

Transcriptomic Analysis Identifies New Non-Target Site Glyphosate-Resistance Genes in *Conyza bonariensis*

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Table 1. Summary of the Illumina sequencing and *de novo* assembly statistics of *Conyza bonariensis* transcriptome.

Description	Assembly Stats*
Total assembled bases	157,784,873
Total of paired-end reads	23,383,488
Total of transcripts	203,054
Total of contigs “gene” level	90,124
Contig N50	1,118
Average contig length (bp)	777.06
GC (%)	40.23

* Assembled from all biotypes and treatments, a total of 12 libraries.

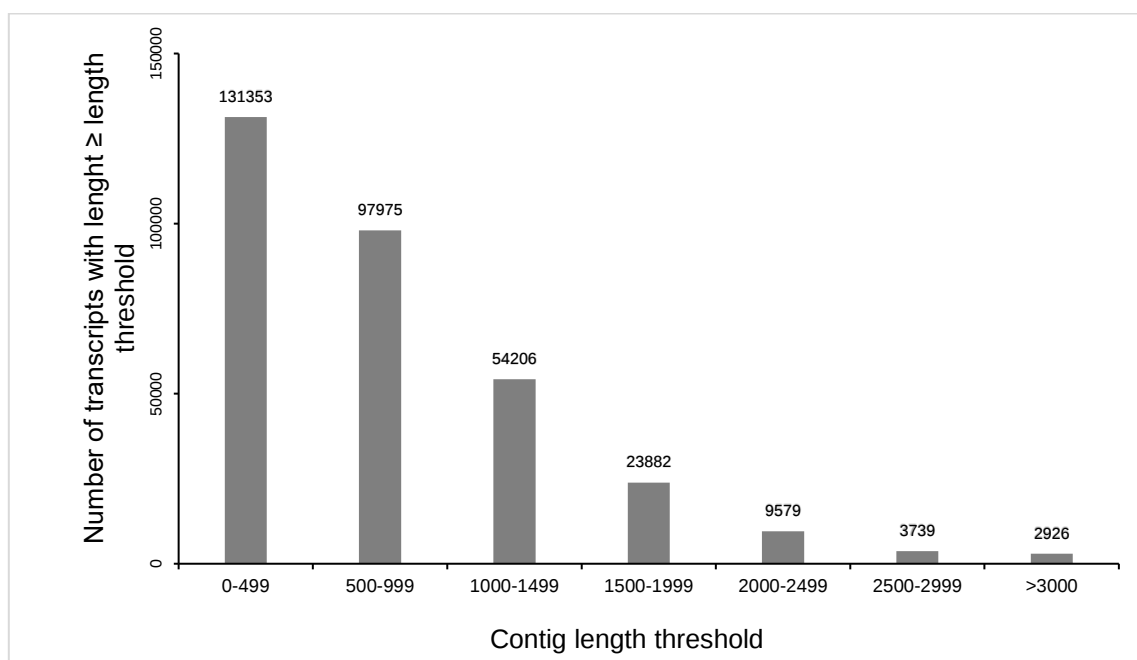


Figure 1. Length distribution of transcripts assembled from transcriptome libraries of hairy fleabane.

