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Overexpression of a cytochrome P450 gene, *CYP81Q32*, endows resistance to HPPD-inhibiting herbicides in *Leptochloa chinensis*

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ABSTRACT

Leptochloa chinensis (L.) Nees is one of the most troublesome weeds in paddy fields in China, and has developed resistance to multiple herbicides, including cyhalofop-butyl and metamifop, severely threatening rice production. In this study, we identified one TC53-R accession evolved 4.2-fold resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide tripyrasulfone, and displayed 2.6 to 4.6-fold cross-resistance to two newly registered HPPD herbicides pyraquinate and flusulfinam. *HPPD* sequencing indicated that target-site resistance was not involved in this resistant accession. P450 inhibitor piperonyl butoxide significantly increased the sensitivity to tripyrasulfone in the TC53-R accession. RNA-Sequencing and quantitative real-time polymerase chain reaction characterized a *LcCYP81Q32* gene that was constitutively up-regulated in the TC53-R accession. The *LcCYP81Q32* gene was overexpressed in a known tripyrasulfone-susceptible indica rice line (HHZ). The transgenic rice line overexpressing *LcCYP81Q32* exhibited increased tolerance to tripyrasulfone, with the fresh weight inhibition rate of 41.0%–66.3% at the dose of 360 and 720 g ai ha^{-1}, respectively, compared to 70.3%–82.4% for the wild type. These findings indicate that *LcCYP81Q32* plays an important role in the evolution of tripyrasulfone resistance in *L. chinensis*, and provide a promising candidate gene for improving herbicide-tolerant crop varieties.

1. Introduction

Chemical control is an important component of integrated weed management system. However, the heavy use of herbicides has led to the burgeoning of herbicide-resistant weed biotypes worldwide (Peterson et al., 2018). For the effective control of resistant weeds, it is imperative to gain an in-depth understanding the molecular mechanisms of resistance. Weeds resist herbicides mainly through two pathways, known as target-site resistance (TSR) and nontarget-site resistance (NTSR) (Gaines et al., 2020). TSR is involved in the gene mutation and amplification in target proteins, such as 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), acetolactate synthase (ALS), and acetyl-CoA carboxylase (ACCase), which have been discovered in various resistant weeds (Powles and Yu, 2010). NTSR refers to any mechanisms other than TSR, including enhanced metabolism, reduced uptake or translocation, and sequestered in vacuoles, etc. (Jugulam and Shyam, 2019; Rigon et al., 2020). Despite NTSR was common in resistant weeds, it remains poorly understood, and the exploration of resistance-related genes is still limited.

4-Hydroxyphenylpyruvate dioxygenase (HPPD), a relatively new herbicide target, is an essential enzyme in the biosynthesis of plastoquinone (Yang et al., 2024). Inhibition of the HPPD enzyme will affect the synthesis of carotenoid, thus leading to plant whitening and subsequent death. There are three herbicide groups targeting at HPPD enzyme, including triketones, pyrazolones, and isoxazoles (Wang et al., 2018). These herbicides, collectively referred to as HPPD herbicides, have been widely used to control annual weeds in various crops due to the characteristics of high efficiency, safety, and broad spectrum (Jhala et al., 2023). In addition, resistance risk of weeds to HPPD herbicides is relatively low. To date, there are only four broadleaf weeds and one grass weed that have developed resistance to HPPD herbicides (Heap, 2026), and rapid herbicide detoxification and overexpression of *HPPD* gene are mainly responsible for resistance in these resistant weeds (Nakka et al., 2017; Lu et al., 2020, 2023; Concepcion et al., 2021).

Leptochloa chinensis (L.) Nees is a tetraploid (2n = 4× = 40) and partly cross-pollinated (~4.7%) troublesome weed, mainly occurring in

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Table 1
The resistance level of the TC53-R *L. chinensis* accession to three HPPD-inhibiting herbicides by the whole-plant dose-response assays, and the effect of PBO and NBD-Cl on tripyrasulfone resistance.

Accession	Treatment	Dose-response model parameters			GR_{50} (SE)[a] (g ai ha^{-1})	RI[b]
		D (SE)	*b* (SE)	R^2		
TC53-R	Tripyrasulfone	95.36 (4.83)	1.87 (0.43)	0.97	103.21 (13.33)	4.2
	Tripyrasulfone+PBO	102.81 (5.25)	1.40 (0.22)	0.98	50.05 (6.75) *	2.2
	Tripyrasulfone+NBD-Cl	96.97 (3.13)	1.91 (0.29)	0.99	104.77 (8.58)	4.0
	Pyraquinate	101.70 (3.49)	2.89 (0.54)	0.99	98.53 (7.03)	4.6
	Flusulfinam	103.84 (7.76)	1.59 (0.36)	0.98	30.85 (5.63)	2.6
YZ120-S	Tripyrasulfone	100.93 (5.83)	1.07 (0.18)	0.98	24.74 (4.42)	–
	Tripyrasulfone+PBO	100.90 (6.00)	1.06 (0.19)	0.98	22.38 (3.17)	–
	Tripyrasulfone+NBD-Cl	101.70 (7.48)	1.34 (0.28)	0.97	26.49 (5.05)	–
	Pyraquinate	100.76 (10.78)	5.41 (1.57)	0.98	21.41 (3.38)	–
	Flusulfinam	100.89 (7.10)	1.46 (0.33)	0.99	11.68 (2.06)	–

