**Supporting information**

**A Trp-574-Leu mutation in acetolactate synthase confers** **imazamox resistance in *Echinochloa crus-galli* from China**

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**Table S1**

Herbicide doses applied in whole-plant dose-response bioassays

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Herbicide target | Groupa | Herbicide | Doses applied to each populationc (g a.i. ha-1 ) | | Recommended field dose (g a.i. ha-1 ) |
| 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 |

aAbbreviations: SCT, sulfonylamino-carbonyl-triazolinone; TP, triazolopyrimidine; SU, sulfonylurea; PTB, pyrimidinylthiobenzoate; APP, aryloxy phenoxy propionate.

**Table S2**

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 R: CCTGGAGTCTCGAGCATCTT | 231 |
| β-actin | F: CACACTGGTGTCATGGTAGG  R: AGAAAGTGTGATGCCAGAT | 129 |

**Table S3**

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 | 030 | 5 | 95 |

**Table S4**

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 |

**Table S5**

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

|  |  |  |
| --- | --- | --- |
| Organic compound | Calibration curve | R2 |
| Imazamox | y=6.11611x+59.5322 | 0.95 |

**Table S6**

Primers used for RT-qPCR

|  |  |  |  |
| --- | --- | --- | --- |
| Gene ID | Function annotation | Pimer 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: TGATGGCGTCCTTTTTGCCT | 106 |
| BH01.3861 | CYP709B2-1 | F: CATTTCGTGAAGACGGACGC R: TGGCGAAAGCTGGATTGACT | 118 |
| CH01.4123 | CYP709B2-2 | F: ACATCATGCCCATCCTGCTG R: ATTGGGCCGATCCAGAACAG | 82 |
| AH01.3113 | GSTU6 | F: ATCGTGCTCAACCTCAAGGG R: GGTACTGGACGATGACCTGC | 115 |
| AH01.4114 | GSTZ5 | F: GTCCGTTCTTCCTCGGTCAG R: TTCTGAAGGTTGGGTCTGCC | 127 |
| AH05.456 | GSTZ1 | F: TTTGCATGAGGGCAGCATGA R: CTCTCGCATCCAGCCAAAAG | 105 |
| AH01.132 | GSTU1 | F: ACAAGAAGATCCCCGTGCTC R: AAGGCCTCGTCGATGTACTG | 82 |
| BH01.4420 | GSTZ5-1 | F: TCGAAGGCCCATCATTGCTT R: AGGGCAGCAACAGATTCCTC | 145 |
| BH07.3899 | GSTZ1-1 | F: GCACCCCAGATTTATGCAGC R: CATGGAGCCTCGCAAGAGTT | 76 |
| CH01.3708 | GSTU6-1 | F: GCGGAGCGATTTGGGAAAAG R: ATTAACGTACTGCACGGCCT | 75 |
| HQ395760 | *Echinochloa crus-galli* β-actin | F: CACACTGGTGTCATGGTAGG R: AGAAAGTGTGATGCCAGAT | 129 |