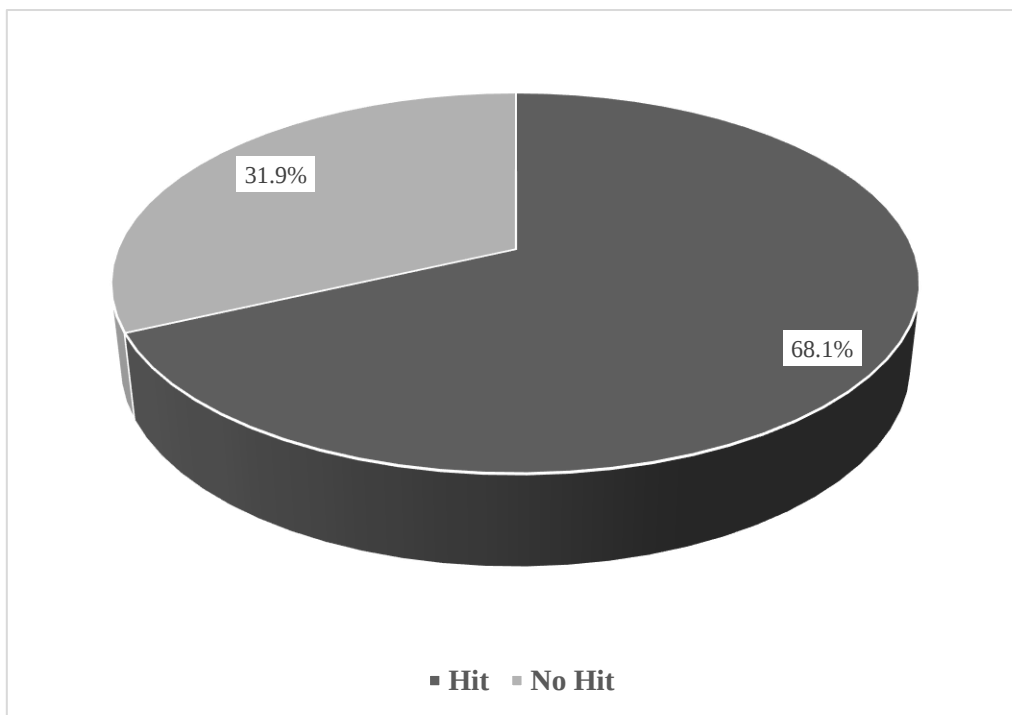


Figure 2. Sequence comparison to other organisms from the distribution of BLASTx hits (e-value < 1e-10) against the non-redundant protein database of the National Center for Biotechnology Information.

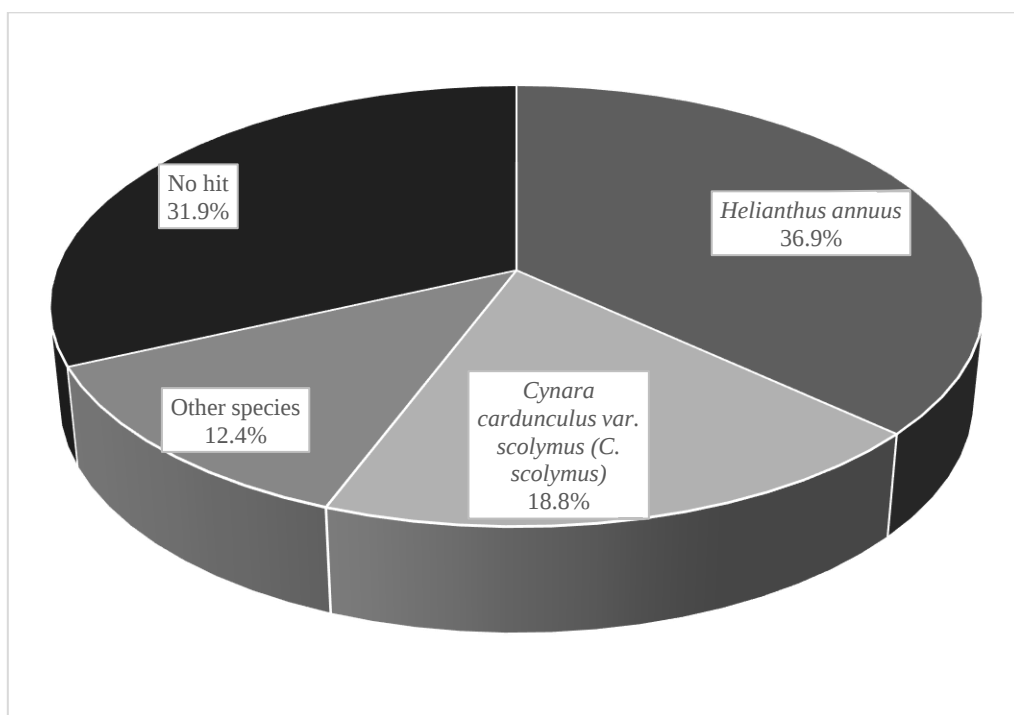


Figure 3. Sequence comparison to other plants (hit ≥ 1%) from the distribution of BLASTx hits (e-value < 1e-10) against the non-redundant protein database of the National Center for Biotechnology Information (NCBI).

