219	Leucine-rich protein kinase	Signal transduction
19115	-0.34	11.67	43.59	0.01	DT459639	Protein kinase ATMRK1	Signal transduction
21323	-0.4	13.57	43.59	0	DT527646	Receptor protein kinase	Signal transduction
21615	-0.39	11.79	43.59	0.01	DT458986	AT1G07150	Signal transduction
23410	-0.53	10.97	43.59	0	BG445840	DSPTP1 protein	Signal transduction
7485	-0.36	10.36	43.59	0.01	N/A	Legumin A precursor	Storage protein
15604	-0.37	10.95	43.59	0.02	N/A	Seed maturation protein PM38 protein	Storage protein
17098	-0.38	10.51	43.59	0.02	N/A	Legumin A precursor	Storage protein
2880	-0.37	11.33	43.59	0.03	DT457195	Immediate-early fungal elicitor protein CMPG1	Stress response-disease resistance
4705	-0.31	10.68	43.59	0.04	DV848993	Anthocyanin 5-aromatic acyltransferase	Stress response-disease resistance
5149	-0.75	10.25	43.59	0.01	BG444002	Pore-forming toxin-like protein HFR-2	Stress response-disease resistance
10955	-0.4	11.21	43.59	0.04	BG446359	Anthocyanin 5-aromatic acyltransferase	Stress response-disease resistance
13490	-0.38	10.5	43.59	0.01	DT458129	Disease resistance response protein	Stress response-disease resistance
6966	-0.34	11.27	43.59	0.03	DT460168	Heat-shock protein 40	Stress response-heat shock
18780	-0.46	14.2	43.59	0.01	DT526946	Heat-shock protein	Stress response-heat shock

Table 1. Continued

GEO ID	Log ₂ ratio	Ave A value	q value –%–	P value	GenBank accession no.	BLAST hit	Functional category
4183	–0.34	12.66	43.59	0.01	DV849409	Farnesylated proteins ATFP4	Stress response–heavy metal
5398	–0.41	11.76	43.59	0.04	DT457682	Calcineurin-like protein	Stress response–osmotic
6730	–0.26	14.11	43.59	0.02	DV848981	Thioredoxin H-type	Stress response–osmotic
9496	–0.42	10.53	43.59	0.02	DT460181	Calcineurin B	Stress response–osmotic
13798	–0.28	11.98	43.59	0.02	DV849325	Cer3 protein	Stress response–osmotic
18041	–0.4	12.36	43.59	0.03	DT458478	Histidine kinase-like protein	Stress response–osmotic
10983	–0.35	10.05	43.59	0.01	BQ409091	Ubiquinol–cytochrome c reductase complex	Stress response–oxidative
12478	–0.39	12.54	43.59	0.02	DT458136	Monooxygenase precursor (skewed roots)	Stress response–oxidative
12736	–0.27	13.84	43.59	0.03	DT527366	Thaumatococin	Stress response–oxidative
13334	–0.46	10.59	43.59	0	BF270006	Iron superoxide dismutase 3	Stress response–oxidative
15094	–0.22	10.16	43.59	0.03	CD486545	Peroxidase precursor	Stress response–oxidative
18890	–0.4	10.62	43.59	0.05	DV849552	Oxidoreductase, zinc-binding	Stress response–oxidative
19617	–0.42	10.73	43.59	0.04	DT460099	AT1G06240	Stress response–oxidative
20220	–0.36	13.2	43.59	0	N/A	Superoxide dismutase [Cu–Zn] 2	Stress response–oxidative
6593	–0.55	9.43	43.59	0.01	BE054141	4-coumarate-coA ligase-like protein	Stress response–wounding
15175	–0.38	10.17	43.59	0.01	BQ407055	GDSL-motif lipase/hydrolase-like protein	Stress response–wounding
4121	–0.34	12.3	43.59	0.01	DT527598	NAM-like protein	Transcription
9013	–0.42	10.61	43.59	0.05	BQ401852	Transcription factor II homolog	Transcription
12717	–0.27	13.79	43.59	0	DT455782	BHLH transcription factor-like protein	Transcription
15598	–0.26	10.6	49.13	0.03	DT460920	WRKY transcription factor 23	Transcription
