

1 **Supplementary information**

2 **A Trp-574-Leu mutation in acetolactate synthase confers imazamox**  
 3 **resistance in barnyardgrass (*Echinochloa crus-galli*) from China**

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8 **Table S1**

9 Herbicide doses applied in whole-plant dose-response bioassays

Herbicide target	Group <sup>a</sup>	Herbicide	Doses applied to each population (g a.i. ha <sup>-1</sup> )		Recommended field dose (g a.i. ha <sup>-1</sup> )
			S	R	
acetolactate synthase	SCT	flucarbazone-sodium	0, 1.925, 3.85, 7.7, 15.4, 30.8	0, 7.7, 15.4, 30.8, 61.6, 123.2	30.8
	TP	penoxsulam	0, 1.875, 3.75, 7.5, 15, 30	0, 15, 30, 60, 120, 240	30
	SU	propyrisulfuron	0, 4, 8, 16, 32, 64	0, 16, 32, 64, 128, 256	64
	PTB	bispyribac-sodium	0, 2.3375, 4.675, 9.35, 18.7, 37.4	0, 9.35, 18.7, 37.4, 74.8, 149.6	37.4
acetyl-CoA carboxylase	APP	metamifop	0, 6.5625, 13.125, 26.25, 52.5, 105	0, 13.125, 26.25, 52.5, 105, 210	105
		cyhalofop-butyl	0, 8.4375, 16.875, 33.75, 67.5, 135	0, 16.875, 33.75, 67.5, 135, 270	135

10 <sup>a</sup>Abbreviations: SCT, sulfonylamino-carbonyl-triazolinone; TP, triazolopyrimidine; SU,  
 11 sulfonylurea; PTB, pyrimidinylthiobenzoate; APP, aryloxy phenoxy propionate.

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13 **Table S2**

14 Primers used to detect ALS expression in *Echinochloa crus-galli* by RT-qPCR

Gene	Primer sequence (5' to 3')	Product size (bp)
ALS	F: CCCAGTGAAGGTCTTTGTGC	231
	R: CCTGGAGTCTCGAGCATCTT	
$\beta$ -actin	F: CACACTGGTGTGCATGGTAGG	129
	R: AGAAAGTGTGATGCCAGAT	

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17 **Table S3**

18 Gradient elution conditions with HPLC-MS/MS

t/min	Flow rate (mL/min)	Acetonitrile (%)	Water solution (%)
0	0.30	5	95
0.5	0.30	5	95
2.0	0.30	95	5
3.0	0.30	95	5
4.0	0.30	5	95
5.0	0.30	5	95

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20 **Table S4**

21 Multiple Reaction Monitoring (MRM) setting conditions

Retention time (min)	Confirmation product ion (m/z)	Quantitation ion product (m/z)	Fragmentor (V)	Collision energy (eV)
1.53	306.145/261	306.145/261	35	25
	306.145/246			20

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23 **Table S5**

24 The calibration curve of imazamox using QuEChERS and HPLC-MS/MS.

Organic compound	Calibration curve	R <sup>2</sup>
Imazamox	y=6.11611x+59.5322	0.95

