Differential gene expression in SuCMoV-tolerant and susceptible sunflower lines

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ABSTRACT

Sunflower chlorotic mottle virus (SuCMoV) is one of the most widely distributed viruses affecting sunflower (*Helianthus annuus* L.) in Argentina. Symptoms include yellow blotches, reduced and distorted leaves and plant stunting. SuCMoV-susceptible (20016) and tolerant (B-133) sunflower lines were mechanically inoculated with SuCMoV and total RNA was isolated from infected leaf tissue, amplified and hybridized to an *Arabidopsis* oligonucleotide-microarray containing 22,000 genes. The gene expression profile in inoculated plants from both lines was statistically different from non-inoculated leaf samples. Eighty-eight genes were differentially expressed in the tolerant sunflower line.

Key words: *Arabidopsis* – genomics – *Helianthus annuus* – microarrays – oligonucleotide – *Sunflower chlorotic mottle virus*

INTRODUCTION

Several viruses infect commercial sunflower hybrids, including *Cucumber mosaic virus*, *Tobacco streak virus*, *Potato virus Y* and *Tomato spotted wilt virus,* whereas *Tobacco ringspot virus* and *Sunflower mosaic virus* have been found in wild sunflowers (Gulya et al., 2002). In Argentina, *Sunflower chlorotic mottle virus* (SuCMoV) has been associated with sunflower chlorotic mottling and plant stunting symptoms and it has been reported in several provinces including Entre Ríos, Santa Fé, Buenos Aires and Córdoba. This virus has been fully characterized (Dujovny et al., 1998, 2000) and has recently been classified as a strain of *Potato virus Y* (Berger et al., 2005). Yield losses associated with this disease can be important if epidemics break up at an early stage of plant development (Lenardon et al., 2001).

Recently, a sunflower line tolerant to SuCMoV infection has been found and the resistance gene Rcmo-1 was mapped (Lenardon et al., 2005). Oxidative stress has been reported in compatible interactions between SuCMoV and a susceptible sunflower line (Arias et al., 2003) and sunflower lines with different responses to SuCMoV infection differed in chlorophyll loss, oxidative generation and antioxidant enzyme activity (Arias et al., 2005). The current work compares transcripts from infected plants of a SuCMoV tolerant-line and a susceptible one using oligonucleotide microarrays from *A. thaliana* in order to analyze the known biological pathways that are well developed for this species.

MATERIALS AND METHODS

Plant inoculations

SuCMoV was maintained on sunflower hybrid CF-7 and used as inoculum source for the whole experiment. The virus was mechanically inoculated (Dujovny et al., 1998) to sunflower SuCMoV-tolerant (B-133) and susceptible (20016) lines (Advanta Semillas S.A.I.C). The susceptible line showed severe systemic chlorotic mottling and yellow blotches 5-7 days after infection, whereas the tolerant line showed scant isolated chlorotic mottling 10-12 days after inoculation. Control plants of both lines were mock inoculated and did not show any type of symptom.

Sunflower total RNA isolation
Total RNA from infected sunflower leaves was obtained by a modified Schneitz-Lab protocol (www.unizh.ch/Cyto_website/Schneitzlab). Leaf tissue (0.5–1 g) was ground in liquid nitrogen and transferred into a 50 ml Falcon tube containing 10 ml of trizol reagent (Invitrogen Inc, CA, USA). After adding 1 ml 2M NaOAc pH 4.1, 10 ml phenol and 2 ml chloroform/isoamylalcohol (49:1) the tube was stirred for 10 sec and placed on ice for 15 min, centrifuged at 3,500 rpm at 4ºC for 20 min, then the aqueous phase was transferred into a new tube and 10 ml isopropanol were used to precipitate the RNA at -20ºC overnight. The RNA pellets were precipitated after centrifugation at 8,000 rpm during 30 min at 4ºC, washed with ice-cold ethanol 75% and air dried for 10 min. The pellet was dissolved in 1.5 ml sterile DEPC-water and total RNA was quantified using the Nanodrop (Nanodrop Techology, Inc). RNA from healthy sunflower leaves was isolated with the same procedure and used as control.

