

Supporting Information

Target-site and metabolic resistance mechanisms to penoxsulam in barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv)

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Table S1. Herbicide doses applied in dose–response tests

Target	Group ^a	Herbicide ^b	Recommended field dose (g a.i. ha ⁻¹)	Doses applied to each population ^c (g a.i. ha ⁻¹)	
				R	S
Acetolactate synthase	PTB	Pyribenzoxim	37.5	0,4.69,9.38,18.75,37.5,75	0,2.35,4.69,9.38,18.75,37.5
	IMI	Imazapic	108	0,6.75,13.5,27,54,108	0,3.38,6.75,13.5,27,54
	SCT	Flucarbazone-sodium	30	0,3.75,7.5,15,30,60	0,1.88,3.75,7.5,15,30
	TP	Pyroxsulam	14	0,1.75,3.5,7,14,28	0,0.88,1.75,3.5,7,14
	SU	Flucetosulfuron	30	0,3.75,7.5,15,30,60	0,1.88,3.75,7.5,15,30
Acetyl-CoA carboxylase	APP	Propyrisulfuron	82.5	0,10.81,20.63,41.25,82.5,165	0,5.41,10.81,20.63,41.25,82.5 82.582.5
		Cyhalofop-butyl	105	0,13.13,26.25,52.5,105,210	0,6.56,13.13,26.25,52.5,105
		Metamifop	120	0,15,30,60,120,240	0,7.5,15,30,60,120
Unknown	PPZ	Pinoxaden	75	0,9.38,18.75,37.5,75,150	0,4.69,9.38,18.75,37.5,75
	QCA	Quinclorac	750	0,187.5,375,750,1500,3000	0,46.88,93.75,187.5,375,750
	PCA	florpyrauxifen-benzyl	22.5	0, 2.81,5.62,11.25,22.5,45	0,1.41,2.81,5.62,11.25,22.5

^aAbbreviations: PTB, pyrimidinylthiobenzoate; IMI, imidazolinone; SCT, sulfonylaminocarbonyltriazolinone; TP, triazolopyrimidine; SU, sulfonyleurea; APP, aryloxy phenoxy propionate; PPZ, phenylpyrazoline; QCA, quinoline carboxylic acids, PCA, pyridine carboxylic acids.

^bPyribenzoxim (5% emulsifiable concentrate; Korea's LG Life Science Co. Ltd., Shanghai, China), Imazapic (240 g L⁻¹ aqueous suspension; Rotam, Suzhou, China), Flucarbazone-sodium [70% water-dispersible granules (WDG); Arysta LifeScience, Shanghai, China], Pyroxsulam (7.5% WDG; Dow AgroSciences, Beijing, China), Flucetosulfuron [10% wettable powder; FMC, Suzhou, China], Propyrisulfuron (100 g L⁻¹ suspension concentrate; Sumitomo Chemical Co. Ltd., Japan), Cyhalofop-butyl(100 g L⁻¹ emulsifiable concentrate; Dow AgroSciences, Beijing, China) ;Metamifop(10% emulsifiable concentrate, FMC, Suzhou, China), Pinoxaden(5% emulsifiable concentrate, Syngenta Biotechnology Co., Ltd. Beijing,China); Quinclorac(50% wettable powder, Xinyizhongkai Agricultural Chemical Co., Ltd., Xinyi, China);florpyrauxifen-benzyl(25g L⁻¹ emulsifiable concentrate, Dow AgroSciences, Beijing, China)

^cPopulation R: AXXZ-6; Population S: JLGY-3.

Table S2. Primer sequences used for the qRT-PCR relative quantification of gene expression in *Echinochloa crus-galli*

