frontiers

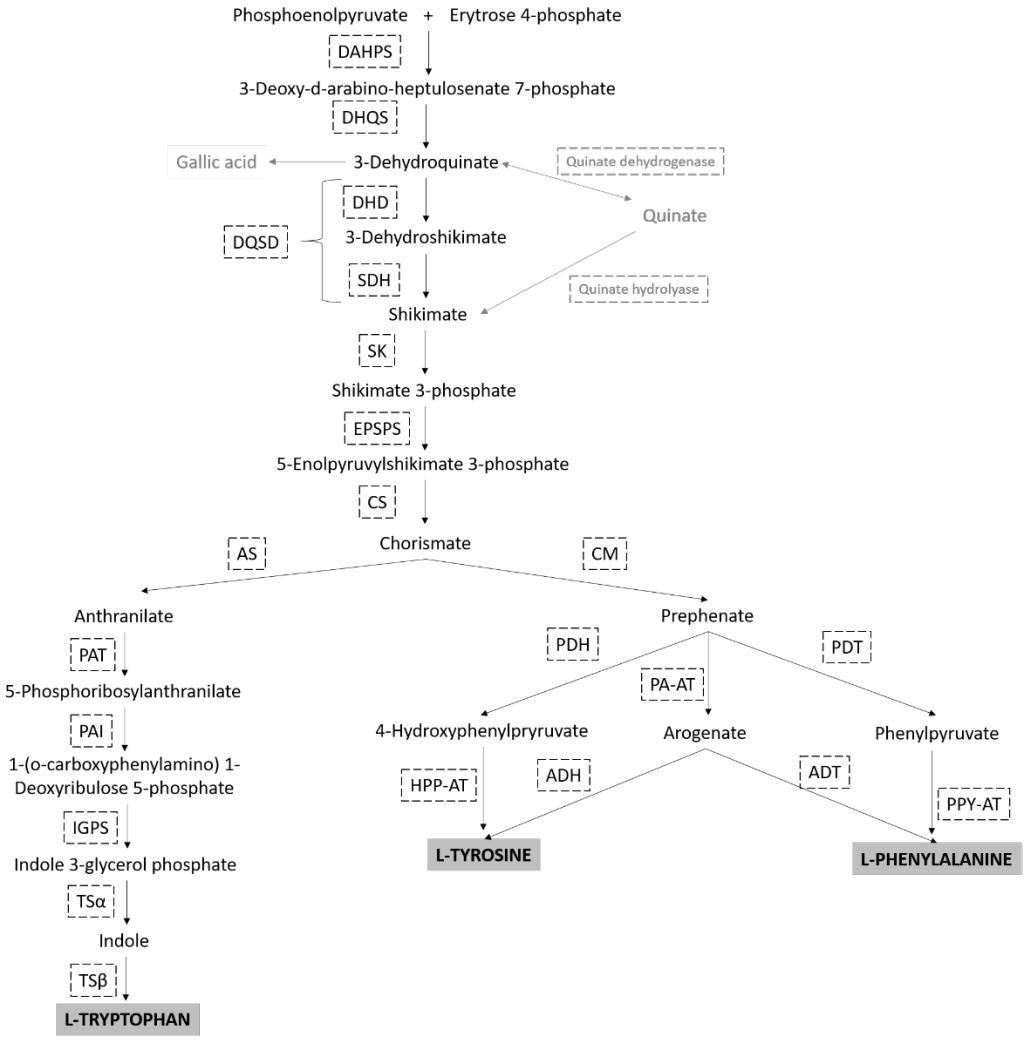
Supplementary Material

Glyphosate-induced increase in gene expression in the shikimate pathway is abolished in the presence of aromatic amino acids and mimicked by shikimate