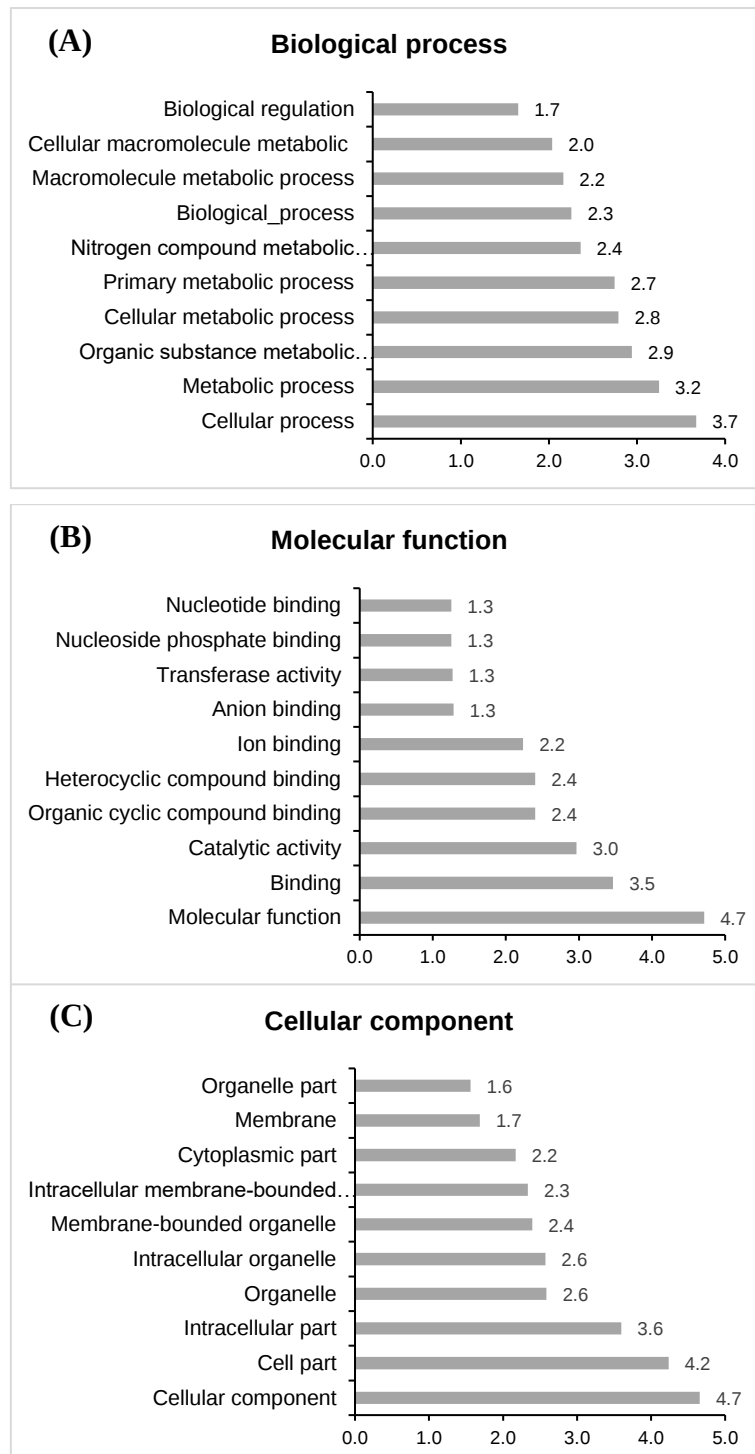


Figure 4. Top ten Gene Ontology (GO) terms identified in the hairy fleabane transcriptome assembly summarized in three main categories: (A) biological process, (B) molecular function, and (C) cellular component.