Gene ID	Function annotation	Sequence (5' to 3')		Product size(bp)
EC_v6.g024971	CYP74A2	F:AGGGCCAGGACAAGTACTTC	R:TCCACCTTGTCATGTCGAA	161
EC_v6.g021245	CYP93A1	F:ATCATGATCACGGCCAAGGA	R:GGACGAAGGCCTTGATGTTT	171
EC_v6.g088422	CYP74B2	F:CTTCACCTTCTCGACGTCT	R:CCAGCACGAAGAGGATGTTG	246
EC_v6.g024973	CYP74A2	F:AGGGCCAGGACAAGTACTTC	R:CCACCTTGTTGACGTCGAA	159
EC_v6.g045480	CYP78A9	F:AACATGTGGGCCATCACGCA	R:TGAGCACCTCCGACAGGTCA	264
EC_v6.g073605	CYP90A1	F:CCCAGGCTATGAGCTTGCCC	R:GCATCAGGTCGTGTCGGAG	160
EC_v6.g082320	CYP734A1	F:GGTTCTCCAGCTTTCAACC	R:ATCAAGACGACGACCTCCTC	235
EC_v6.g091909	CYP93A1	F:GGACATCCTCATGGACGCGG	R:GTGTCGGAACCAGCGGTGAA	106
EC_v6.g105576	CYP98A1	F:CTACATCAAGTCCGCAAGC	R:CGTGATGTTGTTGAAGGCCA	196
EC_v6.g099783	CYP71C2	F:GAAGAGGTGAGCAGGGTGAT	R:GAACTCCTCGACGTTGAAGC	214
EC_v6.g030813	CYP72A15	F:AACGTCGTTCACTTGGTTTCG	R:CCTGTGCTTTGCCCATTTCT	178
EC_v6.g041677	CYP714B1	F:TACGAGAGGTGGAGGAAGGA	R:CTTGTCGGGTTAGAACTGGG	246
EC_v6.g010864	CYP72A15	F:GAATCCAGCCTTCCATGCAG	R:ACACGGACAAGTTGTTTCAGC	215
EC_v6.g096099	CYP81E1	F:CGAGTTCAAGCAGATCGTGG	R:GATGAGCCGATTCAGGAACG	158
EC_v6.g100667	Pectinesterase 2.2	F:GTTTCGACTCAGCCACCCTAG	R:GAAGATGAAGTCGACGGTGC	233
EC_v6.g099076	palmitoyl-protein thioesterase 1	F:AGCTTCAGTGCCACTTTGTG	R:ACCAAGTTTTTCCAAGCTGCT	240
EC_v6.g024163	1-aminocyclopropane-1-carboxylate oxidase	F:GACATGGACTGGGAGGACAT	R:GAGGTTCTCGTCCATGACCT	150
EC_v6.g073396	Oxygen-dependent coproporphyrinogen-III oxidase	F:CATTCAACCGAGGAGCACAAG	R:TGCACGCATCGAGAAGTTTC	212
EC_v6.g000922	zeaxanthin epoxidase, chloroplastic isoform X2 [Setaria italica]	F:TGTGTCAGTGTTTGGAGGGT	R:GCAGACCCATACTAAAGCC	177
EC_v6.g100130	polyamine oxidase-like	F:AACTCCACCCTCAAGCTCAG	R:GCTAGGAGGGAGATGTCGTC	197
EC_v6.g098075	probable L-ascorbate peroxidase 6	F:GACCTCTTTCAGCTTGCCAG	R:TCCTTGTCATTCAGGCCCAT	195
EC_v6.g107834	peroxidase A2-like	F:CCTCACCCGTCTGCATTTTC	R:AGGATATCGGCACAGGAGAC	202