16246	–0.3	11.99	43.59	0.02	BG441590	MYB transcription factor	Transcription
16270	–0.36	10.98	43.59	0.04	BG442627	DNA-binding protein	Transcription
18597	–0.33	10.79	43.59	0.04	BQ411289	ATP-dependent RNA helicase	Transcription
19042	–0.18	13.92	46.29	0.03	DT456608	Zinc finger (C2H2 type)	Transcription
19909	–0.51	10.3	43.59	0.03	BG445970	WRKY6-like protein	Transcription
21667	–0.59	11.15	43.59	0.01	DT461306	Zinc finger (CCCH-type)	Transcription
21670	–0.44	11.2	43.59	0.01	DT460965	SNF5, transcription regulatory protein homolog BSH	Transcription
23136	–0.38	11.23	43.59	0.04	DT458631	BZIP-like protein	Transcription
2361	–0.4	11.04	43.59	0.01	DT457792	AT1G70280	Unknown
2375	–0.44	11.04	43.59	0.04	DT457090	AT4G35220	Unknown
2380	–0.36	10.44	43.59	0.05	BQ409658	AT3G44190	Unknown
2433	–0.37	10.99	43.59	0.05	BQ402642	AT3G04680	Unknown
2449	–0.37	9.65	43.59	0.05	N/A	AT5G13300	Unknown
2867	–0.37	11.46	43.59	0.05	DT457789	AT3G06290	Unknown
2882	–0.45	10.12	43.59	0.04	DT456972	Protein	Unknown
3154	–0.44	10.46	43.59	0.05	BG444762	AT2G44870	Unknown
4142	–0.19	12.97	49.13	0.04	BG443748	Pterophorin-DZ1 protein precursor	Unknown
4182	–0.38	11.25	43.59	0.02	BG445887	AT2G38570	Unknown
4191	–0.38	9.56	43.59	0.05	BG445728	Endosomal protein-like	Unknown
4563	–0.42	10.54	43.59	0.03	N/A	AAA-type ATPase	Unknown
4701	–0.33	9.6	43.59	0.02	BG445307	AT4G27460	Unknown
4707	–0.28	14.29	43.59	0.01	DV848892	AT1G14370	Unknown
4928	–0.37	9.37	43.59	0.01	BG447409	AT4G00150	Unknown
4985	–0.47	10.3	43.59	0.02	BQ404224	KH domain-containing protein-like	Unknown
5115	–0.34	11.15	43.59	0.02	DT527028	AT5G66200	Unknown
5266	–0.49	9.98	43.59	0.01	BF271341	AT4G37100	Unknown
5395	–0.46	11.26	43.59	0.04	DT457987	AT4G17080	Unknown
5397	–0.48	12.39	43.59	0.03	DT457796	AT2G20190	Unknown
5527	–0.6	10.67	43.59	0	BQ406814	AT1G02816	Unknown
6462	–0.21	13.54	46.29	0.02	DT459930	AT3G57080	Unknown
6621	–0.53	9.82	43.59	0	BG440378	AT3G05510	Unknown
6739	–0.4	9.43	43.59	0.04	BE052602	Vegetative cells of pollen VEX1	Unknown
6767	–0.3	10.33	43.59	0.04	BF268175	AT1G12020	Unknown
6810	–0.36	10.3	43.59	0.01	BF273337	AT5G50150	Unknown
6912	–0.44	11.66	43.59	0.02	DT458101	AT2G28380	Unknown
6932	–0.47	11.54	43.59	0.04	BQ409924	AT2G35550	Unknown
7259	–0.52	9.62	43.59	0.04	BG446009	AT3G25030	Unknown
8444	–0.29	12.59	43.59	0.03	DT457482	AT3G18770	Unknown
8485	–0.33	13.38	43.59	0.05	DT460059	AT1G14440	Unknown
8750	–0.3	10.19	43.59	0.04	BG445302	AT5G25754	Unknown
8940	–0.35	12.63	43.59	0.04	DT457709	Expressed protein	Unknown
9293	–0.42	9.93	43.59	0.03	BF268720	AT3G19460	Unknown
9409	–0.46	10.72	43.59	0.04	BF277609	Membrane protein COV-like	Unknown
9504	–0.47	11.42	43.59	0.04	BQ410156	Seed coat burp domain protein 1	Unknown
10712	–0.35	12.07	43.59	0.03	DT527365	AT3G14830	Unknown
10976	–0.39	12.02	43.59	0.04	DT457233	AT2G28430	Unknown
10986	–0.34	12.44	43.59	0.01	DT459287	Glycosylasparaginase-like protein	Unknown
11011	–0.41	10.07	43.59	0.01	BQ410633	Soluble inorganic pyrophosphatase	Unknown
11012	–0.36	10.89	43.59	0	BQ410648	AT2G17670	Unknown
11096	–0.45	10.42	43.59	0.03	BQ406768	BTB/POZ domain-containing protein	Unknown
11346	–0.3	13.82	43.59	0.03	DT462873	AT4G28060	Unknown