[a] GR_{50}, herbicide dose causing a 50% growth reduction; SE, standard error.
[b] RI, GR_{50} value of TC53-R divided by that of YZ120-S.

rice ecosystems (Phongphitak et al., 2014; Wang et al., 2022). In China, this weed spread rapidly across rice fields due to strong environmental adaptability and competition, and now it has become the second most malignant weed behind *Echinochloa* spp. in most rice fields. Since 2006, ACCase inhibitors cyhalofop-butyl has become the main herbicide for controlling L. *chinensis* in Chinese rice fields (Deng et al., 2023). Long-term and over-dosage utilization has resulted in the widespread occurrence of cyhalofop-butyl-resistant accessions (Deng et al., 2023; Jiang et al., 2025; Xu et al., 2025; Chen et al., 2025a). In 2020, an alternative HPPD herbicide, tripyrasulfone, was registered to manage the resistant L. *chinensis*, and indeed it showed a good efficacy against many weeds in rice fields (Wang et al., 2021). However, the tripyrasulfone-resistant *L. chinensis* accession has emerged, which has raised concerns about resistance and application prospect of this herbicide (Ju et al., 2023).

In our previous study, we sampled 124 *L. chinensis* accessions in China, and determined their resistance status to ACCase inhibitor. Results showed that over 56% accessions evolved resistance to ACCase inhibitor (Deng et al., 2023). Tripyrasulfone has been frequently used to control L. *chinensis* for about 5 years, and it is unclear whether there are accessions developing resistance to tripyrasulfone. Here, we investigated the resistance of 124 accessions to tripyrasulfone, and found that one accession has developed resistance to tripyrasulfone. Thus, in this study, the underlying molecular mechanism was characterized.

2. Materials and methods

2.1. Plant materials and growth conditions

A total of 124 *L. chinensis* accessions were originated from Jiangsu Province in China as described previously (Deng et al., 2023). About 30 seeds of each accession were directly sown into plastic pots and cultivated in an incubator with a 16-h light (35 °C) / 8-h dark (30 °C).

2.2. Single dose resistance testing

When L. *chinensis* were grown to around 3–4 leaf stage, plants were thinned to 15 seedlings per pot, and foliar-sprayed with tripyrasulfone at the recommended field-use rate (100 g a.i. ha^{-1}) via a regular sprayer delivering 400 L ha^{-1} at a pressure of 0.2 MPa. The survival percentage of treated plants was visually evaluated at 21 days after treatment (DAT). Three replications (about 45 plants) from each accession were used. Resistance was identified if the survival rate was over 20% based on our previous classification criteria (Deng et al., 2023).

2.3. Dose-response to HPPD herbicides

Based on the results of single dose testing, one known susceptible (S) accession (YZ120-S) and one resistant (R) *L. chinensis* accession (TC53-R) were grown as described above to determine the level of resistance to three HPPD-inhibiting herbicides, including tripyrasulfone, pyraquinate, and flusulfinam. Plants at the 3–4 leaf stage were sprayed with different doses of herbicides. Doses for tripyrasulfone included 0, 12.5, 25, 50, 100 and 200 g a.i. ha^{-1}. Doses for pyraquinate included 0, 14, 28.1, 56.3, 112.5 and 225 g a.i. ha^{-1}. Doses for flusulfinam included 0, 8.4, 16.9, 33.8, 67.5 and 135 g a.i. ha^{-1}. At 21 DAT, the fresh weight of the aboveground tissue per pot was recorded. The dose-response experiments were performed twice with three replicates per treatment.

2.4. Effect of PBO and NBD-Cl on resistance to tripyrasulfone

The metabolic enzyme inhibitor assays were conducted as described previously (Deng et al., 2021). Briefly, plants at the 3–4 leaf stage were treated with tripyrasulfone with and without the P450 inhibitor PBO or the GST inhibitor NBD-Cl. The doses of PBO and NBD-Cl were 4000 and 270 g a.i. ha^{-1}, respectively, and applied 1 h and 48 h prior to tripyrasulfone treatment. Doses for tripyrasulfone were the same as those described above. At 21 DAT, the fresh weight of the aboveground part of the plant was cut and weighed. The experiments were conducted twice with three replicates per treatment.