EC_v6.g107836	peroxidase 54 precursor	F: CACCCGTCTCCATTTTCACG R:CTCAACGGAAATCTCAGCGG	226
EC_v6.g021711	peroxidase 11	F: GCCAGATGCGGAGAACTTCAG R: GAGCGTCTCGTAGTAGGCAT	190
EC_v6.g065624	peroxidase A2-like	F:CAACTTCAGCGGGACAAACA R:GGAAGCCCCTGTTCAITTCG	177
EC_v6.g002096	peroxidase 2-like	F: TCACCATCTCCGAGCTGATC R:CGACAGCAGGTTCCGGTA	170
EC_v6.g096321	hydroxyacylglutathione hydrolase	F: GAGGAAGGGCAGGAGATCAT R:GAACCACGGAACTTGAAGC	238
EC_v6.g007915	ubiquitin carboxyl-terminal hydrolase isozyme L3-like	F: GCCGTCCTCTTCCTCTATCC R: CACCAACTGTTCCACATGCA	151
EC_v6.g020710	glutathione S-transferase 2	F: ATGGCTCCGATGAAGCTGTA R:TTGCAAAGCTGGAACCTGAC	177
EC_v6.g027657	glutathione S-transferase T1	F: ATCACTGGTACCCTGCAGAC R:CCTTTGAGCCACACGGATTC	215
EC_v6.g052496	glutathione S-transferase F11-like	F: AAGTCGCCTTCCTTCCTCAA R:TCGGTGGGTAAAAGCTCTGA	223
EC_v6.g052005	UDP-Glycosyltransferase superfamily	F:ACTCCCTCACCAAATCGCTT R:ACACGTTTTGGACCATCACC	166
EC_v6.g031559	UDP-Glycosyltransferase superfamily	F:CAAGCTGTGGACTGTTGACA R:TGCTCACCCGAAGATGCATA	175
EC_v6.g050762	ABC transporter G family member 11-like	F:TGACACTCTGCATTGGGACT R: AGAGGGTGTTGCTGATGACA	214
EC_v6.g018324	ABC transporter I family member 11I family member 11	F: GCTGGTCTAGATTGGAAGGC R:TTACACGGGCACAGATTCCT	174
EC_v6.g030014	ABC transporter C family member 1	F: AGAACAACAGAGGCTTGGGA R:ACATAGCTCCCAGTTGCCTT	227
HQ395760	<i>Echinochloa crus-galli</i> β -actin	F: GTGCTGTTCCAGCCATCGTTCAT R: CTCCTTGCTCATACGGTCAGCAATA	171

Table S3 Base information statistics table before and after filtering of RNA-seq

Sample	Before Filter					After Filter				
	Clean Data(bp) ^a	Q20(%) ^b	Q30(%) ^c	N(%) ^d	GC(%)	HQ Clean Data(bp) ^e	Q20(%)	Q30(%)	N(%)	GC(%)
R-24h	8672682600	8526476811 (98.31%)	8291510228 (95.60%)	1161276 (0.01%)	4718288780 (54.40%)	8446980213	8354649030 (98.91%)	8156793779 (96.56%)	1015793 (0.01%)	4593207292 (54.38%)
R-6h	7745348100	7618413017 (98.36%)	7409595263 (95.67%)	278636 (0.00%)	4331044351 (55.92%)	7542017617	7461581252 (98.93%)	7285678482 (96.60%)	84934 (0.00%)	4215980657 (55.90%)
R-72h	7168947600	7044855897 (98.27%)	6843978216 (95.47%)	955238 (0.01%)	3983218443 (55.56%)	6980959032	6901895190 (98.87%)	6732491565 (96.44%)	840079 (0.01%)	3877111245 (55.54%)
R-ck	9122407800	8970105187 (98.33%)	8717675551 (95.56%)	330147 (0.00%)	5223585215 (57.26%)	8881100699	8784207883 (98.91%)	8571460349 (96.51%)	99803 (0.00%)	5085023339 (57.26%)
S-24h	11117073900	10962931972 (98.61%)	10713426806 (96.37%)	1361682 (0.01%)	6024472638 (54.19%)	10852776036	10758049353 (99.13%)	10552014651 (97.23%)	1242240 (0.01%)	5876960487 (54.15%)
S-6h	8626077000	8520652113 (98.78%)	8344542972 (96.74%)	1053532 (0.01%)	4773269334 (55.34%)	8438607664	8372907173 (99.22%)	8225406496 (97.47%)	965671 (0.01%)	4666990308 (55.31%)
S-72h	7642848300	7548820133 (98.77%)	7393405837 (96.74%)	933596 (0.01%)	4158001803 (54.40%)	7470869124	7412922750 (99.22%)	7283554231 (97.49%)	854853 (0.01%)	4061790733 (54.37%)
S-ck	7770204000	7635821757 (98.27%)	7414473680 (95.42%)	1025888 (0.01%)	4470273656 (57.53%)	7564566590	7477886435 (98.85%)	7290311357 (96.37%)	902393 (0.01%)	4351939810 (57.53%)

^aClean data (bp): The total number of reads bases that have been initially filtered after the machine is down.

^b Q20 (%): Number and percentage of bases with data quality up to Q20 (ie 99% sequencing accuracy).