RNA amplification
Total RNA was amplified using BLR-PCR methodology (Balogh et al., 2006). The procedure involved three steps: 1. cDNA synthesis using the RT enzyme (Superscript from Invitrogen), 2. Amplification based on dT-T7 primer, and 3. *In vitro* transcription reaction of cDNA into RNA. Two rounds of amplifications were performed.

Fluorescent probe synthesis
The fluorescent probe synthesis for the aaRNA from BLR method used an indirect methodology. At the same time, a fluorescent probe from healthy sunflower leaves aRNA (control) was synthesized, in order to use it in the green channel to hybridize with tolerant or susceptible SuCMoV-infected sunflower leaves. For indirect probe-labeling, 5-10 µg of amplified RNA was used as starting material. For labeling, the total RNA and the aaRNA were combined with their respective primers and the mix was incubated at 70°C for 10 min, then chilled on ice for 10 min. Primer–RNA solution was added to the reverse transcriptase mix [5x first-strand buffer, 6 µl; 50x aa-dUTP/dNTPs (25 mM dATP, dGTP, and dCTP, 15 mM dTTP, and 10 mM aminoallyl-dUTP), 0.6 µl; DTT 0.1 M, 3 µl; Superscript II reverse transcriptase (Invitrogen/Life Technologies), 2 µl] and incubated at 42°C for 2 h. The reaction was terminated by adding EDTA (0.5 M, 10 µl) and the RNA was hydrolyzed with NaOH (1 M, 10 µl) at 65°C for 30 min. After cDNA precipitation with ethanol 100% in the presence of glycogen (20µg/µl) and NaOAc 3M (pH 4.5), it was centrifugated at 13,000rpm for 30 min and washed twice with ETOH 70%. The cDNA pellet was resuspended in 7. 5 µl of coupling buffer, adding 2.5 µl of prepared ester-Cy3 (for the control) or ester-Cy5 (for the virus treated-samples) fluorescent dyes (Amersham) and incubated for 1 h at RT in dark. After purification of the fluorescent probes with QIAquick PCR purification kit (Qiagen), they were quantified using the Nanodrop and 40 pmols of fluorescent cDNA was employed for each channel in each microarray.

Arabidopsis-oligonucleotide microarray hybridization
For microarray hybridization, Cy5-fluorescence dye was used for the SuCMoV treated plants (susceptible 20016 or tolerant B133 lines) and Cy3-fluorescence dye for the control non inoculated plants. 750 ng of Cy3-labeled control probe and 750 ng of Cy5-labeled sample probes were used to hybridize an Agilent 60-mer *Arabidopsis thaliana* oligo microarrays slide containing 22,000 features. Hybridization was performed in SureHyb chambers (Agilent Technlogies) at 60ºC for 18 hours and washed following the manufacturer's protocol. The arrays were scanned and analyzed with Feature extraction software (Agilent Technologies) to verify the hybridization quality. Oligonucleotide microarrays from *A. thaliana* were employed in order to analyze the known biological pathways that are well developed for this species.

Image Analysis
The data were analyzed considering at least three independent biological replicates. The acquired images were analyzed by ImaGene software (BioDiscovery Inc., El Segundo, CA), and statistical analysis was performed using Bioconductor softwares (Biodiscovery, Inc, CA), including Lowess normalization using local background correction. The FatiGo software (www.fatiGo.com) was used in order to establish the putative biological processes in which each gene product is involved.