**Table S7**

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) |

**Table S8**

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

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | BF\_Q20a  (%) | BF\_Q30b  (%) | BF\_Nc  (%) | BF\_GC  (%) | AF\_Q20  (%) | AF\_Q30  (%) | AF\_N  (%) | AF\_GC  (%) | Clean Data (bp)d |
| 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-3-0h-2 | 6552800750 (97.67%) | 6298457381 (93.88%) | 530627 (0.01%) | 3567128770 (53.17%) | 6434031495 (98.13%) | 6205473509 (94.64%) | 505740 (0.01%) | 3481064922 (53.09%) | 6556937627 |
| GY-3-0h-3 | 6638768742 (97.75%) | 6385115008 (94.01%) | 467092 (0.01%) | 3596504455 (52.95%) | 6530572988 (98.17%) | 6301594848 (94.73%) | 441557 (0.01%) | 3518693470 (52.89%) | 6652420944 |
| GY-3-12h-1 | 6834828218 (97.98%) | 6601119521 (94.63%) | 768232 (0.01%) | 3706972905 (53.14%) | 6724998016 (98.37%) | 6514741620 (95.29%) | 719533 (0.01%) | 3628567303 (53.08%) | 6836577118 |
| GY-3-12h-2 | 6191604920 (97.97%) | 5980880394 (94.64%) | 701375 (0.01%) | 3336511777 (52.79%) | 6091188725 (98.38%) | 5902384210 (95.33%) | 653542 (0.01%) | 3265305539 (52.74%) | 6191490537 |
| GY-3-12h-3 | 6485721112 (98.03%) | 6268357537 (94.74%) | 727080 (0.01%) | 3480091536 (52.60%) | 6388146593 (98.40%) | 6192033589 (95.38%) | 678790 (0.01%) | 3410416840 (52.53%) | 6491832373 |
| GY-3-24h-1 | 5784890147 (98.38%) | 5616072839 (95.51%) | 228557 (0.00%) | 3088356044 (52.52%) | 5716304712 (98.64%) | 5560585012 (95.95%) | 225149 (0.00%) | 3041079663 (52.48%) | 5795193275 |
| GY-3-24h-2 | 5995260400 (98.15%) | 5802934915 (95.00%) | 513952 (0.01%) | 3225375733 (52.80%) | 5903517305 (98.49%) | 5729156833 (95.58%) | 481263 (0.01%) | 3161866222 (52.75%) | 5994118645 |
| GY-3-24h-3 | 5430727960 (98.36%) | 5269299473 (95.43%) | 214391 (0.00%) | 2902911531 (52.57%) | 5359262698 (98.63%) | 5210934594 (95.90%) | 210912 (0.00%) | 2854152151 (52.52%) | 5433936212 |
| XY-2-0h-1 | 6418194814 (98.14%) | 6212523192 (95.00%) | 547752 (0.01%) | 3491575174 (53.39%) | 6331600804 (98.48%) | 6144994285 (95.58%) | 514452 (0.01%) | 3430002177 (53.35%) | 6429045463 |
| XY-2-0h-2 | 6512941548 (98.37%) | 6328887679 (95.59%) | 552651 (0.01%) | 3520334403 (53.17%) | 6425849264 (98.67%) | 6258247994 (96.09%) | 522443 (0.01%) | 3460059572 (53.13%) | 6512565457 |
| XY-2-0h-3 | 6363274327 (98.32%) | 6174450532 (95.40%) | 538391 (0.01%) | 3475098080 (53.69%) | 6275856579 (98.61%) | 6103549322 (95.90%) | 507948 (0.01%) | 3414310175 (53.65%) | 6364375359 |
| XY-2-12h-1 | 7829386178 (97.76%) | 7533137255 (94.06%) | 584889 (0.01%) | 4223492265 (52.74%) | 7705124383 (98.17%) | 7436957289 (94.76%) | 539221 (0.01%) | 4132698175 (52.66%) | 7848379057 |
| XY-2-12h-2 | 6406084294 (97.74%) | 6164794000 (94.06%) | 458038 (0.01%) | 3469100363 (52.93%) | 6288178726 (98.21%) | 6072801259 (94.85%) | 434730 (0.01%) | 3384369173 (52.86%) | 6402755582 |
| XY-2-12h-3 | 6067490863 (97.87%) | 5841299821 (94.22%) | 322704 (0.01%) | 3269170785 (52.73%) | 5960875000 (98.26%) | 5756111550 (94.89%) | 312722 (0.01%) | 3195180174 (52.67%) | 6066399358 |
| XY-2-24h-1 | 6506351238 (97.95%) | 6279027624 (94.53%) | 723254 (0.01%) | 3528792414 (53.13%) | 6410652126 (98.33%) | 6204769375 (95.17%) | 677645 (0.01%) | 3459804660 (53.07%) | 6519563179 |
| XY-2-24h-2 | 7012036885 (98.28%) | 6797548133 (95.27%) | 460273 (0.01%) | 3747406766 (52.52%) | 6892308486 (98.59%) | 6698107154 (95.81%) | 451200 (0.01%) | 3666466366 (52.44%) | 6991206733 |
| XY-2-24h-3 | 6998073766 (98.29%) | 6782931867 (95.27%) | 456528 (0.01%) | 3791626265 (53.26%) | 6899441678 (98.57%) | 6701844760 (95.74%) | 449069 (0.01%) | 3724152467 (53.20%) | 6999740248 |
| 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 |

aQ20 (%): Number and percentage of bases with data quality up to Q20 (ie 99% sequencing accuracy).

bQ30 (%): the number and percentage of bases whose data quality reaches Q30 (ie, the sequencing accuracy is 99.9%).

cN (%): number and percentage of N bases.

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

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

**Table S9**

Reads filter information statistics of RNA-seq

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | Clean Reads Numa | HQ Clean Reads Num(%)b | Adapter(%)c | LowQuality(%)d | polyA(%)e | N(%)f |
| 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%) |

aClean 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.

cAdapter (%): number and percentage of reads with adapter.

dLow Quality (%): the number and percentage of reads with a mass value of Q ≤ 20, which account for more than 50% of the total reading.

ePoly A (%): all the number and percentage of reads of A bases.

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

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

The classification of resistance level standard in this study: Sensitivity (RI ≤ 2), decreased sensitivity (2 < RI ≤ 4), low resistance (4 < RI ≤ 10), medium resistance (10 < RI ≤ 30), high resistance (30 < RI ≤ 150).