^c Q30 (%): the number and percentage of bases whose data quality reaches Q30 (ie, the sequencing accuracy is 99.9%).

^d N (%): number and percentage of N bases.

^e HQ Clean data (bp): total number of reads bases obtained after further filtering of clean data.

Table S4 Reads filter information statistics of RNA-seq

Sample	Clean Reads Num ^a	HQ Clean Reads Num(%) ^b	Read Length ^c	Adapter(%) ^d	Low Quality(%) ^e	Poly A(%) ^f	N(%) ^g
R-24h	57817884	57008162 (98.6%)	150 + 150	286806 (0.5%)	513410 (0.89%)	20 (0%)	4733 (0.02%)
R-6h	51635654	50910720 (98.6%)	150 + 150	234372 (0.45%)	482350 (0.93%)	5 (0%)	4101 (0.02%)
R-72h	47792984	47125880 (98.6%)	150 + 150	224350 (0.47%)	435300 (0.91%)	13 (0%)	3714 (0.02%)
R-ck	60816052	59942392 (98.56%)	150 + 150	279032 (0.46%)	585048 (0.96%)	21 (0%)	4769 (0.02%)
S-24h	74113826	73133068 (98.68%)	150 + 150	310866 (0.42%)	666368 (0.9%)	37 (0%)	1725 (0%)
S-6h	57507180	56825598 (98.81%)	150 + 150	259872 (0.45%)	418992 (0.73%)	1 (0%)	1358 (0%)
S-72h	50952322	50321156 (98.76%)	150 + 150	238298 (0.47%)	390506 (0.77%)	8 (0%)	1173 (0%)
S-ck	51801360	51080362 (98.61%)	150 + 150	245482 (0.47%)	467452 (0.9%)	9 (0%)	4023 (0.02%)

^a Clean Reads Num: The number of reads that have been initially filtered after the machine is off.

^b HQ Clean Reads Num (%): the number and percentage of reads obtained by further filtering after clean reads.

^c Read Length: length of reads.

^d Adapter (%): number and percentage of reads with adapter.

^e Low Quality (%): the number and percentage of reads with a mass value of $Q \leq 20$, which account for more than 50% of the total reading.

^f Poly A (%): all the number and percentage of reads of A bases.

^g N (%): the number and percentage of reads with a ratio of N greater than 10%.

Table S5. Genes identified for the establishment of a metabolizing enzyme in *Echinochloa crus-galli*.

Phase	Family	Total gene numbers	Different expression genes numbers
I	Cytochrome P450	176	136
	Esterases	7	3
	Peroxidases	191	156
	Oxidases	310	284
	Hydrolases	38	32
II	Glutathione S-transferases	76	63
	UDP-glycosyltransferases	4	3
III	ATP-binding cassette transporter family	31	17

Table S6. 36 Information of candidate genes used in qRT-PCR.