Data Analysis and statistical interpretation
The Limma Package from Smyth (2005) software version was used for data analysis. All genes with p value below a threshold of 0.001 are selected as differentially expressed ones. The meaning of adjusted p-values is the expected proportion of false discoveries in the selected group should be less than the

threshold value, in this case less than 0.005. The B-statistic (odds or B) is the log-odds that the gene is differentially expressed. For example, if B = 1.5, then the odds of differential expression is exp (1.5)=4.48, i.e., about four and a half to one. The probability that the gene is differentially expressed is 4.48/(1+4.48)=0.82, i.e., about 82%. A B-statistic of zero corresponds to a 50-50 chance that the gene is differentially expressed. The B-statistic is automatically adjusted for multiple testing by assuming that 1% of the genes, or some other percentage specified by the user in the call to eBayes (Smyth, 2005; Smyth et al., 2005; Ritchie et al., 2006), are expected to be differentially expressed. The p-values and B-statistics will normally rank genes in the same order. In fact, if the data do not contain any missing values or quality weights, then the order will be precisely the same. As with all model-based methods, the p-values depend on normality and other mathematical assumptions, which are never exactly true for microarray data. It has been argued that the p-values are useful for ranking genes even in the presence of large deviations from the assumptions. The B-statistic probabilities depend on the same assumptions but require in addition a prior guess for the proportion of differentially expressed genes. The p-values may be preferred to the B-statistics because they do not require this prior knowledge. The eBayes function computes one more useful statistic. The moderated F-statistic (F) combines the t-statistics for all the contrasts into an overall test of significance for that gene (Benjamini and Hochberg, 1995).

RESULTS AND DISCUSSION

Genes whose expression significantly changed in inoculated vs. control plants are shown in Tables 1 and 2 for the SuCMoV-susceptible and the tolerant lines, respectively. Twenty two genes were differentially expressed in the susceptible line and 84 genes in the tolerant one.

Table 1. Genomic expression in susceptible-SuCMoV sunflower leaves[1]

Gene ID	Average-A	M	t	P.Value	adj.P.Val	B
A_84_P302790 expressed protein	7,4120	1,0950	9,8602	0,0000	0,0284	2,9315
A_84_P22706 protein kinase, putative contains protein kinase domain.	6,2267	0,8527	5,8979	0,0007	0,0481	0,1192
A_84_P87029 expressed protein contains Pfam profile PF04146	6,8442	0,7828	6,4191	0,0004	0,0320	0,5925
A_84_P20218 elongation factor P (EF-P) family protein similar to SP|P33398	6,9067	0,7319	5,9250	0,0007	0,0473	0,1448
A_84_P15683 myb family transcription factor	6,4775	0,7179	6,5879	0,0004	0,0293	0,7377
A_84_P186034 NLI interacting factor (NIF) family protein contains Pfam profile PF03031	7,2159	0,6916	6,8517	0,0003	0,0284	0,9574
A_84_P231939 glucose-6-phosphate/phosphate translocator	7,2044	0,6190	6,8201	0,0003	0,0284	0,9316
A_84_P15223 P-glycoprotein, putative similar to P-glycoprotein	6,8034	0,6128	10,9633	0,0000	0,0284	3,4647
A_84_P10874 dehydrin xero2 (XERO2) / low-temperature-induced protein LTI30 (LTI30) identical to dehydrin Xero 2	6,9251	0,6098	6,8311	0,0003	0,0284	0,9405
A_84_P222719 hypothetical protein [At3g09130.1]	6,8787	0,6087	6,5327	0,0004	0,0299	0,6907
A_84_P16415SSXT protein-related / transcription co-activator-related similar to SYT/SSX4 fusion protein	6,8820	0,5589	8,1311	0,0001	0,0284	1,9058
A_84_P22786 rhomboid family protein contains PFAM domain PF01694	6,7587	0,5292	6,0665	0,0006	0,0419	0,2765
A_84_P307770 KH domain-containing protein strong similarity to unknown protein	6,7846	0,5239	8,5221	0,0001	0,0284	2,1610
A_84_P179684 expressed protein [At5g09310.1]	6,3477	0,5042	7,3434	0,0002	0,0284	1,3438
A_84_P224379 expressed protein [At4g29590.1]	6,8426	0,5035	6,4543	0,0004	0,0313	0,6231
A_84_P15375 photosystem I reaction center subunit IV, chloroplast, putative / PSI-E, putative (PSAE2)	6,2624	0,4908	7,1903	0,0002	0,0284	1,2266
A_84_P17102 somatic embryogenesis receptor-like kinase 2 (SERK2)	6,8134	0,4804	5,9722	0,0007	0,0453	0,1891
A_84_P187084 peroxidase, putative similar to cationic peroxidase	5,9467	0,3793	6,1172	0,0006	0,0403	0,3230
A_84_P17469 expressed protein [At3g45830.1]	6,4204	0,3778	7,1371	0,0002	0,0284	1,1852
A_84_P10131640S ribosomal protein S15A (RPS15aD) cytoplasmic ribosomal protein S15a.	5,9350	0,3378	6,8354	0,0003	0,0284	0,9440
A_84_P13444452-phosphoglycerate kinase-related contains weak similarity to 2-phosphoglycerate kinase	6,3818	0,3014	6,1188	0,0006	0,0403	0,3244
A_84_P273760 expressed protein predicted proteins, Arabidopsis thaliana [At3g55600.1]	6,0882	0,2985	5,9758	0,0006	0,0453	0,1924