2.5. HPPD gene sequencing

Total RNA was extracted from leaves of YZ120-S and TC53-R *L. chinensis* accessions using RNAsimple Total RNA Kit (TIANGEN, China), and cDNA was synthesized using the FastKing gDNA Dispelling RT SuperMix (TIANGEN). Primers for *HPPD* gene amplification were described by Ju et al. (2023). PCR was performed in a 40 μL mixture, containing cDNA (1 μL), 2 × Taq PCR MasterMix II (20 μL) (TIANGEN), each primer (1 μL), and ddH_2O (17 μL). The PCR protocol was: 94 °C for 3 min, 32 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, and 72 °C for 10 min. The PCR products were checked on a 1% agarose gel, and were sequenced at Sangon Biotech Co. Ltd. (Shanghai, China). Ten individual plants from each accession were used for *HPPD* gene sequencing.

2.6. RNA-seq analysis

Seedlings of YZ120-S and TC53-R accessions were grown to the 5–6 leaf stage, then the plant leaves were cut for at 0.2 g, which were collected from ten plants and pooled as a biological replicate. The YZ120-S and TC53-R accessions had three replicates. The samples were immediately used for total RNA extraction. The cDNA library construction, transcriptome sequencing, and analysis of differentially expressed genes (DEGs) were conducted as previously described (Yao et al., 2024).

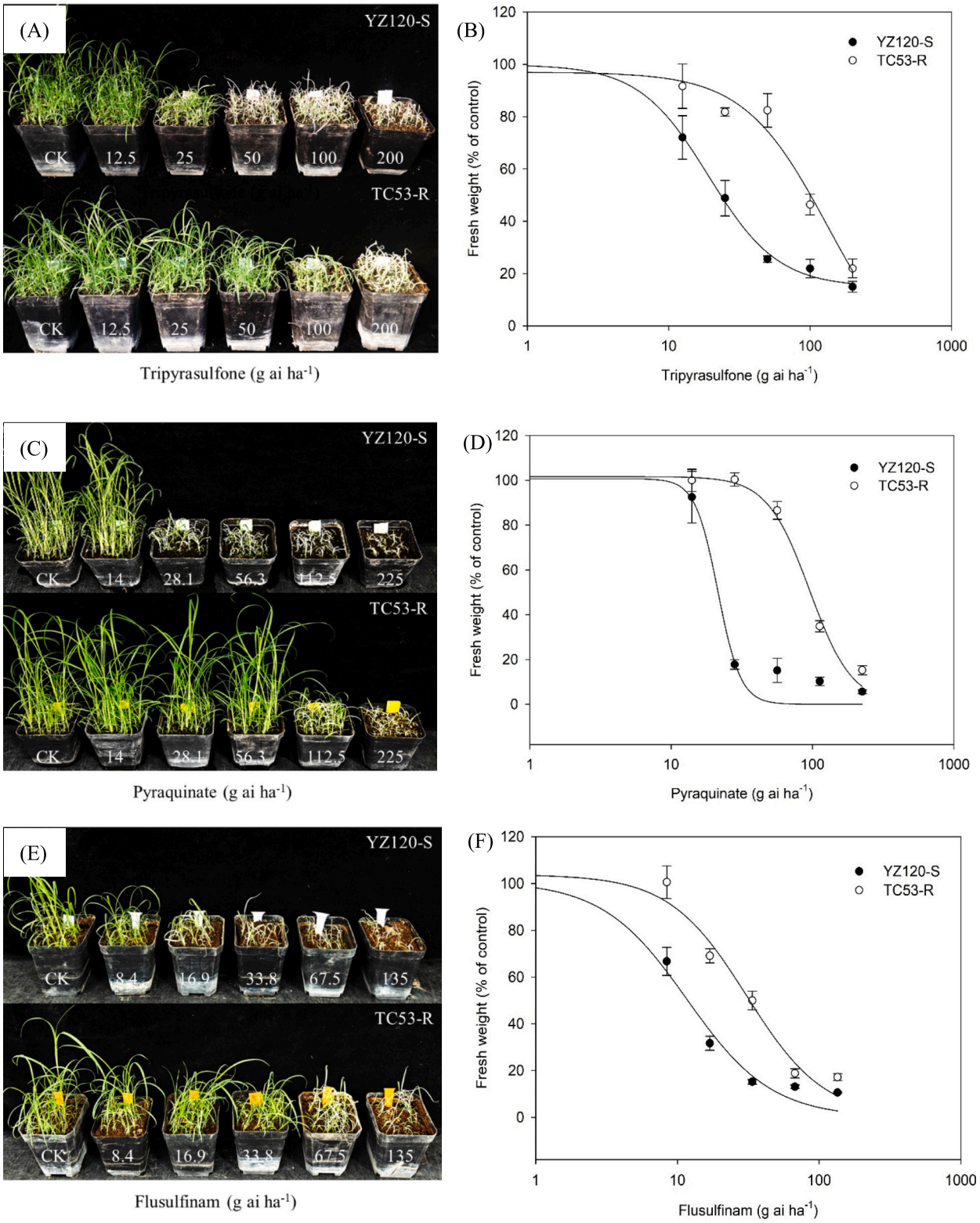

Fig. 1. Dose response to HPPD-inhibiting herbicides tripyrasulfone (A and B), pyraquinate (C and D), and flusulfinam (E and F). The photos were taken 21 days after treatment.