Table 1. Continued

GEO ID	Log ₂ ratio	Ave A value	q value -%-	P value	GenBank accession no.	BLAST hit	Functional category
12855	-0.32	9.56	43.59	0.02	BF271744	Formin homology 2 domain-containing protein 5	Unknown
12982	-0.42	11.05	43.59	0.05	DT458342	AT5G27280	Unknown
12988	-0.33	13.27	43.59	0.01	DT457732	AT2G39440	Unknown
13025	-0.34	12.2	43.59	0.01	DT460801	AT2G35330	Unknown
13059	-0.31	12.09	43.59	0.04	BQ402483	YBAB family COG0718	Unknown
13105	-0.29	10.88	43.59	0.04	BQ405405	Nascent polypeptide-associated alpha subunit	Unknown
13106	-0.25	10.01	43.59	0.03	BQ405445	AT1G62390	Unknown
13203	-0.4	10.6	43.59	0.04	BG441993	AT5G22950	Unknown
13328	-0.44	9.71	43.59	0	BE055611	Selenoprotein O	Unknown
13335	-0.35	9.88	43.59	0.01	BF269772	AT5G03540	Unknown
13517	-0.31	12.41	43.59	0.05	DT459190	AT3G04480	Unknown
13576	-0.5	11.99	43.59	0.02	CD486540	MLP-like protein 31	Unknown
14432	-0.42	9.66	43.59	0.01	BF273944	AT5G50740	Unknown
14555	-0.29	12.08	43.59	0.03	DT460325	Lactose operon repressor	Unknown
14585	-0.25	11.33	43.59	0.03	DT461049	Protein transport protein SEC24-like CEF	Unknown
14642	-0.41	9.98	43.59	0.03	BQ406475	AT3G13050	Unknown
15066	-0.36	12.84	43.59	0	DT459734	AT4G22310	Unknown
15078	-0.34	11.04	43.59	0.03	N/A	Pore protein homolog	Unknown
15546	-0.39	12.27	43.59	0.01	DT458583	Integrase (fragment)	Unknown
15625	-0.38	10.54	43.59	0.05	BQ405551	AT1G69210	Unknown
16047	-0.39	12.03	43.59	0.04	DT459186	AT3G56160	Unknown
16252	-0.36	11.16	43.59	0.05	DT526865	AT5G26940	Unknown
16453	-0.32	9.45	43.59	0.03	BF274307	AT5G39865	Unknown
16554	-0.38	9.91	43.59	0.05	DT459092	AT2G46000	Unknown
16734	-0.44	10.88	43.59	0.01	DT462215	YT521-B-like family	Unknown
16813	-0.45	12.19	43.59	0.01	BG444998	AT1G22230	Unknown
16885	-0.37	9.95	43.59	0.04	BF268714	AT4G35920	Unknown
16932	-0.41	10.86	43.59	0.02	BF272959	Armadillo repeat containing protein	Unknown
17074	-0.3	10.88	43.59	0.01	DT460683	AT1G72650	Unknown
17085	-0.43	11.3	43.59	0.02	DT460339	AT2G23390	Unknown
17133	-0.59	9.72	43.59	0.03	BQ403718	Ring-H2 finger protein ATL2M	Unknown
17258	-0.25	11.1	43.59	0.04	BG441531	Integral membrane family protein	Unknown
17558	-0.59	10.36	43.59	0.02	BQ409863	Prefoldin subunit 4	Unknown
18011	-0.44	10.72	43.59	0.04	BF277456	AT4G15600	Unknown
18322	-0.3	11.83	43.59	0.05	BG443421	AT4G25660	Unknown
19069	-0.19	11.34	46.29	0.04	DT457635	AT1G70370	Unknown
19088	-0.33	11.65	43.59	0.03	DT458611	AT2G44380	Unknown
19109	-0.35	11.94	43.59	0.03	DT460341	AT5G08580	Unknown
19114	-0.36	10.92	43.59	0.05	DT459750	AT1G28380	Unknown
19422	-0.5	10.3	43.59	0.02	DV850119	Pentatricopeptide (PPR)	Unknown
19559	-0.31	11.81	43.59	0.02	DT458484	AT3G54020	Unknown
19563	-0.45	10.32	43.59	0.02	DT458037	AT1G60420	Unknown
19572	-0.48	10.69	43.59	0.04	BG447039	NUF2 family domain	Unknown
19591	-0.44	11.21	43.59	0.01	DT458968	AT1G30845	Unknown
19605	-0.29	10.52	43.59	0.04	DT460549	AT1G55980	Unknown
20072	-0.36	12.73	43.59	0.05	DT457744	AT3G27530	Unknown
20350	-0.31	10.73	43.59	0.04	BG442992	AT2G31670	Unknown
20435	-0.44	10.55	43.59	0.03	DV850076	AT1G27530	Unknown
20586	-0.47	11.13	43.59	0.03	BG446898	AT2G17250	Unknown
20652	-0.43	10.77	43.59	0.04	BQ402072	AT3G13460	Unknown
21041	-0.21	12.41	43.59	0.03	DT467874	AT2G44020	Unknown
21156	-0.53	9.35	43.59	0.04	BQ402463	AT1G27190	Unknown
21169	-0.44	10.33	43.59	0.01	N/A	AT2G03780	Unknown
21303	-0.61	9.74	43.59	0	BG441665	AT3G04780	Unknown
21398	-0.55	9.77	43.59	0.01	BG445518	AT3G12920	Unknown
21598	-0.42	10.98	43.59	0.01	BG446804	Nucleolar protein-like	Unknown
21634	-0.12	11.52	51.87	0.41	BQ411231	AT4G01150	Unknown
21642	-0.44	12.46	43.59	0.04	DT459995	Complex 1 family protein	Unknown
21649	-0.45	11.68	43.59	0	BQ403022	AT4G24690	Unknown
21683	-0.46	10.29	43.59	0.01	BQ404065	AT1G12380	Unknown
22544	-0.45	11.06	43.59	0.03	DT466382	AT2G15270	Unknown
22574	-0.34	13.59	43.59	0.03	DT456850	AT3G58490	Unknown
22599	-0.48	11.75	43.59	0.01	DT458073	AT3G16260	Unknown
22622	-0.56	11.04	43.59	0.02	BQ409121	AT1G72970	Unknown
22658	-0.3	10.13	43.59	0.02	DT459559	Autophagy 5-like protein	Unknown
22895	-0.48	14.42	43.59	0.01	DT455989	AT5G14030	Unknown
23108	-0.38	11.48	43.59	0.02	DT457771	Fiber protein FB33	Unknown
23625	-0.42	11.92	43.59	0.04	DT457408	AT2G12400	Unknown
24182	-0.43	10.09	43.59	0.03	N/A	Vicilin C72 precursor (alpha-globulin B)	Unknown