[1]The M-value (M) is the value of the contrast. This represents a log2-fold change between two or more experimental conditions although sometimes it represents a log2-expression level. The A-value (A) is the average log2-expression level for that gene across all the arrays and channels in the experiment. Column t is the moderated t-statistic. Column p-value is the associated p-value after adjustment for multiple testing. The B-statistic is the log-odds that the gene is differentially expressed.

Table 2. Genomic expression of SuCMoV-tolerant sunflower leaves.

Gene ID	Average-A	M	t	P.Value	adj.P.Val	B
A_84_P23884 arabinogalactan-protein (AGP17)	6,0295	-0,3572	-7,4609	0,0002	0,0284	1,4319
A_84_P20246 basic helix-loop-helix (bHLH) family protein contains	6,2236	-0,3772	-7,2189	0,0002	0,0284	1,2487
A_84_P20534ATP-dependent Clp protease proteolytic subunit	5,9659	-0,3795	-6,8156	0,0003	0,0284	0,9278
A_84_P16654 ankyrin repeat family protein / BTB/POZ domain-containing protein contains Pfam domain	6,0625	-0,3863	-6,3605	0,0005	0,0336	0,5411
A_84_P19128 WRKY family transcription factor contains Pfam profile	5,9804	-0,3927	-5,9904	0,0006	0,0450	0,2060
A_84_P10346 cysteine proteinase-related low similarity to cysteine proteinase	6,0577	-0,4107	-6,0972	0,0006	0,0408	0,3047
A_84_P198504 leucine carboxyl methyltransferase family protein contains	6,2713	-0,4120	-6,6227	0,0004	0,0291	0,7673
A_84_P10447 sugar transporter, putative similar to monosaccharide transporter PaMst-1	6,0660	-0,4131	-7,2179	0,0002	0,0284	1,2479
A_84_P185684 MADS-box family protein	6,1283	-0,4132	-6,2040	0,0005	0,0376	0,4018
A_84_P19906 alanine racemase family protein contains	6,0858	-0,4179	-6,4904	0,0004	0,0306	0,6543
A_84_P24164 OTU-like cysteine protease family protein	6,0449	-0,4194	-6,9337	0,0003	0,0284	1,0239
A_84_P20607 endomembrane protein 70, putative TM4 family	6,0823	-0,4235	-7,3464	0,0002	0,0284	1,3461
A_84_P15263 phosphoribulokinase/uridine kinase family protein	6,0928	-0,4236	-6,2281	0,0005	0,0369	0,4234
A_84_P18442 cation/hydrogen exchanger, putative (CHX28) monovalent cation:proton antiporter family 2 (CPA2)	6,1506	-0,4278	-7,7945	0,0001	0,0284	1,6738
A_84_P22466CBS domain-containing protein contains Pfam profile PF00571: CBS domain	6,2454	-0,4314	-5,8936	0,0007	0,0482	0,1152
A_84_P19135 beta-ketoacyl-CoA synthase family protein	6,0022	-0,4553	-6,4448	0,0004	0,0314	0,6149
A_84_P10794 glycoside hydrolase family 19 protein similar to class I chitinase	6,0612	-0,4602	-7,3277	0,0002	0,0284	1,3319
A_84_P175641 expressed protein	6,0996	-0,4631	-7,4331	0,0002	0,0284	1,4112
A_84_P15723NA	5,9461	-0,4677	-6,9532	0,0003	0,0284	1,0395
A_84_P20270NA	6,0177	-0,4742	-10,4829	0,0000	0,0284	3,2426
A_84_P290374 DNA-binding storekeeper protein-related contains Pfam profile	6,1028	-0,4810	-6,2562	0,0005	0,0363	0,4487