2.7. Candidate genes selection and verification

A total of five DEGs, which were annotated as P450 family genes, were selected for further validation of relative expression levels in additional samples by qRT-PCR. RNA was isolated as described above, and the synthesis of first-strand cDNA were conducted using FastKing gDNA Dispelling RT SuperMix (Tiangen). qRT-PCR was carried out using the CFX96 Real-time PCR system (Bio-Rad, USA), and the L. *chinensis* β-actin gene was used as the reference gene (Deng et al., 2019). Primers (Table S1) for five DEGs were designed using Primer Premier 5.

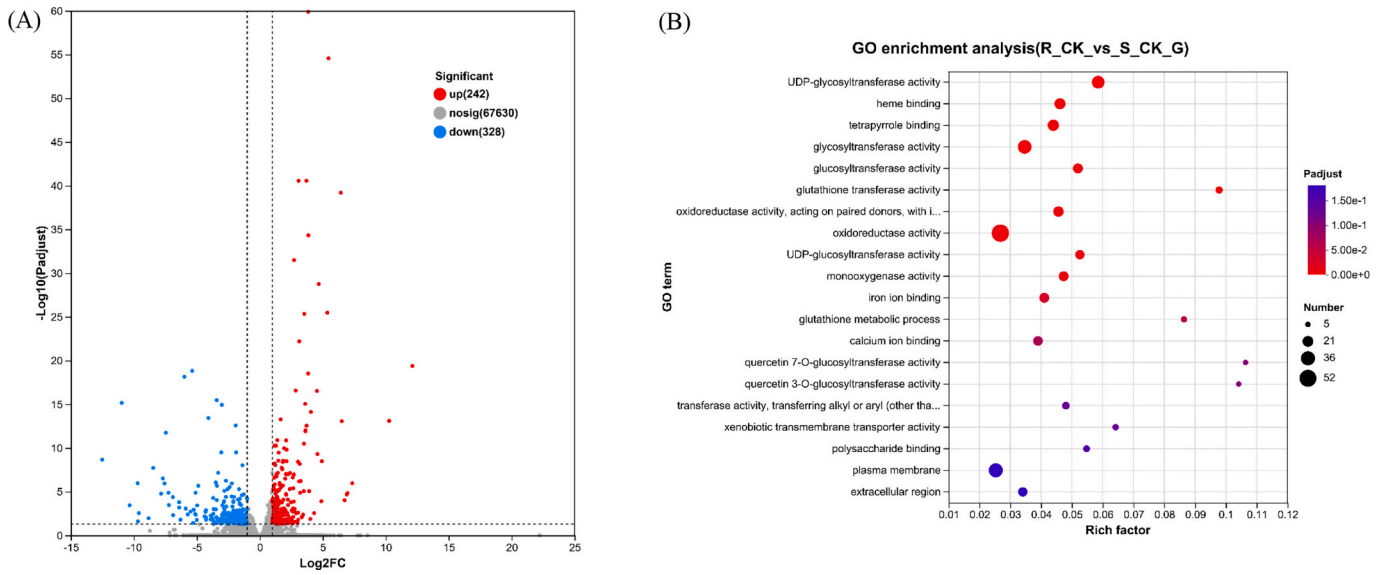

Fig. 2. Volcano plot of differentially expression genes between resistant (TC53-R) and susceptible (YZ120-S) accessions, including 242 up-regulated DEGs and 328 down-regulated DEGs (A). Analysis of Go enrichment analysis of DEGs (B).

Table 2
Identification of the 5 upregulated P450 genes between the TC53-R and YZ120-S *L. chinensis* accession by RNA-Seq and qRT-PCR.

Gene ID	Annotation	RNA-Seq		qRT-PCR
		Fold change[a]	*p*-value	Fold change[b]
DN1249_c0_g1	P450 (CYP709B2)	12.4	1.12×10^{-15}	4.8*
DN14850_c0_g2	P450 (CYP89A9)	3.2	5.05×10^{-17}	2.6*
DN17539_c0_g1	P450 (CYP81Q32)	14.4	5.09×10^{-65}	10.1*
DN4334_c0_g4	P450 (CYP71BE54)	2.9	1.53×10^{-4}	0.6
DN4345_c0_g1	P450 (CYP76C2)	12.3	1.46×10^{-15}	3.5*

[a] The upregulated expressed genes annotated as P450 genes between the tripyrasulfone-resistant TC53-R and susceptible YZ120-S accession by RNA-Seq (p-value<0.05 and $\log_2$ (Fold change) >1).
[b] Results of qRT-PCR, and $p < 0.05$ is represented by * from the SPSS analysis.

The qRT-PCR protocols and data analysis were performed as described previously (Yao et al., 2024), and the experiments had six biological replicates and was repeated twice.