Table 2. Expression data from all genes preferentially expressed in velvetleaf grown in competition with corn (P values less than 0.05 and/or q values of less than 20%). Bold type indicates significant P or q values.

GEO ID	Log ₂ ratio	Ave A value	q value –%–	P value	GenBank accession no.	BLAST hit	Functional category
6416	0.3	10.62	60.33	0.01	BG447123	Acetylglutamate kinase	Amino acid biosynthesis
8238	0.39	10.37	6.13	0.05	DV849114	Aspartate aminotransferase	Amino acid biosynthesis
15853	0.71	11.62	21.85	0.02	DV849507	Arginine decarboxylase	Amino acid biosynthesis
19858	0.43	10.82	11.61	0.12	DT456020	Acetylmethionine aminotransferase	Amino acid biosynthesis
297	0.51	10.26	62.79	0.03	DT467565	NAD-dependent isocitrate dehydrogenase	Carbon utilization
2263	0.68	11.24	9.74	0.03	DT463951	ATP citrate lyase	Carbon utilization
3204	0.61	9.61	49.13	0.01	DV849514	Trehalose-6-phosphate synthase	Carbon utilization
6312	0.7	11.3	4.54	0.02	DT463886	Methylenetetrahydrofolate reductase	Carbon utilization
8128	0.64	10.44	4.54	0.04	BG440679	Succinyl-coA ligase alpha subunit	Carbon utilization
8375	0.43	10.63	6.13	0.07	DT466441	Acyl coA:diacylglycerol acyltransferase	Carbon utilization
8487	0.33	11.22	60.33	0.03	DT459833	3-isopropylmalate dehydratase, small subunit.	Carbon utilization
9304	0.63	10.74	9.74	0.02	DV849994	6-phosphogluconate dehydrogenase	Carbon utilization
9937	0.47	10.45	11.61	0.04	DT468720	Pyruvate decarboxylase 1	Carbon utilization
10004	0.42	11.55	18.82	0.14	DT459931	4-coumarate-coA ligase-like protein.	Carbon utilization
13879	0.68	10.02	0	0.04	DT462539	Acyl-coA oxidase	Carbon utilization
326	0.67	10.62	11.61	0.02	DT468376	Secretory carrier membrane protein	Cellular communication
2348	0.32	11.28	60.33	0.05	DT468591	MRP-like ABC transporter	Cellular communication
3387	0.55	10.27	11.61	0.04	DT457081	Vacuolar protein-sorting protein 33 homolog	Cellular communication
6380	0.54	10.68	15.54	0.03	DT467229	P-glycoprotein-like protein	Cellular communication
6454	0.27	11.22	60.33	0.02	BQ411336	Potyvirus VPG-interacting protein	Cellular communication
7812	0.66	10.71	55.58	0.03	BF273420	Vacuolar sorting receptor 1 precursor (ATVSR1)	Cellular communication
9377	0.26	10.47	58.86	0.04	DT466667	?????	Cellular communication
13215	0.63	10.71	4.54	0.06	DT526827	Nontransporter ABC protein ABCF1	Cellular communication
1097	0.56	11	61.71	0.02	DT527346	Cyclin D3-2	Cell division
2021	0.35	11.4	61.71	0.03	DT461476	Double-strand break-repair protein MRE11	Cell division
2070	0.49	12.02	60.33	0.05	DT527175	Histone H2A	Cell division
5438	0.53	10.6	62.03	0.02	DT460396	Cyclin D3-1 protein	Cell division
10118	0.47	11	16.67	0.02	DT461474	Chromosome condensation regulator	Cell division
10418	0.45	10.76	60.33	0.05	DT467487	Microtubule organization 1 protein	Cell division
11220	0.53	12.29	53.52	0.02	DT527229	Histone H2A	Cell division
11373	0.69	10.56	4.54	0.01	DT463832	Kinesin-related protein (kinesin protein)	Cell division
11842	0.29	10.43	65.83	0.03	BF271605	Histone H2A	Cell division
12204	0.5	12.68	15.54	0.02	DT526864	Kinesin-like protein; 73641-79546	Cell division
2023	0.39	10.61	61.71	0.02	DT469038	Mitochondrial dicarboxylate carrier protein	Energy production
9723	0.45	10.13	62.8	0.03	BG442854	H+-transporting ATP synthase chain 9	Energy production
15212	1.49	11.47	11.61	0.07	BG440620	ADP/ATP carrier protein 1, mitochondrial precursor	Energy production
5087	0.42	11.08	60.33	0.01	BG440872	Chalcone synthase 2	Flavanoid biosynthesis
8308	0.49	13.49	51.87	0.02	DT463093	4-dihydroxy-2-butanone 4-phosphatesynthase	Flavanoid biosynthesis
1971	0.89	9.82	11.61	0.02	CD486189	Proline-rich cell wall protein	Growth
2527	0.52	10.28	11.61	0.1	CD485657	Beta xylosidase	Growth
14027	0.48	10.19	11.61	0.02	DT458696	Endo-1,4-beta-glucanase	Growth
16086	0.42	10.46	62.46	0.04	N/A	Polygalacturonase	Growth
8883	0.55	10.21	6.13	0.09	DT466187	TIR/NBS/LRR protein	Hormone response-auxin
11272	0.59	10.87	51.87	0.01	DV849161	Brassinosteroid-regulated protein bru1 precursor	Hormone response-BZ
3175	0.63	10.49	11.61	0.01	DV849169	Senescence-associated protein DH	Hormone response-ethylene
4306	0.26	9.38	21.85	0.02	DT464835	Ethylene response factor 1	Hormone response-ethylene
7538	0.76	10.44	6.13	0.02	CD485820	Ethylene response factor 2	Hormone response-ethylene
10284	0.44	10.58	53.52	0	DV849817	Ethylene-responsive transcriptional coactivator	Hormone response-ethylene
2107	0.36	10.31	64.67	0.03	DT527495	GASA4 (SNAKIN-1)	Hormone response-GA
2771	0.71	11.13	4.54	0	DT463815	GASA4 (SNAKIN-1)	Hormone response-GA
15854	0.47	11.55	60.33	0.01	DV849493	Phytochrome A	Hormone response-phytochrome
3275	0.67	11.73	9.74	0.01	DT463945	Sterol-C-methyltransferase	Lipid metabolism
3499	0.7	11.63	11.61	0.03	N/A	Lipid transfer protein	Lipid metabolism
10230	0.82	11.79	49.13	0.04	BG443004	NSLTP1	Lipid metabolism
10404	0.54	11.91	49.13	0.04	DT465861	Allene oxide synthase	Lipid metabolism
3095	0.56	10.88	13.63	0	DT526822	U2 small nuclear ribonucleoprotein A	Nucleic acid metabolism
9195	0.43	10.95	51.87	0.02	DT527241	RUV DNA-helicase	Nucleic acid metabolism
9389	0.61	10.44	9.74	0.06	DT466181	ATP-dependent RNA helicase	Nucleic acid metabolism
13372	0.52	11.31	60.33	0.02	DT462654	Symplekin	Nucleic acid metabolism
1835	0.59	10.89	60.33	0.04	BG446577	CND41, chloroplast nucleoid DNA binding protein	Photosynthesis
2700	0.59	14.78	60.67	0.04	DV849468	Ribulose biphosphate carboxylase small chain	Photosynthesis
3147	0.6	13.28	60.33	0.03	DV848766	Chlorophyll a-b binding protein 151	Photosynthesis
3189	0.94	13.55	9.74	0.01	DV848867	Plastocyanin A, chloroplast precursor	Photosynthesis
10574	0.44	13.2	62.03	0.03	CD485895	Type I (26 KDa) CP29 polypeptide	Photosynthesis
13352	0.77	13.19	11.61	0.01	DV849999	Oxygen-evolving enhancer protein 1 precursor	Photosynthesis
13827	0.64	10.56	6.13	0.03	DV849619	FTSZ protein	Photosynthesis
15822	0.4	11.38	60.33	0.01	DV849326	Chlorophyll a-b binding protein CP293	Photosynthesis
15823	0.4	12.8	65.48	0.05	N/A	Ribulose biphosphate carboxylase small chain	Photosynthesis
18129	0.34	10.78	66.34	0.02	CD485707	Chlorophyll a-b-binding protein	Photosynthesis
11635	0.53	11.84	18.82	0.04	DT461412	Potassium transporter 2	Potassium response