A_84_P11724 L-asparaginase, putative / L-asparagine amidohydrolase, putative similar to Swiss-Prot:P30364	6,0750	-0,4910	-6,1961	0,0005	0,0378	0,3947
A_84_P198874 myb family transcription factor contains Pfam profile	6,1290	-0,4922	-8,2374	0,0001	0,0284	1,9767
A_84_P19242NA	5,9641	-0,4974	-5,9192	0,0007	0,0473	0,1394
A_84_P10999 AP2 domain-containing transcription factor	5,9247	-0,5115	-6,5830	0,0004	0,0293	0,7336
A_84_P18950 glycine-rich protein	6,2429	-0,5159	-6,5620	0,0004	0,0296	0,7157
A_84_P23966 expressed protein	6,0259	-0,5217	-9,9400	0,0000	0,0284	2,9730
A_84_P17637 AP2 domain-containing transcription factor	6,0975	-0,5380	-9,1113	0,0001	0,0284	2,5188
A_84_P23205 mitochondrial ribosomal protein L51/S25/CI-B8 family protein	6,2225	-0,5382	-7,7502	0,0001	0,0284	1,6424
A_84_P18471 60S ribosomal protein-related contains weak similarity to 60S ribosomal protein L10A	6,0710	-0,5384	-7,3067	0,0002	0,0284	1,3160
A_84_P15363 F-box family protein contains F-box domain	5,9733	-0,5585	-10,3789	0,0000	0,0284	3,1925
A_84_P21494 pseudo-response regulator 7 (APRR7) identical to pseudo-response regulator 7	6,2043	-0,5588	-11,4663	0,0000	0,0284	3,6818
A_84_P13552 ribosomal protein S14 mitochondrial family protein identical to ribosomal protein S14	5,9936	-0,5659	-8,4420	0,0001	0,0284	2,1100
A_84_P10460 polyubiquitin, putative similar to polyubiquitin	6,0980	-0,5674	-6,2467	0,0005	0,0365	0,4401
A_84_P1684 armadillo/beta-catenin repeat family protein contains Pfam profile	6,1843	-0,5715	-6,8277	0,0003	0,0284	0,9378
A_84_P183904mitochondrial transcription termination factor-related / mTERF-related contains Pfam profile PF02536: mTERF [At5g54180.1]	6,0477	-0,5960	-6,1229	0,0006	0,0403	0,3282
A_84_P18169 polygalacturonase, putative / pectinase	6,0073	-0,6003	-5,8890	0,0007	0,0482	0,1108
A_84_P11898 KOW domain-containing protein contains Pfam PF00467	6,2659	-0,6143	-6,2367	0,0005	0,0367	0,4312
A_84_P22675FAD-binding domain-containing protein similar to SP|P30986 reticuline oxidase precursor (Berberine-bridge-forming enzyme)	6,0213	-0,6196	-9,7199	0,0000	0,0284	2,8576
A_84_P21736 expressed protein [At3g06895.1]	6,0732	-0,6251	-12,6469	0,0000	0,0249	4,1369
A_84_P108252S-locus protein kinase, putative similar to receptor protein kinase	6,2286	-0,6531	-9,0587	0,0001	0,0284	2,4882
A_84_P22508 expressed protein [At5g27730.1]	6,0478	-0,6557	-6,9455	0,0003	0,0284	1,0333
A_84_P19649 protein kinase-related contains protein kinase domain	6,1390	-0,6709	-8,7420	0,0001	0,0284	2,2982
A_84_P17183 phosphomannose isomerase, putative (DIN9) contains Pfam profile: PF01238 phosphomannose isomerase type I	6,1764	-0,6715	-6,9083	0,0003	0,0284	1,0034
