Multiple resistance to thifensulfuron-methyl and fomesafen in redroot pigweed (*Amaranthus retroflexus* L.) from China

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ABSTRACT

Redroot pigweed (*Amaranthus retroflexus* L.) is a troublesome weed infesting soybean (*Glycine max* [L.] Merr.) productions in China. One redroot pigweed population, collected from Heilongjiang (HLJ) Province, China, was suspected to be resistant to thifensulfuron-methyl and fomesafen. The other one redroot pigweed population, collected from Shandong (SD) Province, was susceptible. The study aimed to characterize the level of thifensulfuron-methyl and fomesafen resistance using HLJ population and identify the potential resistance mechanisms to thifensulfuron-methyl. The sensitivity to other herbicides with and without the same target site was also evaluated. Acetolactate synthase (ALS) gene sequencing revealed that $Trp_{574}Leu$ or $Ala_{205}Val$ amino acid substitution were present in the HLJ population. Whole-plant herbicide bioassays showed that, compared with SD population, HLJ population displayed high level of resistance to thifensulfuron-methyl and moderate resistance to fomesafen. The 50% growth reduction (GR_{50}) value of thifensulfuron-methyl with malathion pretreatment was reduced by 23%, suggesting that both target-site resistance and non-target-site resistance mechanisms were present in thifensulfuron-methyl resistance of redroot pigweed. Cross-resistant patterns showed that the HLJ population evolved resistance to pyrithiobac-sodium, pyroxsulam, imazethapyr and fluoroglycofen, but susceptible to bentazone.

Key words: Acetolactate synthase, gene mutation, protoporphyrinogen oxidase, multiple resistance.

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Received: 26 July 2017.
Accepted: 17 October 2017.
doi:10.4067/S0718-58392017000400311

INTRODUCTION

Redroot pigweed (*Amaranthus retroflexus* L.) is one of the most troublesome annual C_4 dicotyledonous weeds in the world and is widely distributed in many farmlands (Sheibany et al., 2009). It can grow to a height of 2 m and produce a million seeds that have prolonged seed longevity and extended germination period, which allow redroot pigweed to build a persistent soil seed bank (Karimmojeni et al., 2014). It competes with crops for water, light and nutrients and severely reduces yield and quality of several crops, such as soybean (*Glycine max*) (Bensch et al., 2003), beans (*Phaseolus* spp.) (Amini et al., 2014), and corn (*Zea mays*) (Sheibany et al., 2009; Ghanizadeh et al., 2014). Unfortunately, Chen et al. (2015) confirmed redroot pigweed to have evolved resistance to acetolactate synthase (ALS) inhibitors in China, which makes it difficult its control in cropping system.

ALS- and protoporphyrinogen oxidase (PPO)-inhibiting herbicides are the most commonly used postemergence herbicides in soybean fields in northeast China. Acetolactate synthase (ALS; EC 2.2.1.6), also known as acetohydroxyacid synthase (AHAS), is a key enzyme involved in biosynthesis of valine, isoleucine and leucine with four catalytic and four regulatory subunits (Duggleby et al., 2008). ALS inhibitors can bind the enzyme, affecting the biosynthesis of valine, isoleucine and leucine, and eventually resulting in the death of plants (Corbett and Tardif, 2006). There are five herbicide classes targeting at AHAS enzyme: imidazolinones (IMI), sulfonylurea (SU), triazolopyrimidine (TP), sulfonylaminocarbonyl triazolinone (SCT) and pyrimidinylthiobenzoate (PTB) (Duggleby et al., 2008).

It is well known that weeds are prone to produce resistance to ALS inhibitors. Two prominent mechanisms of resistance to ALS-inhibiting herbicides are altered target site and strengthened herbicide metabolism by cytochrome P450 monooxygenases (CYP) (Powles and Yu, 2010). ALS target-site resistance (TRS) is caused by amino acid substitution in ALS, prohibiting herbicides from binding to ALS active site channel effectively. Over the past 20 yr, ALS-TSR have developed rapidly and total 26 amino acid substitutions at 8 positions of the ALS gene have been identified: Ala122(3), Pro197(12), Ala205(2), Asp376(1), Arg377(1), Trp574(3), Ser653(3) and Gly654(2) (Powles and Yu, 2010; Beckie and Tardif, 2012; Tranel et al., 2015). However, ALS non-target-site resistance (NTSR) has been much less identified and studied, especially in dicotyledon. NTSR are mechanisms that minimize the amount of herbicides reaching the target site, resulting from reducing herbicides absorption and/or

translocation, enhancing metabolism (Délye, 2013). Unlike TSR, only conferring resistance to herbicides targeting enzyme concerned, NTSR confers unpredictable resistance levels to herbicides with different modes of action (Petit et al., 2010). Up to now, the typical cases in NTSR include *Bromus rigidus* (Owen et al., 2012), *Lolium rigidum* (Yu et al., 2013; Fernández-Moreno et al., 2017) and *Echinochloa phyllopogon* (Iwakami et al., 2014).