Table 2. Continued

GEO ID	Log ₂ ratio	Ave A value	q value %–	P value	GenBank accession no.	BLAST hit	Functional category
952	0.31	9.68	66	0.02	BQ405606	Translation factor	Protein metabolism
3712	0.45	13.82	60.84	0.04	DV849423	Ribosomal protein S3A	Protein metabolism
4173	0.63	10.58	4.54	0.05	N/A	Ubiquitin conjugating enzyme	Protein metabolism
5098	0.65	13.92	18.38	0.02	DT462085	Ribosomal protein L2	Protein metabolism
6386	0.28	11.85	60.33	0.02	BF278028	Proteasome subunit alpha type 3	Protein metabolism
6500	0.62	12	55.58	0.02	BQ404722	Ribosomal protein L2	Protein metabolism
7249	0.61	9.68	13.63	0.02	DV849649	20S proteasome beta subunit PBB2	Protein metabolism
8155	0.2	14.86	65.48	0.05	DT526873	26S proteasome subunit 3	Protein metabolism
8661	0.52	10.88	11.61	0.08	DT526848	T-complex polypeptide 1 homologue	Protein metabolism
9784	0.53	12.13	49.13	0.01	DV849425	50S ribosomal protein L3-1, chloroplast precursor	Protein metabolism
9911	0.98	10.12	0	0.02	DT467590	ABC1 protein	Protein metabolism
10176	0.25	15.59	65.48	0.01	DT527067	40S ribosomal protein S23.	Protein metabolism
10334	0.51	12.68	11.61	0.03	DT462881	Methionyl-tRNA synthetase	Protein metabolism
11302	0.17	9.69	60.33	0.04	DV849449	Beta7 proteasome subunit	Protein metabolism
11669	0.6	12.23	60.33	0.04	BG440585	26S proteasome subunit 8	Protein metabolism
11696	0.25	10.26	60.33	0.03	DT526888	60S ribosomal protein L19	Protein metabolism
11935	0.23	11.28	66.09	0.04	DT467593	26S protease regulatory subunit 6b homolog	Protein metabolism
13165	0.66	11.06	53.52	0.02	DT461729	Proteasome subunit alpha type 3	Protein metabolism
13295	0.63	11.63	60.33	0	DV849175	Ribosome-like protein	Protein metabolism
13351	0.33	10.74	62.8	0.03	DV850013	Ribosomal protein L35	Protein metabolism
13852	0.33	10.17	60.33	0	BF268216	50S ribosomal protein L34, chloroplast precursor	Protein metabolism
13880	0.78	12.02	4.54	0.01	DT462430	50S ribosomal protein L3-1, chloroplast precursor	Protein metabolism
15264	1.32	11.95	11.61	0.09	DT527441	Elongation factor 1-gamma	Protein metabolism
18915	0.57	9.44	11.61	0.01	DV850166	60S ribosomal protein L9 (gibberellin-regulated)	Protein metabolism
20790	0.39	11.08	62.8	0.03	BG440364	Translation elongation factor-TU	Protein metabolism
21921	0.63	14.8	60.84	0.04	DV849739	Chloroplast 50S ribosomal protein L16	Protein metabolism
13461	0.43	10.61	60.67	0.05	BF276276	Retroelement	Retro virus
11345	0.55	11.54	11.61	0.06	DT462982	(+)-Delta-cadinene synthase	Secondary metabolism
11371	0.48	10.91	11.61	0.02	DT463966	(+)-Delta-cadinene synthase	Secondary metabolism
538	0.36	10.29	6.13	0.24	BG440641	Serine/threonine protein kinase	Signal transduction
3248	0.74	10.9	0	0	DT463054	Casein kinase i	Signal transduction
3466	0.49	11.53	62.03	0.05	BQ404418	LOB domain protein 16	Signal transduction
3487	0.16	14.13	66.34	0.04	N/A	14-3-3 H-1 protein	Signal transduction
11740	0.51	10.33	60.84	0.04	DT455821	Pheromone receptor, (AR401)	Signal transduction
13166	0.36	11.08	13.63	0.15	DT461717	NAC domain protein NAC2	Signal transduction
13518	0.39	10.16	53.52	0.02	DT459058	Cleft lip and palate associated protein-like	Signal transduction
13929	0.59	10.28	4.54	0.04	DT466909	Mitogen-activated protein kinase homolog NTF3	Signal transduction
14179	0.57	9.73	9.74	0.03	DT461682	Glycogen synthase kinase-3 homolog MSK-3	Signal transduction
10449	0.51	11.11	60.33	0.04	BG446473	Cyclophilin	Stress response- general
2237	0.46	10.88	11.61	0.07	DT462954	Alpha-galactosidase	Stress response- cold
10337	0.41	10.85	60.33	0.01	DT462537	Phenylpropanoid:glucosyltransferase 1	Stress response- disease
12598	0.52	10.6	21.85	0.05	CD486058	Aldehyde dehydrogenase 1 precursor	Stress response- drought
2178	0.24	10.38	66.34	0.03	N/A	Stress related-like protein interactor	Stress response- general
11674	0.48	10.04	16.67	0.02	DT462164	Aminopeptidase	Stress response- general
16285	0.76	9.91	11.61	0.03	BG443765	Phenylalanine ammonia-lyase 1	Stress response- general
5654	0.27	11.23	13.63	0.21	DT527199	70-KDa peptidylprolyl isomerase	Stress response- heat shock
6324	0.4	11.4	21.85	0.04	DT465419	Heat shock 70-KDa protein, mitochondrial precursor	Stress response- heat shock
7460	0.18	10.45	66	0.03	DT460619	70-KDa heat shock protein	Stress response- heat shock
1787	0.46	10.61	65.83	0.01	DT466618	NADPH-cytochrome P450 oxydoreductase	Stress response- oxidative
6016	0.53	9.97	60.33	0.05	N/A	Cytochrome B5	Stress response- oxidative
8092	0.25	10.18	65.05	0.02	BQ407019	Ubiquinol-cytochrome c reductase	Stress response- oxidative
9390	0.47	11.27	9.74	0.04	DT466073	Peroxidase	Stress response- oxidative
14242	0.46	10.38	15.54	0.06	DT527591	Peroxiredoxin	Stress response- oxidative
17461	0.64	10.1	11.61	0.03	DT464978	Manganese superoxide dismutase 2	Stress response- oxidative
13567	0.34	10.6	65.05	0.05	BQ401919	Adenosine 5'-phosphosulfate reductase	Sulfate response
279	0.63	11.56	16.67	0	DT466399	AT-hook DNA-binding protein	Transcription
473	0.44	11.18	11.61	0.08	BQ406414	Ring-H2 finger protein ATL4K	Transcription
1815	0.65	10.18	15.54	0.01	DT467553	Ring-H2 finger protein	Transcription
3238	0.57	10.61	61.71	0.04	DT463277	Cys-3-His zinc-finger protein	Transcription
3738	0.41	9.71	62.8	0.02	DV849962	TCP family transcription factor	Transcription
5861	0.6	11.04	9.74	0.04	DT467803	WRKY transcription factor 31	Transcription
7143	1.03	11.88	18.38	0.13	DT526824	MADS-box protein MADS3	Transcription
10403	0.4	10.85	21.85	0.12	DT465980	WRKY family transcription factor	Transcription
11131	0.35	10.85	21.85	0.11	DT469067	BHLH transcription factor BHLH033	Transcription
12142	0.43	9.75	9.74	0.46	DT461326	Glycine-rich, zinc-finger DNA-binding protein	Transcription
12179	0.51	13.26	16.67	0.25	DT462252	Squamosa promoter binding protein 4	Transcription
15696	0.54	10.81	9.74	0.05	DT461695	Zinc-finger protein 1	Transcription
166	0.32	10.87	62.79	0.01	DV849687	Purple acid phosphatase	Unknown
269	0.82	10.88	9.74	0.01	DT466633	Selenium-binding protein	Unknown
317	0.7	10.27	11.61	0.02	BF278989	N-myristoyltransferase 1	Unknown
319	0.37	11.19	62.46	0.02	DT456652	AT5G46020	Unknown