A_84_P236543 phototropic-responsive NPH3 family protein contains NPH3 family domain	6,1433	-0,6807	-6,1101	0,0006	0,0405	0,3165
A_84_P10452 phenazine biosynthesis PhzC/PhzF family protein	6,1594	-0,6837	-8,6650	0,0001	0,0284	2,2507
A_84_P306780 expressed protein [At4g33690.1]	6,0027	-0,6965	-12,2907	0,0000	0,0249	4,0071
A_84_P22097 transducin family protein / WD-40 repeat family protein contains 2 WD-40 repeats	6,0636	-0,7002	-8,8670	0,0001	0,0284	2,3742
A_84_P12748 expressed protein [At1g48450.1]	6,1215	-0,7028	-7,7855	0,0001	0,0284	1,6674
A_84_P12828 multidrug resistance P-glycoprotein, putative similar to multidrug-resistant protein CjMDR1	6,1969	-0,7095	-8,4329	0,0001	0,0284	2,1041
A_84_P23698 gibberellin-responsive protein, putative similar to SP|P46688 Gibberellin-regulated protein 2 precursor	6,0641	-0,7243	-7,1993	0,0002	0,0284	1,2335
A_84_P258730 forkhead-associated domain-containing protein / FHA domain-containing protein	6,1882	-0,7355	-7,9378	0,0001	0,0284	1,7740
A_84_P181184 glycosyl hydrolase family 18 protein contains Pfam profile PF00704	6,1473	-0,7695	-6,4453	0,0004	0,0314	0,6153
A_84_P130796 expressed protein [At3g62580.1]	6,1998	-0,7836	-10,1474	0,0000	0,0284	3,0784
A_84_P17154Q9LY19 (Q9LY19) Pectin methyl-esterase-like protein, complete [TC263781]	6,0633	-0,7987	-7,3220	0,0002	0,0284	1,3276
A_84_P18903 Golgi SNARE 11 protein identical to Golgi SNARE 11 protein	6,3686	-0,8001	-8,3804	0,0001	0,0284	2,0702
A_84_P88749 expressed protein contains Pfam profile PF03080	6,1768	-0,8091	-8,7249	0,0001	0,0284	2,2877
A_84_P107132 F-box family protein contains F-box domain Pfam:PF00646 [At1g32140.1]	6,2277	-0,8256	-9,9183	0,0000	0,0284	2,9618
A_84_P91239 expressed protein [At2g16760.1]	6,1667	-0,8421	-6,8491	0,0003	0,0284	0,9553
A_84_P21687 glyceraldehyde-3-phosphate dehydrogenase B, chloroplast subunit B (GAPB) / NADP-dependent glyceraldehydephosphate dehydrogenase	7,2667	-0,8634	-8,7954	0,0001	0,0284	2,3309
A_84_P216998 expressed protein strong similarity to unknown protein	6,2842	-0,8736	-6,3126	0,0005	0,0349	0,4989
A_84_P154245 expressed protein [At4g33480.1]	6,1892	-0,8785	-6,6138	0,0004	0,0292	0,7597
A_84_P117052 expressed protein contains Pfam profile PF04759	6,3999	-0,8922	-7,9861	0,0001	0,0284	1,8073
A_84_P13513 protein kinase, putative contains protein kinase domain	6,0580	-0,9007	-12,2489	0,0000	0,0249	3,9915
A_84_P13102 transferase family protein similar to anthranilate N-hydroxycinnamoyl/benzoyltransferase	6,6334	-0,9245	-6,4834	0,0004	0,0307	0,6483
A_84_P84909Q9LSN6 (Q9LSN6) Arabidopsis thaliana genomic DNA, chromosome 3	6,2436	-0,9249	-11,7643	0,0000	0,0280	3,8036
A_84_P13924 homeodomain protein (BELLRINGER) several homeodomain proteins	6,0814	-0,9299	-10,9980	0,0000	0,0284	3,4801
A_84_P13746 serine/threonine protein kinase, putative similar to Pto kinase interactor 1 (Pti1)	6,1311	-0,9605	-8,9603	0,0001	0,0284	2,4301