PPO catalyzes the last step in the biosynthesis of heme and chlorophyll (Beale and Weinstein, 1990). The inhibition of PPO enzymes with herbicides causes an accumulation of protogen IX, a substrate of these enzymes (Jacobs and Jacobs, 1993). Protogen IX is transported from these organelles to the cytoplasm where peroxidase enzymes convert it to proto IX, which, in the presence of sunlight, causes the generation of damaging singlet oxygen species, ultimately leading to the demise of the plant (Jacobs and Jacobs, 1993; Lee et al., 2000). As far as we known, there has no report that resistance to PPO-inhibiting herbicides in redroot pigweed in China.

PPO-inhibiting herbicides, such as lactofen, acifluorfen, and fomesafen, provide excellent control of redroot pigweed. Therefore, many soybean farmers mix these herbicides with ALS-inhibiting herbicides to control ALS-resistant biotypes. In recent years, thifensulfuron-methyl and fomesafen control failure on redroot pigweed occurs frequently in soybean fields in northeast China. The objectives of the study were to characterize the level of thifensulfuron-methyl and fomesafen resistance in suspected resistant redroot pigweed populations collected in Heilongjiang province of China, identify the potential resistance mechanisms to thifensulfuron-methyl, and determine the sensitivity to other herbicides with and without the same target site.

MATERIALS AND METHODS

Plant materials

Two redroot pigweed populations were used in this study. One suspected resistant (HLJ, referred to R) population was collected from Heilongjiang province, and the other susceptible population (SD, referred to S) was collected from Shandong province. Resistant redroot pigweed seeds were collected from soybean field with histories of repeated either thifensulfuron-methyl or fomesafen applications and what obtained from the grower was that thifensulfuron-methyl or fomesafen gave bad control effect against the resistant redroot pigweed at two times recommended dose. Susceptible redroot pigweed seeds were collected from a roadside not exposed to any herbicide in Shandong province. Seeds of two population were collected from multiple plants and were mixed to make a composite. After collection, seeds were cleaned, sun dried at room temperature, and stored in paper bags at room temperature (20 ± 5 °C) until use. Before planting, seeds were immersed in distilled water for 6 h to accelerating germination at 25 °C under a 12:12 h photoperiod. After radicle being visible, 25 seeds were sown below the soil surface in plastic pots with a 16-cm diameter and 13-cm height. The soil, with 1.7% organic matter, was passed through a 3-mm sieve. The experiment was conducted in a greenhouse with day/night temperatures set at 25 ± 5/20 ± 5 °C with a 12-h photoperiod. After emergence, seedlings were thinned to 15 plants per pot. The pots were watered as needed to maintain moisture.

ALS gene amplification and sequencing

DNA was extracted from S populations and 20 individual seedling redroot pigweed plants surviving a field-rate application of thifensulfuron-methyl to ensure only resistant plants were sequenced by CTAB method. One pair of primers (Table 1) (synthetized by Sangon Biotech, Shanghai, China) designed by the Primer Premier 5.0 software (Biosoft, Palo Alto, California, USA), were used to amplify the ALS gene covering eight amino acid residues endowing resistance that have been reported in other resistant species. The polymerase chain reaction (PCR) was conducted in a 25 µL volume containing 1 µL gDNA, 2.5 µL 10 × EasyTaq Buffer (TransGen Biotech, China), 2 µL dNTP Mixture (2.5 mM, TransGen Biotech, China), 1 µL each primer (10 µM), 0.25 µL EasyTaq DNA Polymerase (5 U μL^{-1}, TransGen Biotech, China), 17.25 µL DNase, RNase-free water (Sangon Biotech, Shanghai, China). PCR was run in a MyCycler Thermal Cycler (BioRad, Hercules, California, USA) subjected to the following profile: denaturation at 94 °C for 5 min, 36 cycles of 94 °C for 30 s, 56 °C for 40 s, 72 °C for 2 min, followed by final extension step of 10 min at 72 °C. The PCR products were separated by 1% agarose gel run in 1 × TAE buffer and then purified using the *EasyPure* Quick Gel Extraction Kit (TransGen Biotech, Beijing, China). The purified products were sequenced from both forward and reverse directions commercially (Sangon Biotech, China). Sequence data were analyzed by DNAMAN version 5.2.2 software (Lynnon Biosoft, Quebec, Canada).

Whole-plant herbicide bioassays

Plants from the R and S populations were treated with herbicides to quantify the level of resistance to thifensulfuron-methyl (methyl 3-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate; 75% WG, CYNDA, Weifang, China) and fomesafen (5-(2-chloro-α,α,α-trifluoro-p-tolyloxy)-*N*-mesyl-2-nitrobenzamide; 250 g L^{-1} AS, Jiangsu Jiangnan Agrochemical, Lianyungang, China). Redroot pigweed seedlings at the three- to four-leaf growth stage were treated with thifensulfuron-methyl and fomesafen to study the 50% growth reduction (GR_{50}) using research track sprayer that

Table 1. Primers used in this study.