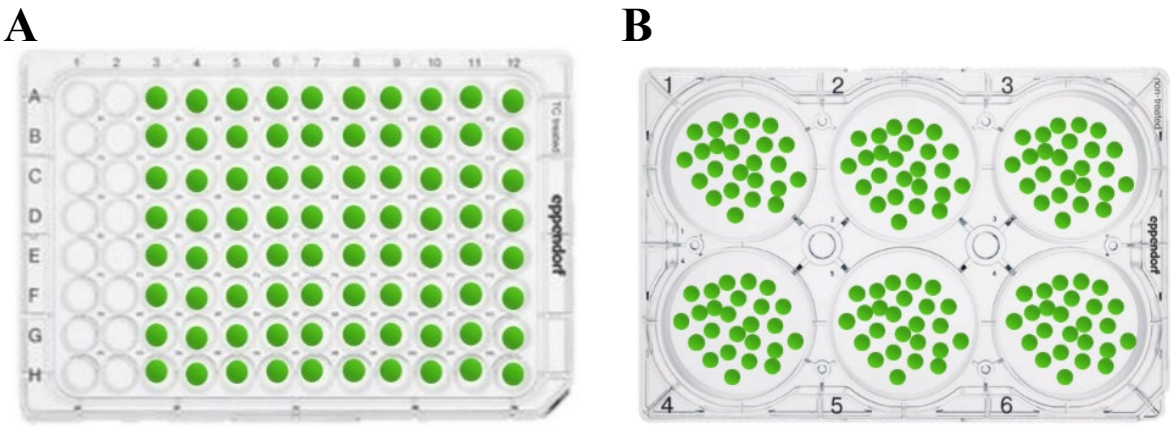
Ainhoa Zulet-González[1], Maria Barco-Antoñanzas[1], Miriam Gil-Monreal[1], Mercedes Royuela[1], Ana Zabalza[1,*]

[1] Institute for Multidisciplinary Research in Applied Biology (IMAB), Universidad Pública de Navarra, Campus Arrosadia s/n, 31006, Pamplona, Spain

*** Correspondence:**
Ana Zabalza
ana.zabalza@unavarra.es



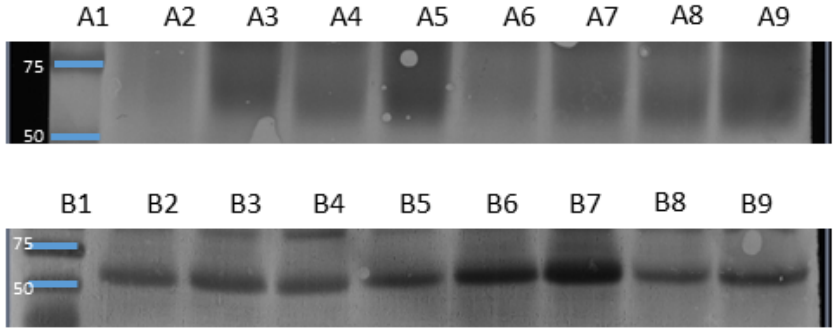
Supplementary Figure 1. Aromatic amino acids biosynthetic pathway in plants. The enzymes belonging the pre-chorismate pathway: D-arabino-heptulosonate 7-phosphate synthase (DAHPS), dehydroquinate synthase (DHQS), 3-dehydroquinate dehydratase (DHD), shikimate dehydrogenase (SDH), the bifunctional DHD-SDH dimer (DQSD), shikimate kinase (SK), 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) and chorismate synthase (CS). The enzymes belonging the post-chorismate pathway, towards the tryptophan synthesis: Anthranilate synthase (AS), phosphoribosylanthranilate transferase (PAT), phosphoribosylanthranilate isomerase (PAI), indole-3-glycerol phosphate synthase (IGPS), tryptophan synthase α subunit (TSα), tryptophan synthase β subunit (TSβ). The enzymes belonging the post-chorismate pathway towards the tyrosine and phenylalanine synthesis: chorismate mutase (CM), prephenate dehydrogenase (PDH), 4-hydroxyphenylpyruvate aminotransferase (HPP-AT), prephenate aminotransferase (PA-AT), arogenate dehydrogenase (ADH), arogenate dehydratase (ADT), prephenate dehydratase (PDT), phenylpyruvate aminotransferase (PPY-AT). Secondary metabolites are represented in gray and final products AAA are represented in bold capital letters and gray squared.