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26 **Table S6**

27 Primers used for RT-qPCR

Gene ID	Function annotation	Primer sequence (5' to 3')	Product size (bp)
BH07.217	CYP	F: TGATGGAGCTCTACGCCAAC R: GCACATGAAGTCCGGGATCA	137
AH01.4462	CYP74A2	F: CCTCCGTTACGACACGTTCA R: TTGGTAATGCCGGTGAGGTT	78
AH03.1780	CYP28	F: ATGCAAGACCTGGCACACTT R: CCATCAAGCTCTAGGCAGGG	133
BH01.1428	CYP71C4	F: CATGGCCTATCTGAGAGCCG R: GATGACTCGAGTGCCAGAGG	133
BH07.64	CYP74B2	F: ATCTGGCCGGGATACTACCT R: TGATGGCGTCCTTTTTCCT	106
BH01.3861	CYP709B2-1	F: CATTTCGTGAAGACGGACGC R: TGGCGAAAGCTGGATTGACT	118
CH01.4123	CYP709B2-2	F: ACATCATGCCCATCTGCTG R: ATGGGCCGATCCAGAACAG	82
AH01.3113	GSTU6	F: ATCGTGCTCAACCTCAAGGG R: GGTACTGGACGATGACCTGC	115
AH01.4114	GSTZ5	F: GTCCGTTCTTCTCGGTGAC R: TTCTGAAGGTTGGGTCTGCC	127
AH05.456	GSTZ1	F: TTTGCATGAGGGCAGCATGA R: CTCTCGCATCCAGCCAAAAG	105
AH01.132	GSTU1	F: ACAAGAAGATCCCCGTGCTC R: AAGGCCTCGTCGATGTACTG	82
BH01.4420	GSTZ5-1	F: TCGAAGGCCATCATTGCTT R: AGGGCAGCAACAGATTCCTC	145
BH07.3899	GSTZ1-1	F: GCACCCAGATTATGCAGC R: CATGGAGCCTCGCAAGAGTT	76
CH01.3708	GSTU6-1	F: GCGGAGCGATTGGGAAAAG R: ATTAACGTACTGCACGGCCT	75
HQ395760	<i>Echinochloa crus-galli</i> β-actin	F: CACTGTGTGCATGGTAGG R: AGAAAGTGTGATGCCAGAT	129

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29 **Table S7**

30 The single nucleotide polymorphisms in multiple sites of ALS sequences in JHXY-2 (R) population.

Sequence Name	Single Nucleotide Polymorphisms			
	Codon (Amino acid)			
	217 Position	265 Position	322 Position	441 Position
ALS1	TCC (ser)	CCC (pro)	GAG (glu)	CAG (gln)
ALS2	TCC (ser)	CCG (pro)	GAG (glu)	CAA (gln)
ALS3	TCA (ser)	CCG (pro)	GAA (glu)	CAC (his)

31 **Table S8**

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

Sample	BF_Q20 <sup>a</sup> (%)	BF_Q30 <sup>b</sup> (%)	BF_N <sup>c</sup> (%)	BF_GC (%)	AF_Q20 (%)	AF_Q30 (%)	AF_N (%)	AF_GC (%)	Clean Data (bp) <sup>d</sup>
GY-3-0h-1	6039197777 (97.72%)	5807529402 (93.97%)	460172 (0.01%)	3283804177 (53.13%)	5937647316 (98.16%)	5729416068 (94.72%)	420890 (0.01%)	3209350964 (53.06%)	6048939337
GY-3-	6552800750	6298457381	530627	3567128770	6434031495	6205473509	505740	3481064922	6556937627