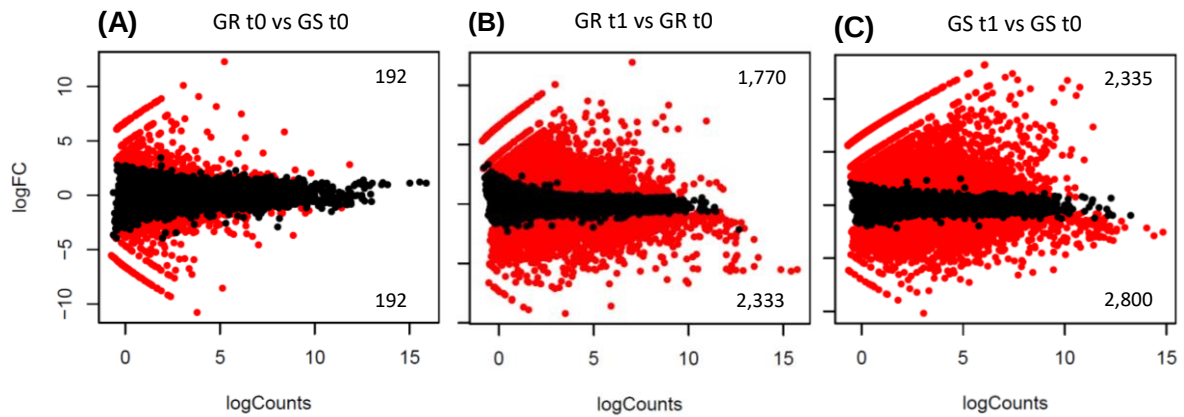


Figure 5. MA plot of differential expression analysis generated by EdgeR from transcriptome study performed in *Conyza bonariensis* glyphosate-resistant (GR) and -sensitive (GS) biotypes in response to glyphosate treatment. Dots above zero are up-regulated, and dots below are down-regulated. Red dots indicate significant expression at an adjusted p -value and false discovery rate (FDR) threshold set at ≤ 0.001 , and $\log_2\text{FC} \geq 2$ (up-regulated) or $\leq \log_2\text{FC}$ (down-regulated). Plots for each contig its $\log_2\text{FC}$ (fold change) (A, Y-axis) vs. its counts (mean of normalized counts) (M, X-axis). t0 = without glyphosate treatment; t1 = with glyphosate treatment – RNA was obtained from plants collected at several timepoints up to 288 h after treatment and pooled. GR t1: RNA sampled at 24, 96, 192, and 288 hours after treatment and pooled; GS t1: RNA sampled at 24, 96, and 192 hours after treatment and pooled.

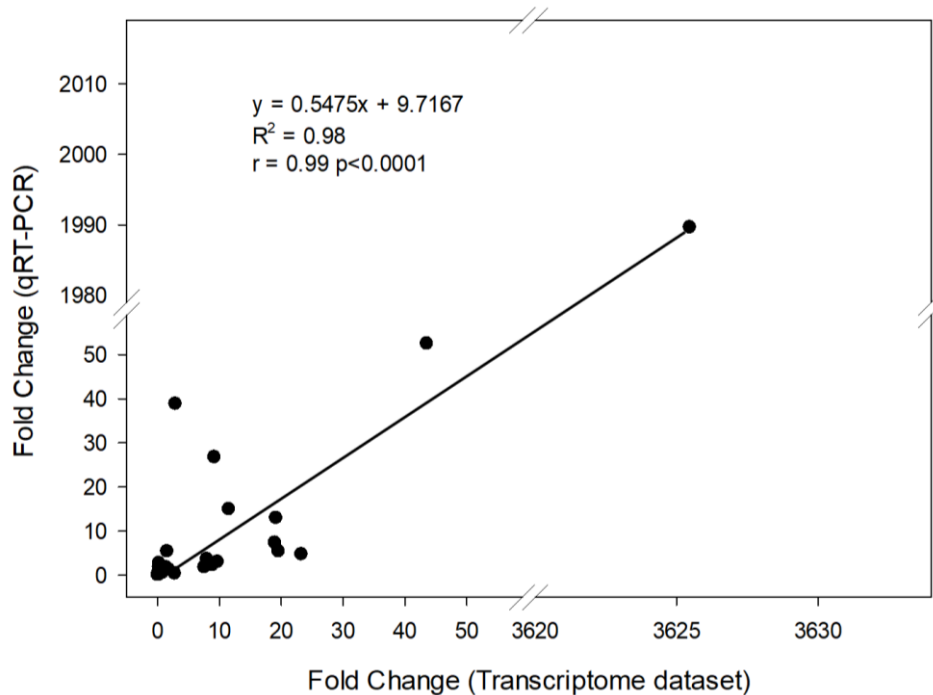


Figure 6. Correlation of transcriptomic and qRT-PCR (average of time-course) expression levels results of 19 genes of glyphosate-resistant (GR) and -sensitive (GS) *Conyza bonariensis* biotypes.

