SUPPORT INFORMATION

Investigation of the target-site resistance of EPSP synthase mutants P106T and T102I/P106S against glyphosate

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Predicted Structures of EPSPS Showed Reliable Stereochemical and Energy Profiles

The protein sequence of sensitive species of *C. sumatrensis*; *E. indica,* and *L. spicata* were obtained from the UniProt database[1] using the accession codes AAY40474.1, Q95AK0, and KP143747, respectively. To select the most suitable reference structure for homology modeling, we performed a sequence alignment using the BLASTp tool. The EPSPS structure of *Vibrio cholerae* (accession code 3NVS) showed 56.57% of similarity with the EPSPS sequence of the three analyzed species. The overall quality of the model was analyzed using the QMEAN scoring function (Figure S1) [2]. Regarding the *Ei*EPSPS, 95.37% of residues were found in the favorable regions, 3.70% of residues in the permitted regions, and 0.93% in non-permitted regions.

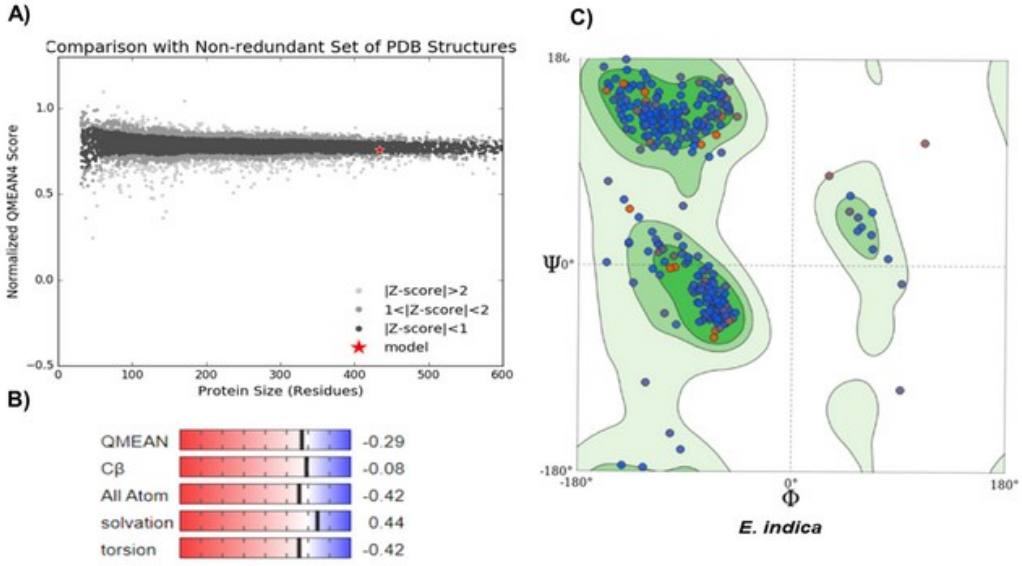

Figure S1. Structural validation of the modeled wild-type *Ei*EPSPS structure. Panel (A) shows the Z-score of *Ei*EPSPS structure comparing it with similar structures elucidated by experimental methods. Panel (B) shows the energy profile evaluated by the QMEAN score. Panel (C) represents the Ramachandran plot of the *Ei*EPSPS structure.

Regarding the *Cs*EPSPS structure, the analysis of the Ramachandran plot showed 94.77% in the most favorable regions, 3.64% of the residues in the permitted regions, and 1.59% in the non-permitted regions.

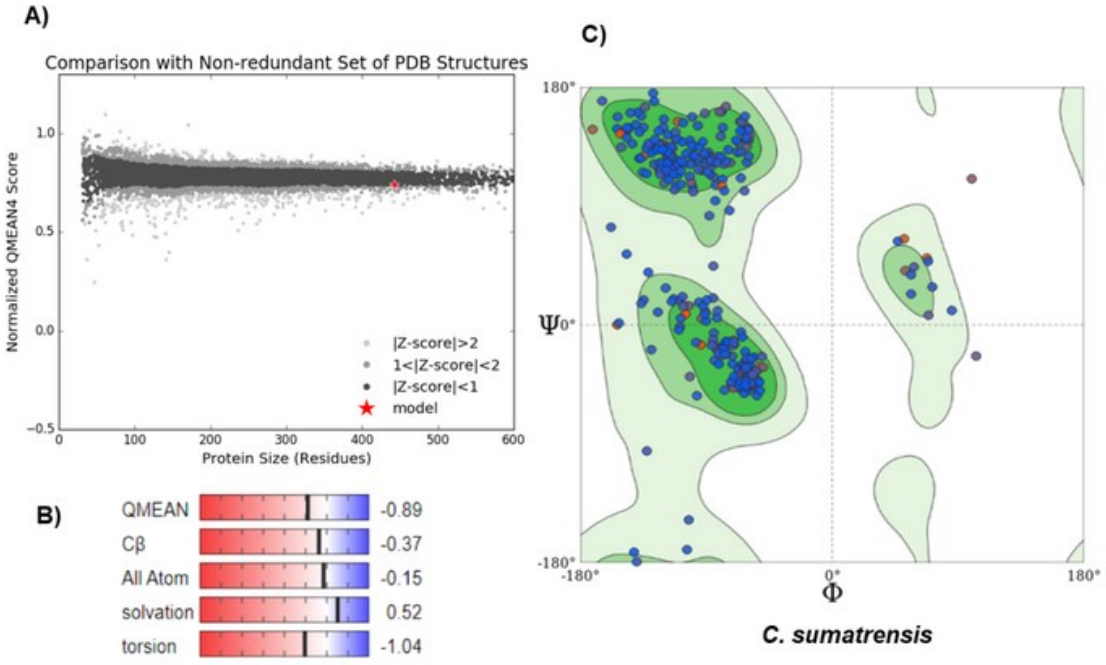

Figure S2. Structural validation of the modeled wild-type *Cs*EPSPS structure. Panel (A) shows the Z-score of the *Cs*EPSPS structure comparing it with similar structures elucidated by experimental methods. Panel (B) shows the energy profile evaluated by the QMEAN energy score. Panel (C) represents the Ramachandran plot of the structure.

C. sumatrensis			
Structural variants	**Total energy**	**van der Waals energy**	**Electrostatic energy**
Sensitve	-95.80 ± 0.47	-18.51 ± 0.22	-65.12 ± 1.16
Resistant	-75.64 ± 0.48	-9.29 ± 0.24	12.50 ± 0.87
E. indica			
Sensitive	-126.04 ± 0.47	-3.23 ± 0.21	-173.11 ± 1.13
Resistant	-75.67 ± 0.56	-3.88 ± 0.26	-152.71 ± 1.79

Table S1. Electrostatic and van der Waals components (kcal.mol^{-1}) of the binding free energy obtained using molecular mechanics Generalized Born surface area (MM/GBSA) method for both *Conyza sumatrensis* 5-enolpyruvyl shikimate-3-phosphate synthase (*Cs*EPSPS) and *Eleusine indica* EPSPS (*Ei*EPSPS) structures complexed with glyphosate.

References

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