2.8. Overexpressing *CYP81Q32* in WT indica rice

The primer (F: ACTAGGGTCTCGCACCATGGATAAGGCCTACC TGGCC, R: ACTAGGGTCTCTACCGTTAGAGCTCCTGCAGAAGCTCAC), containing the *Bsa* I-HF site, was used to amplify the full-length CDS of *LcCYP81Q32* (Fig. S1) using cDNA of the TC53-R *L. chinensis* accession. The PCR products were purified using the TIANquick Midi Purification Kit (TIANGEN). After digestion with *Bsa* I-HF, the products and pEGOEP35S vector (EDGENE BIOT, Wuhan, China) were linked together by T4 DNA ligase at 20 °C for 5 min. The plasmid was transformed into DH5α, then *Escherichia coli* clones containing the right insert were sequenced with the primer (F: CACGGGGGACTCTTGCCACC, and R: GGGTCCTAACCAAGAAAATGAA) at Sangon Biotech Co. Ltd.

The constructed pEGOEP35S-*CYP81Q32* vector (Fig. S2), checked by sequencing and restriction analysis, was introduced into *Agrobacterium tumefaciens* via electroporation. Then, the transformed *A. tumefaciens* was used to transform the wild-type indica rice Huanghuazhan (HHZ) as the methods described by Toki et al. (2006). By this way, rice seedlings (T_0) were obtained and cultivated in the rice field during the growing season of rice (April to October) until the seeds (T_1) matured.

2.9. Sensitivity of transgenic rice seedlings to HPPD-inhibiting herbicides

In order to determine overexpression of *LcCYP81Q32* endowing resistance to HPPD-inhibiting herbicides in transgenic rice seedlings, wild-type and T_1 rice seedlings were used for sensitivity testing. The seedlings were grown to the 3–4 leaf stage, and treated with tripyrasulfone (360 and 720 g a.i. ha^{-1}) respectively. 21 days after herbicide treatment, the aboveground fresh weight of rice seedlings was measured.

2.10. Statistical analysis

The herbicide dose causing the 50% growth reduction (GR_{50}) was calculated using the three-parameter log-logistic equation in SigmaPlot 12.0 (Systat Software, USA) (Seefeldt et al., 1995). Resistance index was expressed as the ratio of GR_{50} (TC53-R accession) / GR_{50} (YZ120-S accession). SPSS 16.0 (IBM, UAS) was employed to analyze the significance of differences in GR_{50} values among different treatments, gene expression in qRT-PCR, and fresh weight inhibition in sensitivity tests of rice seedlings, using a *t*-test ($P < 0.05$).

3. Results

3.1. Tripyrasulfone resistance screening

A total of 124 field-collected L. *chinensis* accessions were used to test their sensitivity to tripyrasulfone. Among them, 123 accessions had a 100% mortality rate, with all plants whitening and dying (data not shown). By contrast, one accession (TC53-R) survived at the field-use dose of tripyrasulfone, showing a 100% survival rate. Thus, this accession (TC53-R) was identified as the 'resistance' category.

3.2. Whole-plant dose response to HPPD-inhibiting herbicides

Dose response experiments confirmed that the dose of tripyrasulfone causing 50% growth reduction (GR_{50} value) of R TC53-R accession was 103.21 g a.i. ha^{-1}, which was greater than the 24.74 g a.i. ha^{-1} for the S