A_84_P10718 two-component responsive regulator-related / response regulator protein-related	6,2384	-0,9667	-6,2855	0,0005	0,0355	0,4748
A_84_P14575 halotolerance protein (HAL3A) identical to GB:AAD51616 from	6,1256	-0,9865	-16,7928	0,0000	0,0220	5,2825
A_84_P19632 arabinogalactan-protein (AGP15)	6,3536	-0,9896	-7,2109	0,0002	0,0284	1,2425
A_84_P1658840S ribosomal protein S13 (RPS13A) AtRPS13A mRNA	6,3063	-1,0191	-10,0159	0,0000	0,0284	3,0119
A_84_P18451RNA polymerase sigma subunit SigC (sigC) / sigma factor 3 (SIG3)	6,2597	-1,1044	-8,3487	0,0001	0,0284	2,0496
A_84_P244595 expressed protein [At5g67620.1]	7,6589	-1,1428	-6,2554	0,0005	0,0363	0,4479
A_84_P19563 L-ascorbate peroxidase, putative similar to ascorbate peroxidase [Gossypium hirsutum]	6,2166	-1,2228	-7,2824	0,0002	0,0284	1,2974
A_84_P21499 aspartyl aminopeptidase, putative similar to SP|Q9ULA0 Aspartyl aminopeptidase	6,3542	-1,2562	-6,4943	0,0004	0,0306	0,6577
A_84_P155285 calmodulin-binding protein-related contains	7,0546	-1,3937	-8,4296	0,0001	0,0284	2,1020
A_84_P307190 integral membrane protein, putative contains 4 transmembrane domains	6,3567	-1,4500	-7,6461	0,0001	0,0284	1,5677
A_84_P18413 lectin protein kinase family protein contains Pfam domains PF00138	6,4838	-1,4833	-7,4521	0,0002	0,0284	1,4254
A_84_P13868 stigma-specific Stig1 family protein low similarity to stigma-specific protein STIG1	6,8161	-1,5079	-8,7475	0,0001	0,0284	2,3016
A_84_P270250 expressed protein [At3g45750.1]	6,5488	-1,5180	-7,9357	0,0001	0,0284	1,7726
A_84_P21185 leucine-rich repeat transmembrane protein kinase	6,6231	-1,5840	-8,7576	0,0001	0,0284	2,3078
A_84_P23790RWP-RK domain-containing protein similar to nodule inception protein	6,3086	-1,6702	-6,7172	0,0003	0,0286	0,8465
A_84_P833592,4-dienoyl-CoA reductase-related low similarity to peroxisomal 2,4-dienoyl-CoA reductase	6,2780	-1,6970	-8,0776	0,0001	0,0284	1,8697
A_84_P11251UDP-galactose/UDP-glucose transporter-related	7,0793	-1,8752	-7,9786	0,0001	0,0284	1,8021
A_84_P15770 methylcrotonyl-CoA carboxylase beta chain, mitochondrial / 3-methylcrotonyl-CoA carboxylase 2 (MCCB)	6,7478	-1,8988	-13,4946	0,0000	0,0249	4,4224
A_84_P159445 invertase/pectin methylesterase inhibitor family protein	6,4597	-2,0236	-7,7064	0,0001	0,0284	1,6111
A_84_P278320 expressed protein [At4g38060.2]	6,4566	-2,0826	-12,2350	0,0000	0,0249	3,9863
A_84_P13029 lipase class 3 family protein low similarity to Triacylglycerol Acylhydrolase	6,4270	-2,1386	-7,5034	0,0002	0,0284	1,4634
A_84_P19134 60S ribosomal protein L23 (RPL23B)	6,5888	-2,8132	-12,9919	0,0000	0,0249	4,2569
A_84_P22874 GDSL-motif lipase/hydrolase family protein similar to myrosinase-associated proteins	7,2955	-3,9183	-5,9822	0,0006	0,0452	0,1984