Table 2. Continued

GEO ID	Log ₂ ratio	Ave A value	q value –%–	P value	GenBank accession no.	BLAST hit	Functional category
453	0.5	11.8	58.86	0.03	CD486238	AT1G21680	Unknown
454	0.6	10.85	60.33	0.03	CD485799	Related to aminopeptidase YSCI, vacuolar	Unknown
481	0.47	11.17	60.33	0.01	N/A	Serin-like protein	Unknown
897	0.47	10.27	18.82	0.02	DT459785	Major sperm protein	Unknown
1071	0.82	11.8	62.8	0.05	DT526820	GDSL-motif lipase/acylhydrolase	Unknown
1178	0.49	13.47	61.71	0.04	DV849626	AT4G24460	Unknown
1211	0.63	10.61	15.54	0.04	DT463618	AT1G53380	Unknown
1813	0.43	10.84	65.83	0.04	DT467783	AT5G40690	Unknown
2190	0.69	12.03	11.61	0.01	DV849688	H-protein	Unknown
2261	0.29	12.27	64.25	0.01	BF272749	AT1G76760	Unknown
2352	0.44	9.62	60.33	0.04	BG446494	AT5G01590	Unknown
3388	0.16	14.56	66.34	0.29	DT456967	AT1G75150	Unknown
3411	0.46	10.37	11.61	0.08	DT460726	AT4G00950	Unknown
4080	0.46	11.18	11.61	0.09	BG440677	AT1G68610	Unknown
4272	0.53	10.38	13.63	0.14	DT464654	Nucleotide sugar epimerase-like protein	Unknown
5059	0.28	12.79	65.83	0.04	DT469014	AT5G62290	Unknown
5131	0.38	11.82	55.58	0.02	DT527638	AT5G61670	Unknown
5230	1.16	10.45	11.61	0.11	DV849447	Light-inducible protein ATLS1	Unknown
6131	0.34	11.18	18.82	0.14	DT526872	Myosin heavy chain-like	Unknown
6284	0.7	11.62	9.74	0.07	DT463080	Signal peptidase	Unknown
6370	0.5	10.45	11.61	0.05	DT467468	AT1G12350	Unknown
6436	0.28	12.55	60.84	0.04	DT458791	110-KDa 4SNC-Tudor domain protein.	Unknown
6458	0.26	11.21	63.7	0.04	BQ410657	AT2G34860	Unknown
6638	0.45	11.22	13.63	0.04	DT526836	AT4G23840	Unknown
7122	0.98	10.43	0	0.01	DT462086	AT2G23140	Unknown
7323	0.25	11.95	60.67	0.03	DT463956	AT1G28170	Unknown
7537	0.59	10.86	51.87	0.02	CD486265	Membrane-related protein CP5	Unknown
7855	1.15	10.65	0	0.02	DT467098	Leucine-rich repeat family	Unknown
7873	0.65	11.05	9.74	0.02	DT465948	Integral membrane protein	Unknown
8179	0.22	15.38	66.34	0.04	DT527498	BTB/POZ domain-containing protein	Unknown
9219	0.46	10.84	18.82	0.03	DV848769	AT3G12570	Unknown
9261	0.64	10.25	60.67	0.04	DV848871	AT1G72020	Unknown
9319	0.46	9.64	60.33	0.02	DT463193	AT1G15200	Unknown
9387	0.7	9.56	11.61	0.01	DT466430	AT2G16460	Unknown
9753	0.44	10.68	55.58	0.02	DV849147	Fiber protein GLP1	Unknown
10067	0.43	10.59	60.33	0.02	CD486285	AT4G01150	Unknown
10246	0.16	15.06	66.34	0.03	DT455993	AT2G47370	Unknown
11243	0.43	9.63	18.82	0.1	DV848770	Phosphatidylserine decarboxylase	Unknown
11298	1.04	11.06	0	0.01	DV849633	AT4G24770	Unknown
11415	0.45	9.81	53.52	0.04	DT465973	AT2G23810	Unknown
11801	0.85	11.18	0	0.01	DV849734	Nucleotide sugar epimerase-like protein	Unknown
12359	0.31	11.02	60.33	0.02	DT462787	RANBP2-type zinc-finger protein	Unknown
13291	0.44	10.59	13.63	0.03	DV849360	ARD-like protein	Unknown
13322	0.73	10.73	60.33	0.02	DV849634	Nucleotide sugar epimerase-like protein	Unknown
13437	0.75	10.73	9.74	0.03	DT466202	Glycosyl transferase	Unknown
14167	0.32	10.08	55.58	0.04	DT468983	AT4G24150	Unknown
14504	0.34	10.76	63.45	0.02	DT457938	AT2G27350	Unknown
15218	0.63	10.6	0	0.11	DT462089	AT3G16910	Unknown
15855	0.38	14.76	63.7	0.05	N/A	AT1G63830	Unknown
15856	0.54	11.83	55.58	0.01	DV849429	AT4G24770	Unknown
16392	0.68	10.36	15.54	0.07	DT463595	AT3G53710	Unknown
20001	0.07	10.56	66.34	0.03	DT466929	AT4G12650	Unknown

22 C in 2× SCC 0.1% SDS and then at least two times at 60 C in 2× SCC 0.1%SDS. Radiolabeling was visualized and quantified using a Packard Instant Imager³.