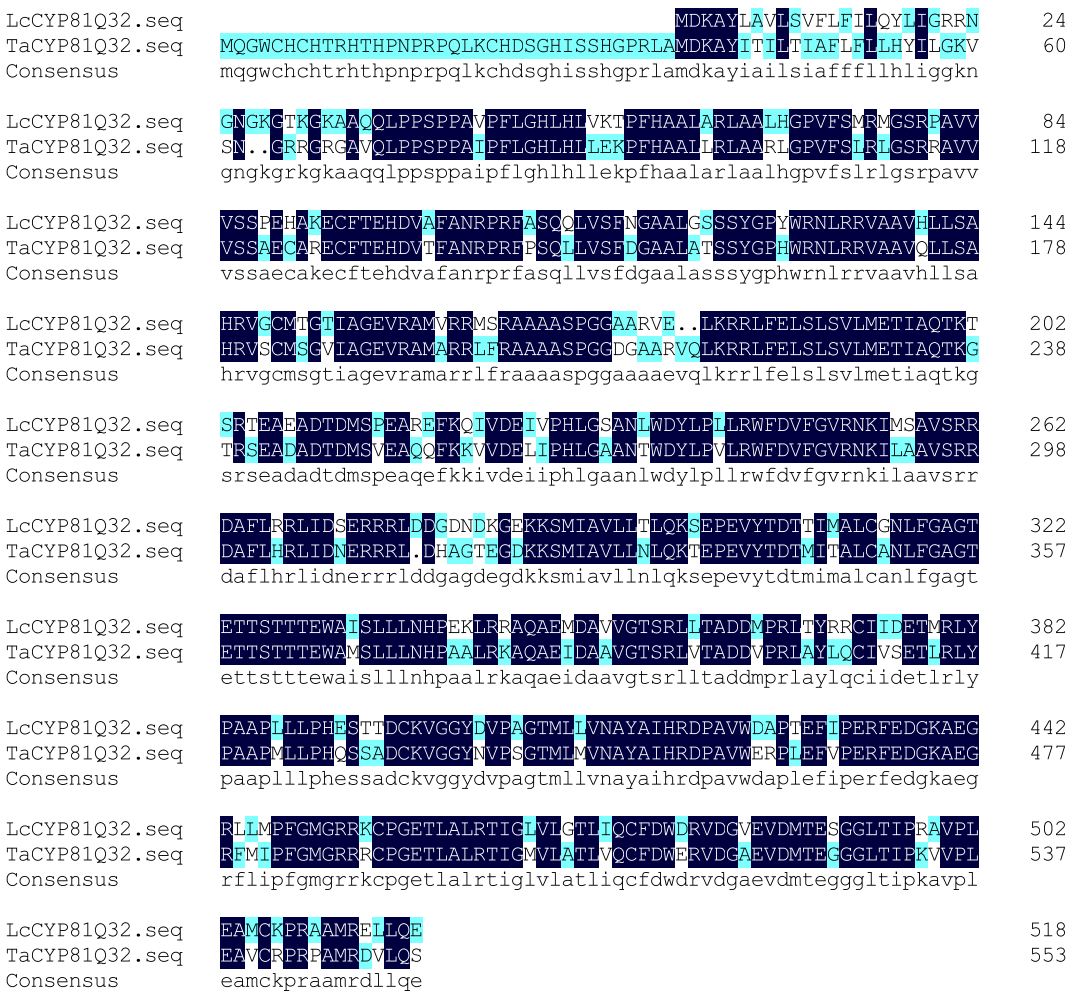

Fig. 3. The amino acid sequence alignment of *LcCYP81Q32* from L. *chinensis* and *TaCYP81Q32* from *Triticum aestivum*.

YZ120-S accession (Table 1), indicating the TC53-R accession had 4.2-fold resistance to tripyrasulfone. The YZ120-S plants died at 1/2 of the recommended TRF dosage, while the TC53-R plants could endure the recommended dose (Fig. 1). Simultaneously, the TC53-R accession showed 4.6- and 2.6-fold resistance to pyraquinate and flusulfinam, respectively (Fig. 1, Table 1).

3.3. Effect of PBO and NBD-Cl on resistance to tripyrasulfone

Pre-experiments confirmed that PBO or NBD-Cl treated separately did not affect the plant growth at the dose of 4000, and 270 g a.i. ha^{-1}, respectively. Pre-treatment of PBO or NBD-Cl had no effect on the GR_{50} value of tripyrasulfone in the YZ120-S accession, ranging from 22.38 to 26.49 g a.i. ha^{-1} ($P > 0.05$). By contrast, the GR_{50} value of PBO plus tripyrasulfone were reduced from 103.21 g a.i. ha^{-1} (tripyrasulfone alone) to 50.05 g a.i. ha^{-1} ($P = 0.016$) in the TC53-R accession (Table 1).

3.4. HPPD gene sequencing

Three fragments of the L. *chinensis HPPD* gene were amplified from ten individual plants of TC53-R and YZ120-S, respectively. The three sequences were assembled to the full length (1317 bp) of *HPPD* gene. The *HPPD* gene from L. *chinensis* TC53-R and YZ120-S showed a 83.97% homology with that of the *Oryza sativa* (GenBank accession NM_001409339.1). No amino acid mutations of *HPPD* gene were observed in TC53-R and YZ120-S plants (Fig. S3).

3.5. Screening of differentially expressed contigs associated with NTSR

The RNA-Seq results displayed an average 50.9% GC content per sample, with an average Q20 value of 98.63% and an average Q30 value of 95.75%. The six samples generated 298,550,696 raw reads and 295,673,538 clean reads. A total of 154,155 transcript with an average length of 1652 bp and a N50 length of 2573 bp were obtained from the assembly, and yielding 68,200 unigenes. Differential expression analysis showed a 570 DEGs, with 242 up-regulated and 328 down-regulated genes (Fig. 2A). The Go enrichment analysis identified significant enrichment in detoxification and defense pathways of DEGs (Fig. 2B). Among these DEGs, they are 5 P450 genes, 6 GST genes, 5 UDPGT genes, 1 ABC genes, and 1 AKR genes (Tables 2, S2). Due to the significant synergistic effect of PBO, 5 P450 genes were selected as the candidate genes for further validation of expression levels.

3.6. qRT-PCR verification

Based on the RNA-Seq screening results, the expression levels of five candidate P450 genes (DN1249_c0_g1, DN14850_c0_g2, DN17539_c0_g1, DN4334_c0_g4, and DN4345_c0_g1) were validated using RT-qPCR. The results confirmed that four of them were upregulated in the TC53-R accession. The candidate P450 gene (DN17539_c0_g1), annotated as *CYP81Q32*, was chosen for functional characterization owing to its reported herbicide resistance-conferring function in other weeds.

