**Unraveling the mechanism of resistance in glufosinate-resistant**

***Amaranthus palmeri* population**

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**Supplementary Table 1.** Read counts and average coverage for putative chloroplastic and cytosolic glutamine synthetase from resistant (Glu-R1) and susceptible (S1) biotypes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Isoform | Accession | Contig Identifier | Read Count | Average Coverage | Percentage of Total Readsa |
| Cytosolic | Glu-R1 | TRINITY\_DN19984\_c1\_g1\_i4 | 5,942 | 668 | 0.026 |
| Cytosolic | Glu-R1 | TRINITY\_DN19984\_c1\_g2\_i4 | 2,834 | 340 | 0.013 |
| Cytosolic | S1 | TRINITY\_DN34761\_c0\_g1\_i3 | 3,857 | 390 | 0.035 |
| Cytosolic | S1 | TRINITY\_DN30605\_c0\_g1\_i1 | 3,012 | 275 | 0.028 |
| Chloroplast | Glu-R1 | TRINITY\_DN20153\_c0\_g1\_i2 | 54,760 | 18,226 | 0.242 |
| Chloroplast | Glu-R1 | TRINITY\_DN24116\_c2\_g2\_i11 | 35,845 | 3,843 | 0.159 |
| Chloroplast | Glu-R1 | TRINITY\_DN24117\_c2\_g2\_i5 | 17,835 | 2,116 | 0.079 |
| Chloroplast | S1 | TRINITY\_DN35099\_c0\_g1\_i1 | 1,985 | 163 | 0.018 |
| aA percent of total reads was calculated to account for differences in read numbers for the resistant (Glu-R1) and susceptible (S1) biotypes. The Glu-R1 biotype sequencing produced 22,582,008 pair-end reads while S1 biotype sequenced produced 10,883,584. | | | | | |

**Supplementary Figure 1.**ClustalW alignment of *GS1* isoform from both Glu-R1 and S1 accession aligned with *GS1* from *Cucumis melo* (NCBI Accession: NP\_001284433.1) and *A. palmeri* (NCBI Accession: GFQG01042326.1). Since there was no difference in translated amino acid sequences of the nucleotide contigs presented in Supplementary Table 1, a consensus sequence was for Glu-R1 and S1 *GS1* amino acid alignements.

**Supplementary Figure 2.** ClustalW alignment of *GS2* isoform from both Glu-R1 and S1 aligned with *GS2* from *Cucumis melo* (NCBI Accession: NP\_001284439.1) and *A. palmeri* (Heap 2022b). Since there was no difference in translated amino acid sequences of the nucleotide contigs presented in Supplementary Table 1, a consensus sequence was for Glu-R1 and S1 *GS1* amino acid alignements.

**Supplementary Figure 3.** HPLC-RAD analysis chromatograms of two susceptible (S1 and S2) and resistant (Glu-R1) *A. palmeri* accessions collected 48 h after 14C-glufosinate treatment.

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