Supplementary Figure 2. Leaf disk incubation system. Leaf disks were excised from glyphosate-sensitive and glyphosate-resistant plants of *Amaranthus palmeri* and incubated for 24 h. One disk per well was incubated for shikimate content determination (A) and 25 or 45 disks were incubated for enzyme content and transcript level determination, respectively (B).

Sensitive population:

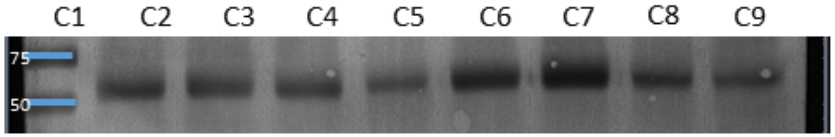
DAHPS



A1. Mw
A2. Control GS Rep1
A3. Glp GS Rep1
A4. AAA GS Rep1
A5. AAA+Glp GS Rep1
A6. Control GS Rep2
A7. Glp GS Rep2
A8. AAA GS Rep2
A9. AAA+Glp GS Rep2

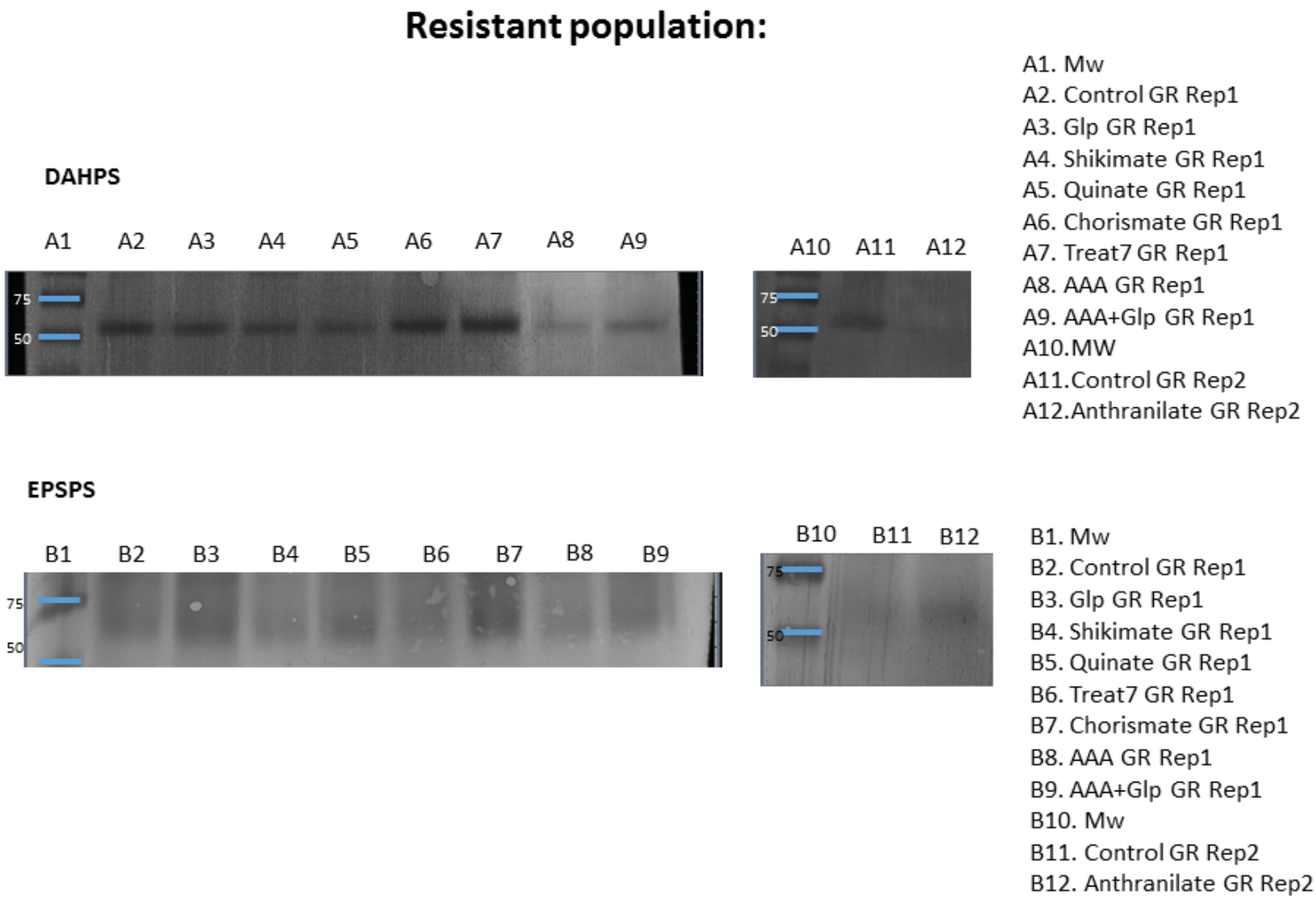
B1. Mw
B2. Control GS Rep3
B3. Glp GS Rep3
B4. Shikimate GS Rep3
B5. Quinate GS Rep3
B6. Chorismate GS Rep3
B7. Treat7 GS Rep3
B8. Anthranilate GS Rep3
B9. Treat8 GS Rep3