0h-2	(97.67%)	(93.88%)	(0.01%)	(53.17%)	(98.13%)	(94.64%)	(0.01%)	(53.09%)	
GY-3-	6638768742	6385115008	467092	3596504455	6530572988	6301594848	441557	3518693470	
0h-3	(97.75%)	(94.01%)	(0.01%)	(52.95%)	(98.17%)	(94.73%)	(0.01%)	(52.89%)	6652420944
GY-3-	6834828218	6601119521	768232	3706972905	6724998016	6514741620	719533	3628567303	
12h-1	(97.98%)	(94.63%)	(0.01%)	(53.14%)	(98.37%)	(95.29%)	(0.01%)	(53.08%)	6836577118
GY-3-	6191604920	5980880394	701375	3336511777	6091188725	5902384210	653542	3265305539	
12h-2	(97.97%)	(94.64%)	(0.01%)	(52.79%)	(98.38%)	(95.33%)	(0.01%)	(52.74%)	6191490537
GY-3-	6485721112	6268357537	727080	3480091536	6388146593	6192033589	678790	3410416840	
12h-3	(98.03%)	(94.74%)	(0.01%)	(52.60%)	(98.40%)	(95.38%)	(0.01%)	(52.53%)	6491832373
GY-3-	5784890147	5616072839	228557	3088356044	5716304712	5560585012	225149	3041079663	
24h-1	(98.38%)	(95.51%)	(0.00%)	(52.52%)	(98.64%)	(95.95%)	(0.00%)	(52.48%)	5795193275
GY-3-	5995260400	5802934915	513952	3225375733	5903517305	5729156833	481263	3161866222	
24h-2	(98.15%)	(95.00%)	(0.01%)	(52.80%)	(98.49%)	(95.58%)	(0.01%)	(52.75%)	5994118645
GY-3-	5430727960	5269299473	214391	2902911531	5359262698	5210934594	210912	2854152151	
24h-3	(98.36%)	(95.43%)	(0.00%)	(52.57%)	(98.63%)	(95.90%)	(0.00%)	(52.52%)	5433936212
XY-2-	6418194814	6212523192	547752	3491575174	6331600804	6144994285	514452	3430002177	
0h-1	(98.14%)	(95.00%)	(0.01%)	(53.39%)	(98.48%)	(95.58%)	(0.01%)	(53.35%)	6429045463
XY-2-	6512941548	6328887679	552651	3520334403	6425849264	6258247994	522443	3460059572	
0h-2	(98.37%)	(95.59%)	(0.01%)	(53.17%)	(98.67%)	(96.09%)	(0.01%)	(53.13%)	6512565457
XY-2-	6363274327	6174450532	538391	3475098080	6275856579	6103549322	507948	3414310175	
0h-3	(98.32%)	(95.40%)	(0.01%)	(53.69%)	(98.61%)	(95.90%)	(0.01%)	(53.65%)	6364375359
XY-2-	7829386178	7533137255	584889	4223492265	7705124383	7436957289	539221	4132698175	
12h-1	(97.76%)	(94.06%)	(0.01%)	(52.74%)	(98.17%)	(94.76%)	(0.01%)	(52.66%)	7848379057
XY-2-	6406084294	6164794000	458038	3469100363	6288178726	6072801259	434730	3384369173	
12h-2	(97.74%)	(94.06%)	(0.01%)	(52.93%)	(98.21%)	(94.85%)	(0.01%)	(52.86%)	6402755582
XY-2-	6067490863	5841299821	322704	3269170785	5960875000	5756111550	312722	3195180174	
12h-3	(97.87%)	(94.22%)	(0.01%)	(52.73%)	(98.26%)	(94.89%)	(0.01%)	(52.67%)	6066399358
XY-2-	6506351238	6279027624	723254	3528792414	6410652126	6204769375	677645	3459804660	
24h-1	(97.95%)	(94.53%)	(0.01%)	(53.13%)	(98.33%)	(95.17%)	(0.01%)	(53.07%)	6519563179
XY-2-	7012036885	6797548133	460273	3747406766	6892308486	6698107154	451200	3666466366	
24h-2	(98.28%)	(95.27%)	(0.01%)	(52.52%)	(98.59%)	(95.81%)	(0.01%)	(52.44%)	6991206733
XY-2-	6998073766	6782931867	456528	3791626265	6899441678	6701844760	449069	3724152467	
24h-3	(98.29%)	(95.27%)	(0.01%)	(53.26%)	(98.57%)	(95.74%)	(0.01%)	(53.20%)	6999740248
GY-3-	6039197777	5807529402	460172	3283804177	5937647316	5729416068	420890	3209350964	
0h-1	(97.72%)	(93.97%)	(0.01%)	(53.13%)	(98.16%)	(94.72%)	(0.01%)	(53.06%)	6048939337

33 <sup>a</sup>Q20 (%): Number and percentage of bases with data quality up to Q20 (ie 99% sequencing  
34 accuracy).

35 <sup>b</sup>Q30 (%): the number and percentage of bases whose data quality reaches Q30 (ie, the sequencing  
36 accuracy is 99.9%).

37 <sup>c</sup>N (%): number and percentage of N bases.

38 <sup>d</sup>Clean data (bp): The total number of reads bases that have been initially filtered after the machine  
39 is down.

40 “GY-3” is the abbreviation of “JLGY-3”, “XY-2” is the abbreviation of “JHXY-2”.