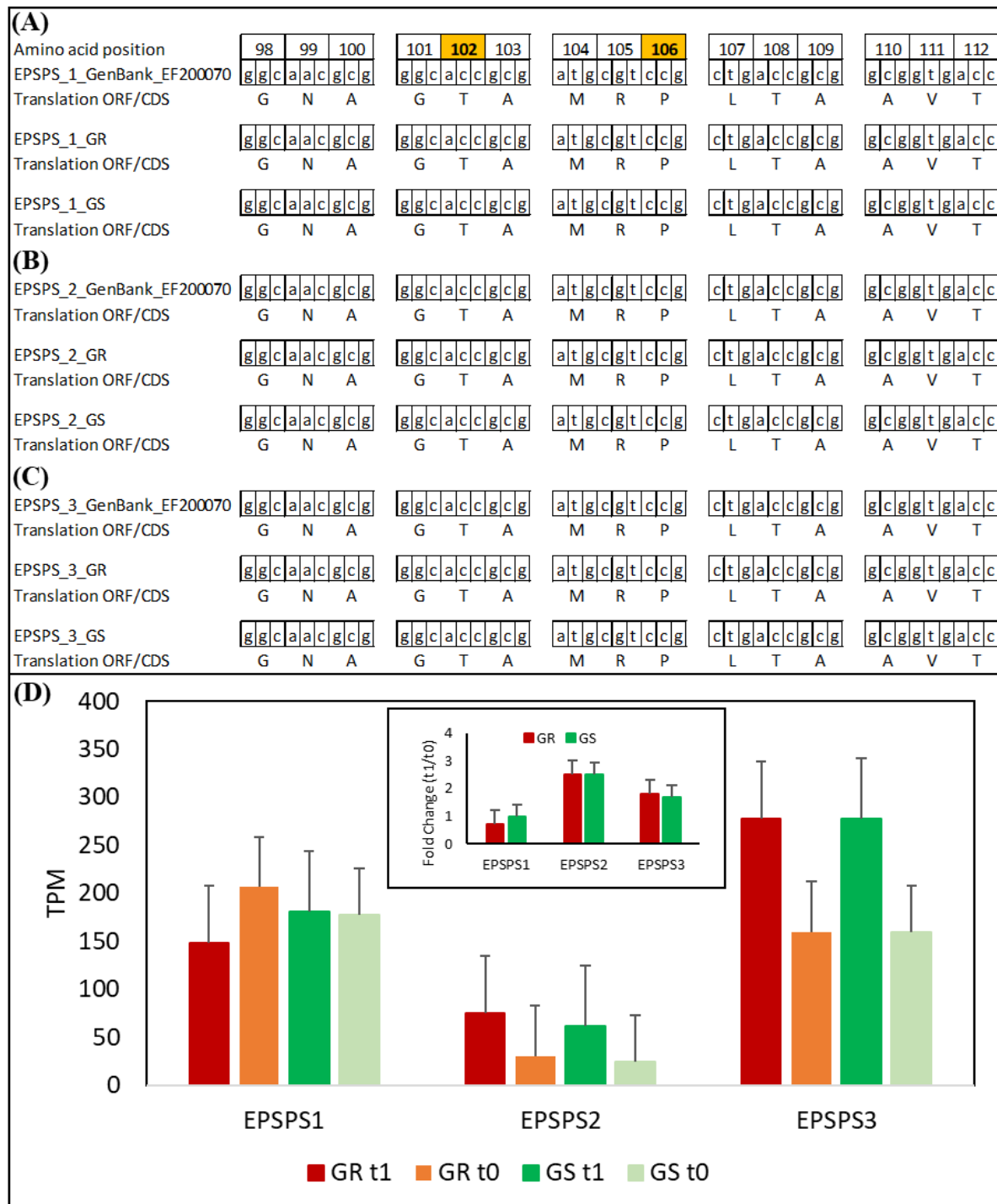


Figure 7. Partial sequence alignment of the EPSPS transcripts and amino acid sequence assembled of glyphosate-resistant (GR) and -sensitive (GS), and a sensitive *C. bonariensis* sequence from GenBank. (A) EPSPS1 (GenBank - accession number EF200070); (B) EPSPS2 (GenBank accession number EF200069); (C) EPSPS3 (GenBank accession number EF200074). The red boxed amino acids show no substitution at positions Threonine 102 and Proline 106. (D) Transcriptome