EPSPS



C1. Mw
C2. Control GS Rep1
C3. Glp GS Rep1
C4. Shikimate GS Rep1
C5. Quinate GS Rep1
C6. Chorismate GS Rep1
C7. Anthranilate GS Rep1
C8. AAA GS Rep1
C9. AAA+G GS Rep1

Supplementary Figure 3: Representative DAHPS and EPSPS immunoblots of glyphosate-sensitive Total soluble protein were fractioned by 12.5% SDS-PAGE and blotted.



Supplementary Figure 4: Representative DAHPS and EPSPS immunoblots of glyphosate-sensitive Total soluble protein were fractioned by 12.5% SDS-PAGE and blotted.

Supplementary Table 1. Oligonucleotide sequences used for the qRT-PCR reactions. Genes of the shikimate pathway: D-arabinoheptulosonate 7-phosphate synthase (*DAHPS*), dehydroquinate synthase (*DHQS*), 3-dehydroquinate dehydratase/shikimate dehydrogenase (*DQSD*), shikimate kinase (*SK*), 5-enolpyruvylshikimate 3-phosphate synthase (*EPSPS*), chorismate synthase (*CS*), chorismate mutase (*CM*) and anthranilate synthase (*AS*). Normalization gene selected for this study was β tubulin. For each primer pair, the annealing temperature is given.

.

GENE	FORWARD	REVERSE	ANNEALING TEMP
AAA biosynthetic pathway			
DAHPS	cctcataggatgataagggc	ctttgcatggcagcataacc	55
DHQS	gcattgttggctagggatcc	aacctcggccttgttttcac	61
DQSD	ggtgtactcaagcaaggagc	tgtggactcttactatggcc	57
SK	gattctgaagcacaaagcagc	cagttgttttcccagagccc	55
EPSPS	aatgctaaaggaggccttcc	tcaatctccacgtctccaag	61
CS	cttgatagaaggaggcctgg	gtttctttcctaggagtagtg	61
AS	tttggagggaaggttgtgcg	ctggtgagctttttccatgc	52
CM1-3	gaatccaagcccgcgtataa	cttcaatccaatcgtctcaacaag	59
CM 2	aagggtactgaagctgttcaag	tgtgctaatgaaggcggtaag	59
ADHα	accctcgctcttctctctatc	cggccgtgttggaattagta	52
ADHβ	cgggaatcttcctttcgtctc	aggttgagctgcgtcaatag	59
Normalization gene			
βTUBULIN	gatgccaagaacatgatgtg	tccacaaagtaggaagagttc	61