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## 42 Table S9

### 43 Reads filter information statistics of RNA-seq

Sample	Clean Reads Num <sup>a</sup>	HQ Clean Reads Num(%) <sup>b</sup>	Adapter(%) <sup>c</sup>	LowQuality(%) <sup>d</sup>	polyA(%) <sup>e</sup>	N(%) <sup>f</sup>
GY-3-0h-1	41200918	40810338 (99.05%)	48392 (0.12%)	340686 (0.83%)	0 (0.00%)	1502 (0.00%)
GY-3-0h-2	44725668	44335440 (99.13%)	53788 (0.12%)	335636 (0.75%)	0 (0.00%)	804 (0.00%)
GY-3-0h-3	45278918	44888486 (99.14%)	42188 (0.09%)	347296 (0.77%)	0 (0.00%)	948 (0.00%)
GY-3-12h-1	46503626	46149652 (99.24%)	39098 (0.08%)	312532 (0.67%)	0 (0.00%)	2344 (0.01%)
GY-3-12h-2	42132902	41796606 (99.20%)	34930 (0.08%)	298996 (0.71%)	0 (0.00%)	2370 (0.01%)
GY-3-12h-3	44107716	43782450 (99.26%)	30106 (0.07%)	292740 (0.66%)	0 (0.00%)	2420 (0.01%)
GY-3-24h-1	39201176	39009332 (99.51%)	21482 (0.05%)	170362 (0.43%)	0 (0.00%)	0 (0.00%)
GY-3-24h-2	40721606	40468180 (99.38%)	23740 (0.06%)	228348 (0.56%)	0 (0.00%)	1338 (0.00%)
GY-3-24h-3	36810110	36626062 (99.50%)	21016 (0.06%)	163032 (0.44%)	0 (0.00%)	0 (0.00%)
XY-2-0h-1	43598916	43317556 (99.35%)	23782 (0.05%)	256192 (0.59%)	0 (0.00%)	1386 (0.00%)
XY-2-0h-2	44136882	43903480 (99.47%)	19250 (0.04%)	212712 (0.48%)	0 (0.00%)	1440 (0.00%)
XY-2-0h-3	43148552	42921358 (99.47%)	20002 (0.05%)	205760 (0.48%)	0 (0.00%)	1432 (0.00%)
XY-2-12h-1	53390578	52926524 (99.13%)	57230 (0.11%)	405092 (0.76%)	0 (0.00%)	1732 (0.00%)
XY-2-12h-2	43694472	43316182 (99.13%)	44360 (0.10%)	333160 (0.76%)	0 (0.00%)	770 (0.00%)
XY-2-12h-3	41328890	41000708 (99.21%)	43834 (0.11%)	284122 (0.69%)	0 (0.00%)	226 (0.00%)
XY-2-24h-1	44281828	43949526 (99.25%)	31174 (0.07%)	298874 (0.67%)	0 (0.00%)	2254 (0.01%)
XY-2-24h-2	47565336	47310824 (99.46%)	46824 (0.10%)	207688 (0.44%)	0 (0.00%)	0 (0.00)
XY-2-24h-3	47464900	47220080 (99.48%)	30066 (0.06%)	214754 (0.45%)	0 (0.00%)	0 (0.00%)
GY-3-0h-1	44725668	44335440 (99.13%)	53788 (0.12%)	335636 (0.75%)	0 (0.00%)	804 (0.00%)

44 <sup>a</sup>Clean Reads Num: The number of reads that have been initially filtered after the machine is off.

45 <sup>b</sup> HQ Clean Reads Num (%): the number and percentage of reads obtained by further filtering after  
46 clean reads.

47 <sup>c</sup>Adapter (%): number and percentage of reads with adapter.

48 <sup>d</sup>Low Quality (%): the number and percentage of reads with a mass value of Q ≤ 20, which account

49 for more than 50% of the total reading.

50 <sup>e</sup>Poly A (%): all the number and percentage of reads of A bases.

51 <sup>f</sup> N (%): the number and percentage of reads with a ratio of N greater than 10%.

52 “GY-3” is the abbreviation of “JLGY-3”, “XY-2” is the abbreviation of “JHXY-2”.

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54 The classification of resistance level standard in this study: Sensitivity ( $RI \leq 2$ ),

55 decreased sensitivity ( $2 < RI \leq 4$ ), low resistance ( $4 < RI \leq 10$ ), medium resistance

56 ( $10 < RI \leq 30$ ), high resistance ( $30 < RI \leq 150$ ).

57