After obtaining the number of genes differentially expressed in the susceptible and tolerant sunflower lines, the biological functions related to those genes were studied. 16.7% of the expressed genes in the tolerant line and only 0 to 2.9% in the susceptible line were related to responses to inorganic substances, osmotic stress, jasmonic acid stimulus, salicylic acid stimulus, water and hormone stimulus. 5.8% gene expression belonged to the cellular catabolic process in the susceptible leaves (Fig. 1).

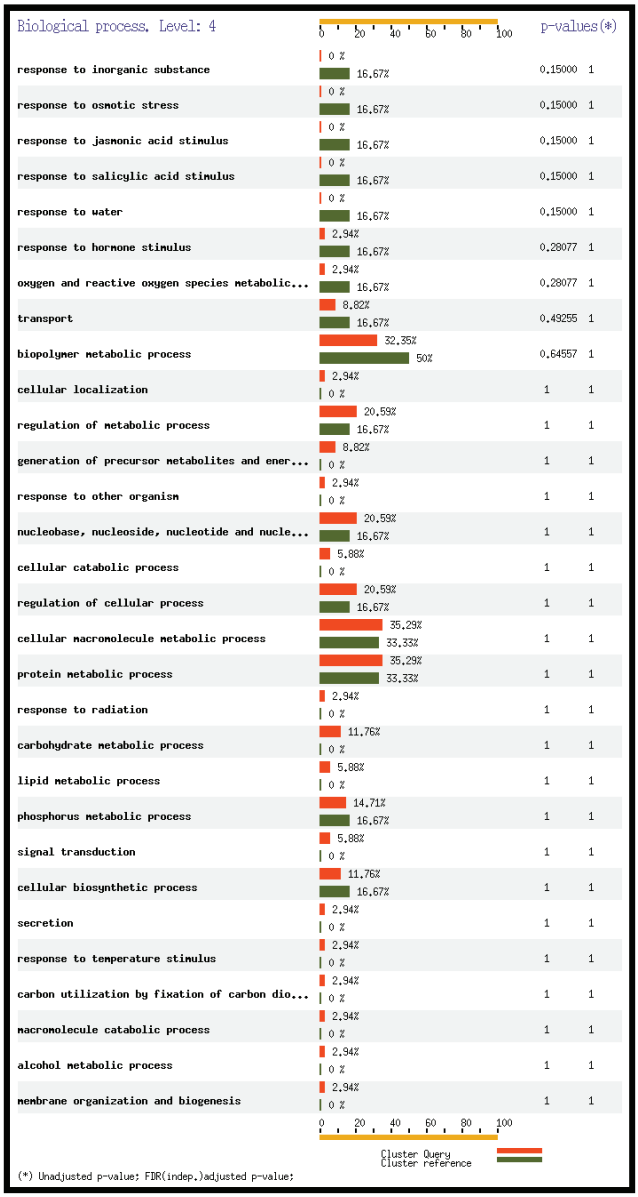

Fig. 1. Percentage of genes related to different biological processes in virus susceptible (upper bars) and tolerant (lower bars) sunflower plants, as expressed in leaf tissue.

The results indicate that the defense mechanisms involving the molecular response were up-modulated in the SuCMoV-tolerant sunflower. Other biological functions did not show significant differences between either group. In the SuCMoV-susceptible sunflower plants we also observed some expression of defense-related genes, but less importantly than in the tolerant ones.

The data suggest that sunflower plants activate different defense mechanisms, from changes in cell wall composition (mechanical protection) to the activation of defense pathways. The energy suppression in the infected-plants, and the increased lignification of the tolerant plants could be other responses to the

pathogen. Defense mechanisms in the sunflower were: activation of SAR, oxidative pathway induced by oxigene, response to salicylic acid (SA) stimulation, gene expression involving specific molecular pathway, activation of local resistant LAR and response to jasmonic acid (JA) stimulation.

These results have been validated by real time-RT-PCR, and by experiments aimed to establish whether the lines differ in SA concentration and if this is related to the different tolerance to SuCMoV. Likewise, it would be interesting to determine whether responses to SA and JA are antagonic.

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