Gene ID	Symbol	R-ck_fpk ^a	R-6h_fpkm	R-24h_fpkm	R-72h_fpkm	S-ck_fpkm	S-6h_fpkm	S-24h_fpkm	S-72h_fpkm
EC_v6.g024971	CYP74A2	117.93	339.66	139.9	508	70.4	242	42.15	125
EC_v6.g021245	CYP93A1	21.71	3.6	33.52	15.5	16.2	0.9	18.54	1.73
EC_v6.g088422	CYP74B2	19.89	14.91	31.09	43.3	25.6	15.9	33.28	26.8
EC_v6.g024973	CYP74A2	13.48	105.13	139.4	330	8.32	120	62.65	63.4
EC_v6.g045480	CYP78A9	24.19	65.38	5.94	16.7	20.3	41.7	3.83	14
EC_v6.g073605	CYP90A1	12.77	24.24	32.4	43.7	9.21	14.8	20.15	25.7
EC_v6.g082320	CYP734A1	8.39	24.88	36.03	20.3	0.99	6.14	15.48	27.2
EC_v6.g091909	CYP93A1	127.85	25.13	185.1	104	63.5	4.41	96.35	11.4
EC_v6.g105576	CYP98A1	27.03	6.67	45.77	26.6	31.7	0.81	42.87	2.66
EC_v6.g099783	CYP71C2	10.58	12.28	18.27	6.81	6.39	8.33	4.31	9.18
EC_v6.g030813	CYP72A15	11.47	20.17	82.4	18.5	1.55	3.6	4.88	7.27
EC_v6.g041677	CYP714B1	4.96	23.32	1.68	6.94	3.21	17.5	0.53	4.27
EC_v6.g010864	CYP72A15	6.57	4.61	15.52	4.12	1.57	1.09	2.26	3.54
EC_v6.g096099	CYP81E1	6.57	20.07	17.04	5.7	5.02	4.04	2.56	1.08
EC_v6.g100667	Pectinesterase 2.2 [Dichanthelium oligosanthes]	11.22	75.55	30.72	62.84	4.5	51.75	13.99	29.1
EC_v6.g099076	palmitoyl-protein thioesterase 1	7.19	12.34	18.34	10.8	5.11	9.65	11.09	6.24
EC_v6.g024163	1-aminocyclopropane-1-carboxylate oxidase	148.33	94.15	71.01	95.7	143	65.2	40.36	28.1
EC_v6.g073396	Oxygen-dependent coproporphyrinogen-III oxidase	136.06	183.54	171.9	272	83.2	87.6	126	65.3
EC_v6.g000922	zeaxanthin epoxidase, chloroplastic isoform X2 [Setaria	123.38	164.37	11.19	39.5	125	129	18.42	77.2
EC_v6.g100130	polyamine oxidase-like	47.04	66.73	27.66	24.7	53	15.6	5.13	11.8
EC_v6.g098075	probable L-ascorbate peroxidase 6	3	13.78	25.08	25	7.78	12.2	22.48	27.6

EC_v6.g107834	peroxidase A2-like	46.38	92.74	276.2	90.1	0.76	7.62	42.93	4.94
EC_v6.g107836	peroxidase 54 precursor	42.85	81.78	881.7	180	3.91	116	420.2	133
EC_v6.g021711	peroxidase 11	7.34	4.73	20.52	13.8	0.77	0.26	6.54	1.18
EC_v6.g065624	peroxidase A2-like	46.52	57.57	192.1	185	22.4	38.4	98.26	51.1
EC_v6.g002096	peroxidase 2-like	64.1	34.2	94.08	94.6	13	5.67	41.27	23
EC_v6.g096321	hydroxyacylglutathione hydrolase	20.12	53.1	28.36	30.82	2.22	9.93	1.43	0.37
EC_v6.g007915	ubiquitin carboxyl-terminal hydrolase isozyme L3-	5.68	8.39	19.14	17.31	3.14	8.15	6.78	8.77
EC_v6.g020710	glutathione S-transferase 2	35.46	27.7	215.1	123	13	8.37	40.69	108
EC_v6.g027657	glutathione S-transferase T1	23.1	34.18	27.19	24	16.9	15.2	12.92	12.3
EC_v6.g052496	glutathione S-transferase F11-like	7.92	24.39	23.98	36.3	4.66	7.22	11.63	12.4
EC_v6.g052005	UDP-Glycosyltransferase superfamily	4.85	4.85	3.91	3.42	3.12	2.53	2.36	1.96
EC_v6.g031559	UDP-Glycosyltransferase superfamilyGlycosyltransferase	0.66	1.68	2.34	1.89	2.28	2.75	1.2	1.91
EC_v6.g050762	ABC transporter G family member 11-like	7.84	6	10.53	10.2	0.1	0.4	2.68	0.1
EC_v6.g018324	ABC transporter I family member 11	8.78	18.03	14.53	28.3	2.21	8.23	9.96	3.98
EC_v6.g030014	ABC transporter C family member 1	10.89	16.33	17.84	20.3	7.69	15.1	13.67	8.17

^afpkm: fragments per kilobase million.

Figures legends

Figure S1. Twelve genes expression level that did not show significant difference between the two population treated with three herbicides in qRT-PCR validations. (A) EC_v6.g024971, (B) EC_v6.g021245, (C) EC_v6.g091909, (D) EC_v6.g105576, (E) EC_v6.g041677, (F) EC_v6.g010864, (G) EC_v6.g096099, (H) EC_v6.g100130, (I) EC_v6.g002096, (J) EC_v6.g007915, (K) EC_v6.g020710, (L) EC_v6.g050762.