Statistical Analysis. Plant dry weight, leaf area, N and P concentrations at the June sampling, and corn yield measured at the end of the season were analyzed using paired *t*-test analysis with a P value of 0.05. As per standard methods of analyzing microarray results, the M value and the A value were determined for each probe. The M value is the measure of differential gene expression and is calculated as the log₂ of the hybridization intensity ratios of velvetleaf with corn over velvetleaf in monoculture. The A value is a measure of how strongly a given gene is expressed and calculated as the log₂ of the square root of the product of the hybridization intensity

values from each probe. The hybridization intensity is determined by the level of fluorescence resulting from the excitation of the labeled velvetleaf cDNA that “sticks” to each DNA-containing probe on the cotton microarray. The resulting MA plots (M value by A value for each probe) were Loess-normalized to produce a normalized M value for each gene using the GeneMath XT1 program.⁴ This procedure removes differences by differential labeling of the sample RNA as opposed to differential expression of individual genes. Because low intensity probes are often difficult to interpret, and are a major source of meaningless variation, expression ratios from these probes are customarily deleted from the data sets prior to analysis. Thus, probes with A values of less than 1 standard deviation over the mean for non-DNA containing controls as well as all probes that did not contain cotton DNA

were deleted. Eight replicate hybridizations were done for each probe. If a given probe was deleted in three or more replications, then the probe was considered unreliable, and all data from that probe was deleted from further statistical analysis. The normalized ratios of hybridization intensities for all of the remaining probes were analyzed for statistical significance using the SAM 1.22 software⁵ set for one class with 100 iterations to assign a q value as an estimate of the false discovery rate for each gene. P values were obtained for each probe by performing a *t* test of the normalized M values of each probe against the combined M values of all probes on the array.

Results and Discussion

Plant Growth and Corn Yield. At the late June sampling date, corn had overtopped velvetleaf (Figure 1). Velvetleaf leaf area, dry weight, P concentration, and N content averaged 640 cm² plant⁻¹, 8.5 g plant⁻¹, 2.65 mg P (g plant⁻¹), and 49 g N (kg plant⁻¹), with values not statistically significant between treatments (*P* > 0.05). Total P content (mg plant⁻¹), in contrast, was reduced by 27% when velvetleaf was grown with corn. Corn leaf area and dry weight per plant were reduced by about 16%, and P concentration and total P were 7 and 20% less, respectively, when corn was grown with velvetleaf compared with weed-free corn (data not shown). Grain yield, measured at physiological maturity of corn, averaged 3,610 kg ha⁻¹ in the weed-free treatment. Corn yield when velvetleaf was present was reduced by 27% compared with the weed-free treatment yield. This yield reduction was similar to the reduction reported in Horvath et al. (2006) for corn grown with velvetleaf at this location in 2005.

Hybridization of Velvetleaf Probe to Cotton Microarray. Cotton microarrays developed by CSIRO contain 24,287 probes including 84 empty and negative control probes, of which 15,426 had significant similarity to sequences in the nonredundant database (GenBank). Of the probes on the arrays, 9,190 (38%) consistently hybridized to labeled velvetleaf cDNA at a level at least 1 standard deviation over background (average of empty and negative control probes) (expression data can be downloaded from GEO accession number GSE6445). The percentage of hybridizing probes was roughly 50% lower than what is usually observed from homologous hybridizations, but was within the low range of what is commonly observed for other nonhomologous hybridizations (Horvath et al. 2003a). The lower hybridization percentage might be due to the fact that most of the cotton cDNAs used to develop the array were from developing ovule and seed fiber libraries. However, data were obtained for 5,522 probes that had significant similarity to other known genes. Consequently, there are still sufficient numbers of characterized genes on the array to identify physiological and developmental processes impacted by competition with corn.

Differential Gene Expression in Velvetleaf Responding to Corn Competition. Of the 9,190 hybridizing genes, 132 were preferentially expressed when velvetleaf was grown in competition with corn based on q values (those with less than a 22% false positive rate) (Tables 1 and 2). No genes were

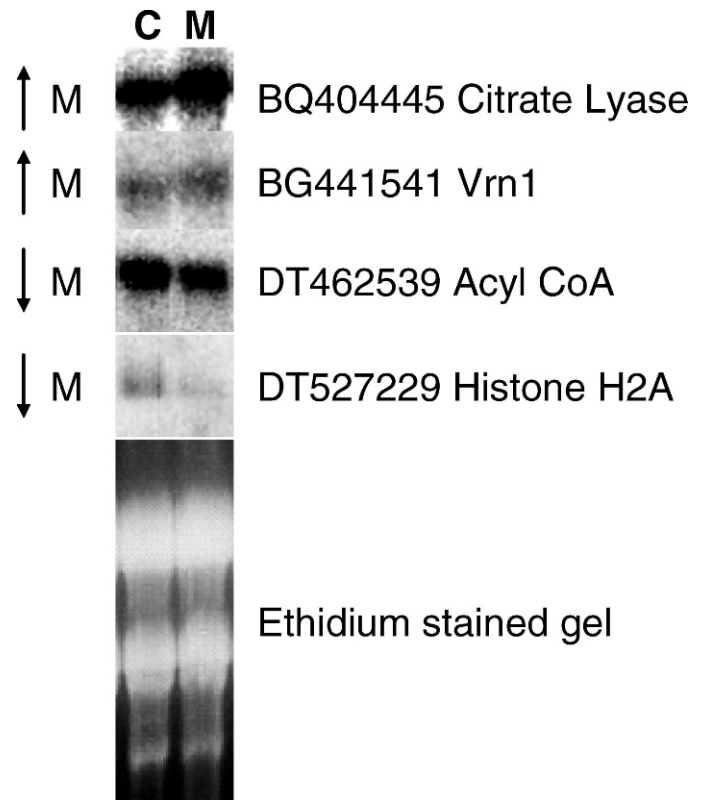


Figure 2. Northern analysis of four representative probes hybridized to total velvetleaf RNA isolated from plant grown in competition with corn (C) or in monoculture (M). Probes were from the designated genes (with both GenBank accession number and top BLAST hit); arrows indicate if the gene was predicted to be up-regulated or down-regulated in monoculture based on microarray data.

preferentially expressed in velvetleaf grown in monoculture based on q value. When a P value cutoff of 0.05 for differential expression was used, 625 genes were preferentially expressed in velvetleaf monocultures and 226 genes were preferentially expressed when velvetleaf was grown in competition with corn. Eighty-six genes had both q values less than 22% and P values less than 0.05, and 899 had either q values less than 22% and/or P values less than 0.05. Surprisingly, there were few obvious classes of genes representing known physiological processes or multiple copies of specific genes that fell within the statistical guidelines for differential expression. However, some patterns of gene expression did emerge.