expression levels (transcript reads per million mapped reads - TPM) of the three EPSPS copies in GR and GS in response to glyphosate treatment and expression difference (Fold change). GR: glyphosate-resistant biotype; GS: glyphosate-sensitive biotype. t0: without glyphosate treatment; t1: with glyphosate treatment – RNA was obtained from plants collected at several timepoints up to 288 h after treatment and pooled.

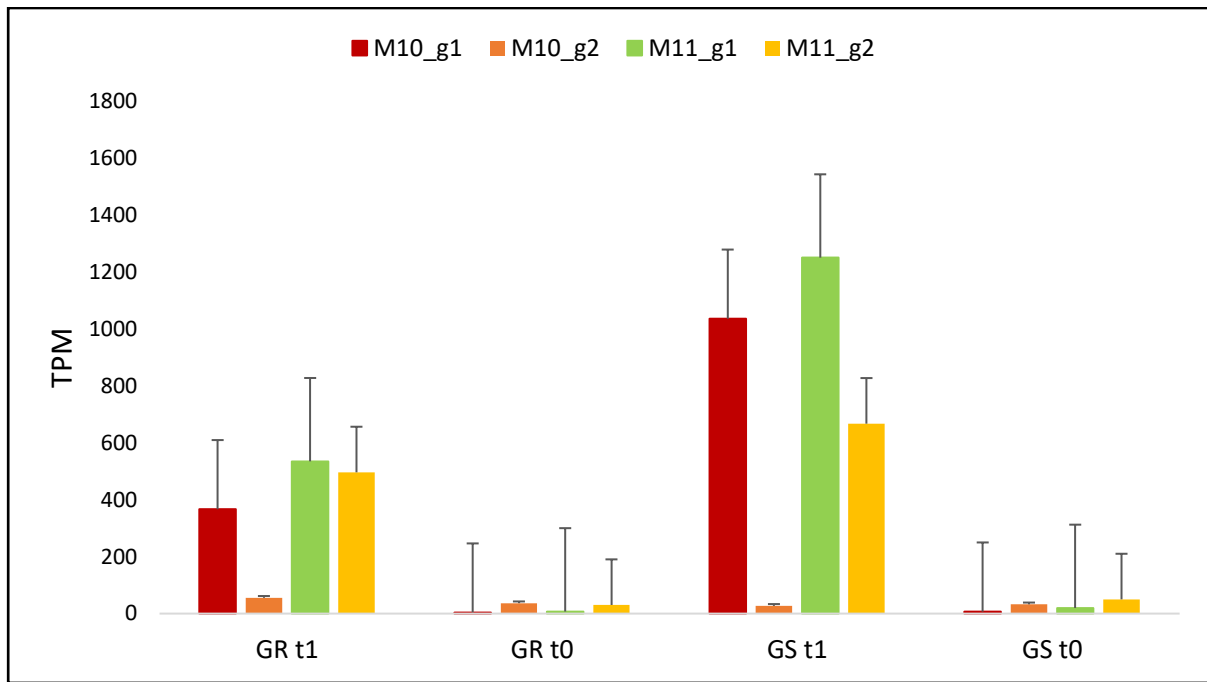
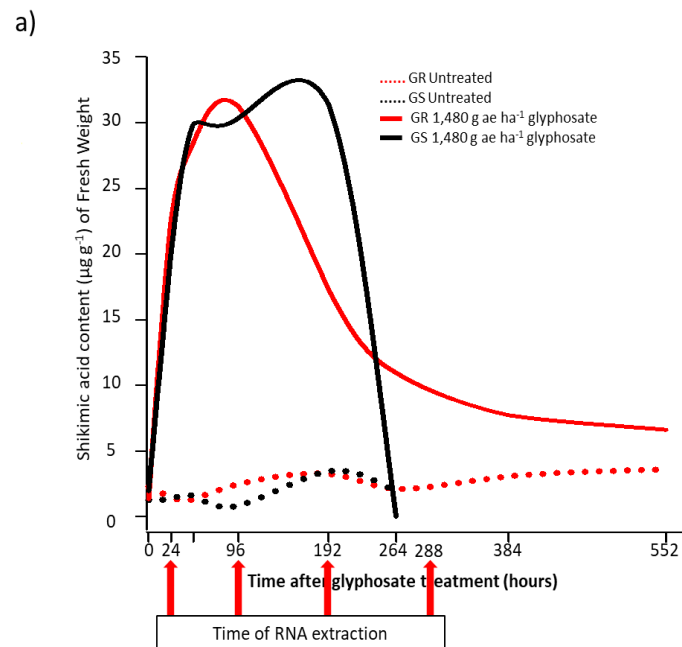