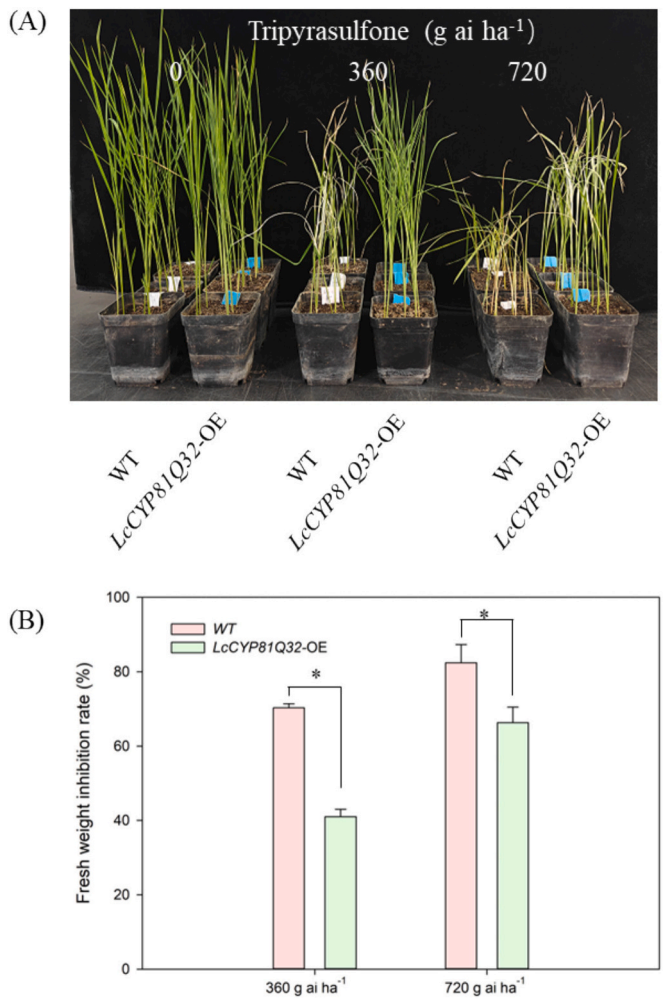

Fig. 4. Comparison of tripyrasulfone sensitivity between the transgenic rice expressing *LcCYP81Q32* (*LcCYP81Q32*-OE) and wild type (WT) plants (A). The fresh weight inhibition rate for *LcCYP81Q32*-OE and WT plants after 21 days of tripyrasulfone treatment.

3.7. Overexpression of LcCYP81Q32 enhances tolerance to tripyrasulfone

The full-length coding sequencing (CDS) of *LcCYP81Q32* was 1560 bp, encoding 520 amino acids. *LcCYP81Q32* showed a high degree (70.7%) of amino acid identity with *CYP81Q32-like* in *Triticum aestivum* (Fig. 3). No single nucleotide polymorphisms were observed in *LcCYP81Q32* between the TC53-R and YZ120-S plants.

As shown in Fig. 4, the WT rice plants was significantly injured at 360 g ai ha^{-1} of tripyrasulfone. In contrast, the *LcCYP81Q32*-overexpressing rice was slightly inhibited at the same dose. Similarly, the WT rice completely died, while the transgenic rice only exhibited albino symptoms at 720 g ai ha^{-1} of tripyrasulfone (Fig. 4A). In addition, the fresh weight inhibition rate (FWIR) of WT at 360 and 720 g ai ha^{-1} of tripyrasulfone was 70.3% and 82.4%, respectively, whereas the FWIR of *LcCYP81Q32*-OE rice was 41.0% and 66.3%, respectively (Fig. 4B).

4. Discussion

In this study, we showed that a L. *chinensis* accession developed 2.6- to 4.6- fold resistance to three HPPD herbicides, tripyrasulfone, pyraquinate and flusulfinam. The last two herbicides were registered to control weeds in Chinese paddy fields from 2025, and the resistant accession has never exposed to the two herbicides. Thus, pyraquinate and flusulfinam resistances in the TC53-R accession were resulted from its cross resistance. The risk of resistance to HPPD herbicides is relatively low in weeds with only five resistant cases (Heap, 2026). Ju et al. (2023) reported a HPPD-resistant L. *chinensis*. The increasing number of resistant L. *chinensis* accessions indicates that HPPD resistance need sustained attention to avoid its continuous development. Especially now, three HPPD herbicides have been widely used to control ACCase-resistant *L. chinensis* in rice fields in China. The increased selection pressure may lead to a surge of HPPD-resistant accessions in the future. Integrated weed management strategy should be adopted to manage the resistant L. *chinensis* accessions, rather than relying solely on herbicides, to extend the usage life of new HPPD inhibitors and avoid the resistance evolution.