Genes involved in photosynthesis (e.g., *Ribulose biphosphate carboxylase* and *chlorophyll a-b binding protein*), carbon metabolism (e.g., *Nucleotide sugar epimerase* and *Acyl-CoA oxidase*), and cell division (e.g., *Histone H2A* and *Cyclin D3*) were preferentially expressed when velvetleaf was competing with corn. This is opposite of what was observed in corn competing with velvetleaf where such genes were down-regulated in response to competition (Horvath et al. 2006). The reason for this difference is unclear. However, it should be noted that in the case of the velvetleaf, the samples were taken earlier in the season and at a time when the corn was overtopping the velvetleaf whereas the corn samples were collected late in the growing season and the corn was considerably taller than the velvetleaf.

Several genes encoding proteins involved in protein metabolism such as cullin and several F-box-related proteins

were preferentially expressed in velvetleaf grown in monoculture. A similar response was observed in comparisons between corn grown in competition with velvetleaf or in monoculture (Horvath et al. 2006). In the corn comparisons, the difference was hypothesized to be due to a response to nitrogen availability. However, N concentration of velvetleaf tissue was similar between treatments (4.8 vs. 4.96%) at this sampling period.

Because velvetleaf was beginning to be shaded by the corn, it was expected that shade-avoidance responses would be activated. One of the hallmarks of the shade-avoidance response is an increase in shoot growth as the shaded plant attempts to outgrow its competitor (Ballaré and Casal 2000; Smith and Whitelam 1997; Vandebussche et al. 2003). It was expected that genes involved in auxin, ethylene, and gibberellic acid (GA) responses affecting cell division and elongation would have increased in velvetleaf competing with corn. Despite having numerous auxin-regulated genes hybridizing at significant levels on the array, few were differentially expressed. However, several ethylene-responsive genes (Sakai et al. 1998; Solano et al. 1998) were preferentially expressed when velvetleaf was competing with corn. Although some GA-induced genes involved in cell division were preferentially expressed due to competition, they were few in number. The only obviously GA-induced genes that were preferentially expressed in velvetleaf competing with corn, besides those involved in cell division (i.e., *Cyclin D3*), were two putative *GASA4* orthologues (Aubert et al. 1998). It is noteworthy that the one *phytochrome A* gene that is present on the array is preferentially expressed in response to corn competition. Phytochrome is known to play a role in shade-avoidance signal transduction (Smith and Whitelam 1997). Also, the putative arabidopsis orthologue of DT467097 (leucine-rich repeat family), which is preferentially expressed in response to competition, is also differentially expressed in shade-avoidance responses in arabidopsis (see <http://genome-www5.stanford.edu/cgi-bin/data/spotHistory.pl?state=parameters;login=no;suid=140354>) (Zimmermann et al. 2004).

There was little evidence of competition for water and most nutrients. However, a phosphate-inducible gene *PHI1* and another phosphate-inducible cell cycle-related protein gene showed significantly higher expression in velvetleaf grown in monoculture suggesting that there was less phosphate available when the velvetleaf was grown with corn. Also, a potassium transporter was preferentially expressed in velvetleaf competing with corn. It was expected that genes preferentially expressed due to nutrient deficiencies in arabidopsis would primarily be preferentially expressed in response to competition in velvetleaf. Thus we looked at the expression of several putative arabidopsis orthologues of velvetleaf genes using the response viewer tool in the GENEVESTIGATOR database (Zimmermann et al. 2004). Interestingly, the putative arabidopsis orthologues of several velvetleaf genes that were preferentially expressed in response to competition are down-regulated in arabidopsis in response to nitrogen deficiency (for example, see report on expression of the arabidopsis orthologue of DV849429 [AT4g24770] at <https://www.genevestigator.ethz.ch/at/index.php?page=tair&option=stress&agi=AT4g24770>). This is opposite of what we would have expected to find assuming that nitrogen levels were limiting, and suggests perhaps that nitrogen levels were actually higher in velvetleaf competing with corn than when in monoculture. However, it should be noted that nitrogen accumulation was

not significantly different between velvetleaf grown alone or in competition with corn.

Hybridization of Northern Blots with Cotton Probes. In order to assess the accuracy of the microarray analysis and to confirm that the cotton probes could be used to identify specific velvetleaf RNAs, we analyzed the expression of selected velvetleaf genes by hybridizing northern blots to cotton cDNAs (Figure 2). Nineteen of the 22 cotton probes hybridized to single bands on northern blots of total velvetleaf RNA. This result suggests that most of the cotton probes hybridizing to labeled velvetleaf cDNAs on the microarrays could be used to isolate the corresponding velvetleaf gene from a library for further analysis. Differential expression could be observed in at least one of the three biological replicates for several of the genes tested. However, there appeared to be a very high false-positive rate, even with genes that had low *q* values. This could be due to the fact that differential expression was very subtle even on the microarray analysis (none of the probes tested had greater than an average 1.5-fold difference in expression between treatments).

Conclusions and Future Perspectives. Combined, these data show that cotton microarrays can be used to follow changes in gene expression in velvetleaf. The data also suggest that there may be significant differences in shade avoidance responses that are dependent on species and timing of sampling. Future work should focus on confirming and then identifying the reasons for these differences in order to understand which signaling pathways are more conserved among various plant species, and how the timing of competition affects gene expression in weeds such as velvetleaf. Such information should greatly enhance design of models needed to predict and manipulate the impact of crop–weed competition.

Sources of Materials

¹ Alexa Fluor cDNA labeling kit (A32755), Invitrogen Life Technologies Inc., 1600 Faraday Ave., Carlsbad, CA 92008.

² Affy428 scanner and Jaguar software, Affymetrix, Inc., 3380 Central Expressway, Santa Clara, CA 95051.

³ Packard Instant Imager, Packard Instrument Company, 2200 Warrenville Road, Downers Grove, IL 60515.

⁴ GeneMath XT 1.5, Applied Maths, Inc. 512 East 11th Street, Suite 207 Austin, TX 78701.

⁵ SAM 1.22 (<http://www-stat.stanford.edu/~tibs/SAM/index.html>), Stanford University Labs, Stanford, CA 94305.

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