Figure 8. *Conyza bonariensis* transcriptome expression analysis (transcript reads per million mapped reads - TPM) of the M10 and M11 ABC Transporters reported being involved in glyphosate resistance in *C. canadensis* by Peng et al. (2010). M10_c1: Score (Bits) 2,682, *E-value* zero; M10_c2: Score 608, *E-value* 1e-72; M11_c1: Score 2,669, *E-value* zero; M11_c2: 1,232, *E-value* zero. GR: glyphosate-resistant biotype; GS: glyphosate-sensitive biotype. t0: without glyphosate treatment; t1: with glyphosate treatment – RNA was obtained from plants collected at several timepoints up to 288 h after treatment and pooled. Contigs were filtered according to the *p-value* and false discovery rate (FDR) threshold set at ≤ 0.001 . Intervals indicate the standard error. Adapted from Piasecki et al. (2019).[27]



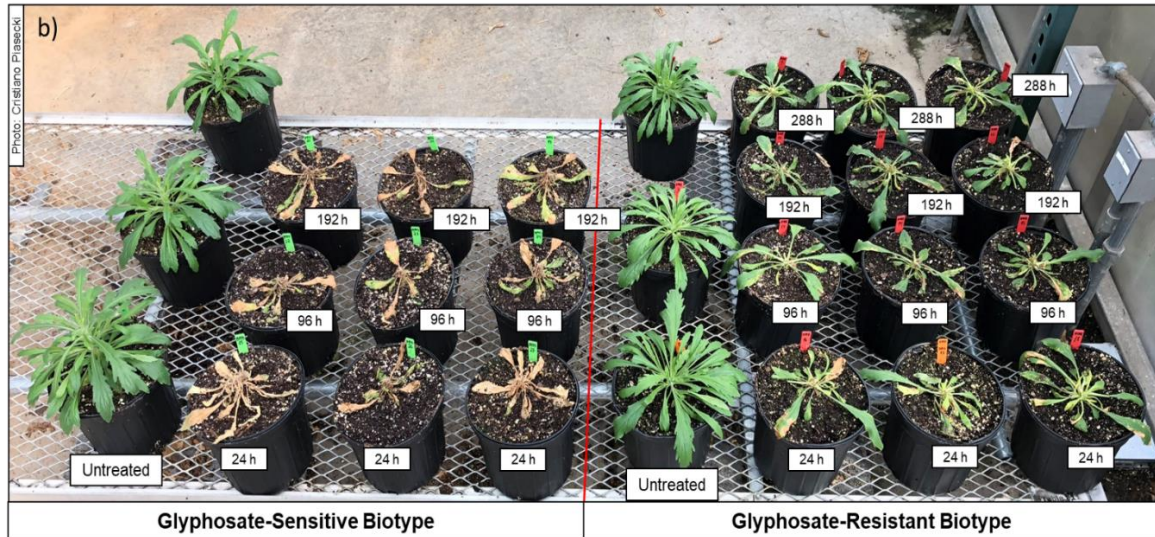


Figure 9. Responses of *Conyza bonariensis* glyphosate-resistant (GR) and -sensitive (GS) biotypes to glyphosate treatment ($1,480 \text{ g ae ha}^{-1}$). a) The shikimic acid content in a time-course experiment - Adapted from Piasecki et al. (2019) [28]; and b) Plants status at 288 hours after glyphosate treatment (12 days). 24 h, 96 h, 192 h, and 288 h illustrated the time after glyphosate treatment that leaves were collected for RNA extraction. GS plants died after 192 h after treatment whereas GR plants survived. Leaf samples were collected for RNA extraction based on shikimic acid content curve in GR biotype. Then RNA was extracted from untreated plants in GR and GS biotypes, and treated GR plants at 24, 96, 192, and 288 h after treatment, and at 24, 96, and 192 h in the GS plants which died after 192 h after treatment.