A lot of evidences have indicated that P450-mediated metabolic resistance is a major mechanism in the evolution of herbicide resistance (Yu and Powles, 2014; Dimaano and Iwakami, 2021; Zou et al., 2023; Guan et al., 2024; Xu et al., 2024; Chen et al., 2025b). In our study, no *HPPD* gene mutation was detected in the TC53-R *L. chinensis* accession, and the expression of *HPPD* gene as shown by RNA-Seq was similar between the TC53-R and YZ120-S accessions (data not shown). Based on these results, the TSR mechanism can be excluded. Furthermore, pretreatment with PBO increased the tripyrasulfone sensitivity of the TC53-R accession, as demonstrated by the reduction of GR_{50} from 103.21 g a.i. ha^{-1} to 50.05 g a.i. ha^{-1}. These findings confirmed that metabolic resistance was responsible for tripyrasulfone resistance in the TC53-R accession. Our result was similar with previous studies. Lu et al. (2020) reported that a wild radish population evolved resistance to HPPD herbicide mesotrione, and found that enhanced rate of mesotrione metabolism was the main reason for resistance. Concepcion et al. (2021) discovered a GSH conjugation detoxification mechanism in HPPD herbicide-resistant waterhemp.

P450s are one of the largest gene superfamilies, and overexpression of certain P450 gene could increase the activity of enzyme, degrade herbicides into low toxicity substances, thereby leading to resistance (Dimaano and Iwakami, 2021). In weeds, some P450 genes have been demonstrated to confer resistance to different herbicides, including *CYP81A12/21* (Iwakami et al., 2019), *CYP709C69* (Suda et al., 2023), and *CYP72A15* (Zhang et al., 2025) in *E. phyllopogon*, *CYP71AF43* (Tian et al., 2025) in *Alopecurus myosuroides*, *CYP81A10v7* (Han et al., 2021) in *Lolium rigidum*, *CYP96A146* and *CYP77B34* (Shen et al., 2022, 2024) in *Descurainia sophia*, *CYP81Q32* (Wang et al., 2023), *CYP709B1*, *CYP704C1* (Yin et al., 2024), *CYP99A44*, and *CYP704A177* (Bai et al., 2022) in *Beckmannia syzigachne*, and *CYP81A104* and *CYP71AK44* (He et al., 2024) in *Eleusine indica*. Moreover, overexpression of *CYP709B1* and *CYP704C1* in *Raphanus raphanistrum* endowed resistance to mesotrione (Lu et al., 2023). Specially, recent study showed that the *CYP81Q32-like* gene overexpression in *T. aestivum* increased the tolerance to HPPD herbicides mesotrione (Sudhakar et al., 2025). In our study, the expression level of a *CYP81Q32* gene in TC53-R accession was significantly up-regulated compared with the YZ120-S accession, and this gene showed the 70.7% amino acid similarity with the *CYP81Q32-like* gene in *T. aestivum*. Hence, we infer that *CYP81Q32* is the functional gene that caused TC53-R accession to develop resistance to tripyrasulfone.

To verify this speculation, *LcCYP81Q32* was overexpressed in indica rice, which was sensitive to tripyrasulfone, and the indica rice overexpressing *LcCYP81Q32* showed significantly increased tolerance to tripyrasulfone. This finding confirmed that *LcCYP81Q32* was responsible for resistance to HPPD herbicides in L. *chinensis*. In addition, the TC53-R accession also showed cross-resistance to pyraquinate and flusulfinam. However, the rice was highly tolerant to the two herbicides. In our experimental observation, rice plants only showed slight whitening symptoms at the 16-fold recommended dosage treatment of pyraquinate and flusulfinam, respectively. Hence, the sensitivity difference of WT and transgenic rice to the two herbicides was not determined. This is a limitation of using crop models for weed gene validation and future study will focus on utilizing a more sensitive heterologous system like

Arabidopsis thaliana or yeast expression systems to definitively prove whether *LcCYP81Q32* possesses broad-spectrum degradative activity against multiple HPPD chemistries. It is worth noting that PBO has not completely reversed the resistance to tripyrasulfone, and the GR_{50} remains approximately twice as high as that of the susceptible YZ120-S accession. This implies that other potential genes, such as GST or UGT, may also involve in the resistance. Of course, this study has still some questions that need to be answered. Whether other overexpressing metabolic genes, such as CYP709B2, GST4, UGT74AC1, and AKR2, play a role in the herbicide resistance or not, and their function need to be further validated. What factor causes the up-regulation of the *LcCYP81Q32*, and whether this gene display cross resistance to other herbicides with different mode of action.

In summary, this study identified that one L. *chinensis* accession showed resistance to three commonly used HPPD herbicides in rice fields, including tripyrasulfone, pyraquinate and flusulfinam. The mechanism was not related to the target-site resistance, and PBO can reverse the resistance to tripyrasulfone. Moreover, this study demonstrated that the overexpression of *LcCYP81Q32* was mainly responsible for the resistance.

CRediT authorship contribution statement

Zhixun Ge: Software, Methodology, Investigation. **Dengqing Lu:** Supervision, Investigation. **Hanqi Yin:** Methodology, Formal analysis, Data curation. **Shuzhong Yuan:** Supervision. **Xia Yang:** Visualization, Supervision, Resources. **Qian Yang:** Resources, Methodology. **Wei Deng:** Writing – review & editing, Validation, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2026.107141.

Data availability

Data will be made available on request.

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