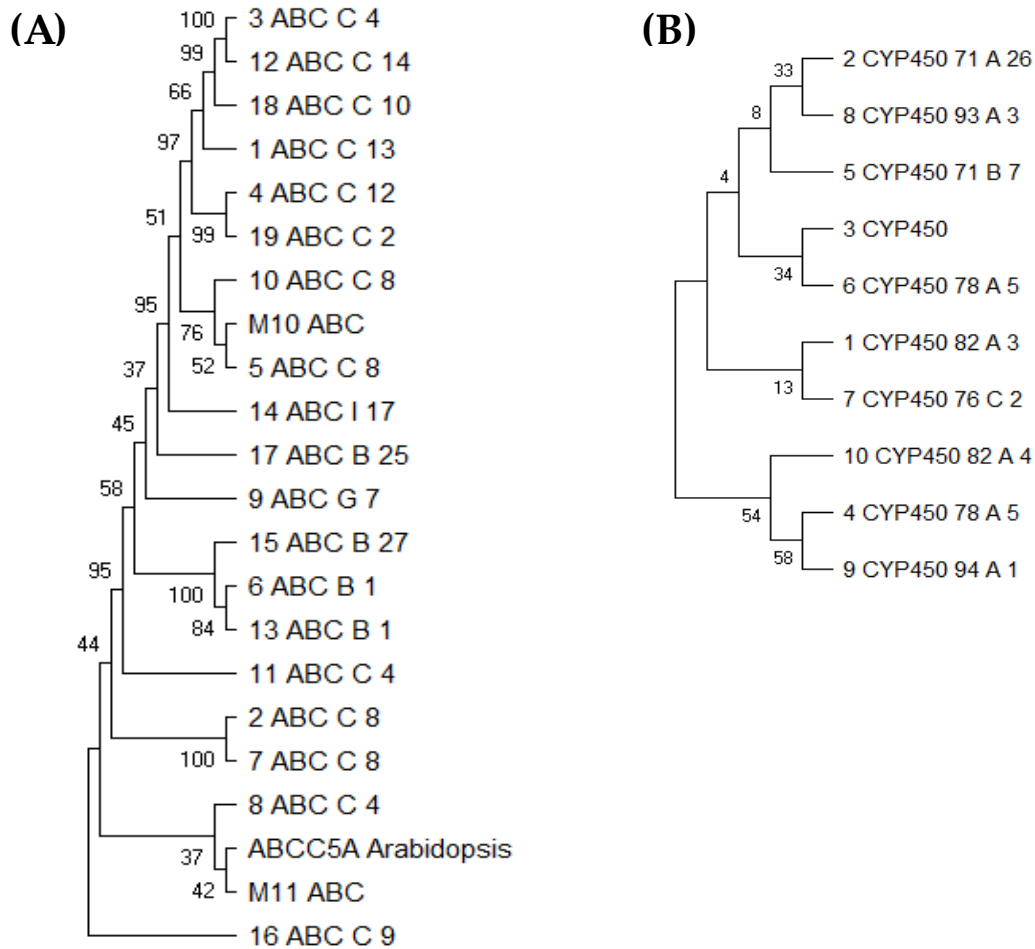


Figure 10. Evolutionary analysis of the candidate contigs sequences similarity to be related to glyphosate-resistance in *C. bonariensis* obtained from a transcriptome. A) ABC transporters; B) cytochrome P450 (CYP450). The number in front of the contig description indicate the order presented in each respective table 1 and 2. ABCC5A *Arabidopsis*: ABC Transporter full sequence obtained from GenBank *Arabidopsis thaliana*; M10 and M11 ABC: sequences of ABC transporters obtained from Peng et al. (2010). The evolutionary history was inferred by using the Maximum Likelihood method and the Tamura-Nei model. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

Supplementary Sequences

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