Primers	Sequence (5'-3')	Annealing temperature (°C)	Amplicon size (bp)
FZAL-F1	TCTCACCGATGATAAACCCTC	56	1859
FZAL-R1	TAAGCCCTTCTTCCATCACC		

delivered a 450 L ha^{-1} spray solution at a spray pressure of 275 kPa; flat-fan nozzles (TeeJet 903EVS, Greenman Machinery Company, Beijing, China) were used in the sprayer. Thifensulfuron-methyl was applied at 0, 1/81, 1/27, 1/9, 1/3, 1 and 3 times the recommended dose for the S population and 0, 1/9, 1/3, 1, 3, 9 and 27 times for R population. Fomesafen was applied at 0, 1/81, 1/27, 1/9, 1/3, 1 and 3 times the recommended dose for the S population and 0, 1/27, 1/9, 1/3, 1, 3 and 9 times for R population. The recommended dose is 22.5-33.75 g ai ha^{-1} for thifensulfuron-methyl, 375-450 g ai ha^{-1} for fomesafen and the lower limit were chosen for the study.

To determine the sensitivity to other herbicides, uniform-sized (three- to four-leaf growth stage) plants of R and S populations were treated with pyrithiobac-sodium at 34.5 g ai ha^{-1} (sodium 2-chloro-6-(4,6-dimethoxypyrimidin-2-ylthio) benzoate; 10% AS, Rainbow, Weifang, China); pyroxsulam at 13.5 g ai ha^{-1} (*N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-a] pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide; 4% OD, Dow Chemical Company, Shanghai, China); imazethapyr at 75 g ai ha^{-1} (5-ethyl-2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid; 5% AS, CYNDA, Boxing, China); bentazone at 748.8 g ai ha^{-1} (3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide; 480 g L^{-1} AS, BASF, Shanghai, China); fluoroglycofen at 75 g ai ha^{-1} (*O*-[5-(2-chloro-α,α,α-trifluoro-*p*-tolyloxy)-2-nitrobenzoyl]glycolic acid; 10% EC, Changqing Agrochemical, Yangzhou, China).

At 21 d after the herbicide application, shoots were harvested and oven dried at 70 °C for 72 h and biomass was recorded. All treatments were replicated three times, and the experiment was conducted twice.

CYP inhibition by malathion

Seedling from the R and S populations at the three- to four-leaf growth stage were treated with malathion, thifensulfuron-methyl, and malathion plus thifensulfuron-methyl (Table 2). Malathion was applied at 1000 g ai ha^{-1}. Malathion was formulated in a mixture of emulsifier (Tween 80, 1 mL L^{-1} in water) and acetone and applied 1 h prior to herbicide application (Preston et al., 1996). At 21 d after the herbicide application, shoots were harvested and oven dried at 70 °C for 72 h and biomass was recorded (Guo et al., 2016). All treatments were replicated three times, and the experiment was conducted twice.

Table 2. Malathion treatment applied for cytochrome P450 monooxygenases (CYP) inhibition.

	Thifensulfuron-methyl (times of the lower limit of recommended dose[b])	
Malathion	R	S
0 g ai ha^{-1}	0, 1/9, 1/3, 1, 3, 9, 27	0, 1/81, 1/27, 1/9, 1/3, 1, 3
1000 g ai ha^{-1}[(a)]	0, 1/9, 1/3, 1, 3, 9, 27	0, 1/81, 1/27, 1/9, 1/3, 1, 3

[a]Recommended dose.
[b]Recommended dose: 22.5 g ai ha^{-1}
R: Resistant population; S: susceptible population.

Statistical analysis

The values from two replicates of the whole- plant herbicide bioassays and CYP inhibition by malathion experiments were analyzed by ANOVA (Version 17.0), and date were combined because of the nonsignificant interaction with two repeated experiments. The combined data were used for further analysis by the four parameter log-logistic curve using SigmaPlot (Version 12.5; Systat Software Inc., San Jose, California, USA):

$$y = c + (d-c)/\{1+exp[b(logx - logED_{50})]\}$$

In this model, b is the relative slope around the herbicide dose resulting in 50% growth inhibition (ED_{50}), c is the lower limit and d is the upper limit. The herbicide dose was the independent variable (x), and the growth response (percentage of the untreated control) was the dependent variable (y) in the regression equation. The resistance index (RI) was calculated by dividing the GR_{50} (the dose causing a 50% dry-weight growth reduction) value of the resistant population by that of the susceptible population.

RESULTS

ALS gene sequencing

The ALS gene sequence data were compared using DNAMAN (Lynnon, San Ramon, California, USA). Comparison of the gene between the R and S populations showed two nucleotide polymorphisms, each leading to an amino acid substitution (Table 3). According to the ALS gene sequencing result of the 20 individual plants in HLJ, there is a TGG-574-TTG substitution that resulting in a $Trp_{574}Leu$ substitution in 8 plants, and a GCT-205-GTT substitution that leading to the substitution of Ala by Val in 12 plants. The corresponding sequence chromatograms revealed double-peaks in all 205 mutation site and no double-peaks in all 574 mutation site, indicating that all 574 mutant was homozygous and the all 205 mutant was heterozygous (Figure 1). The results above indicate that the occurrence of 574 mutant may be earlier than 205 mutant in HLJ population.

Sensitivity bioassay to thifensulfuron-methyl and fomesafen

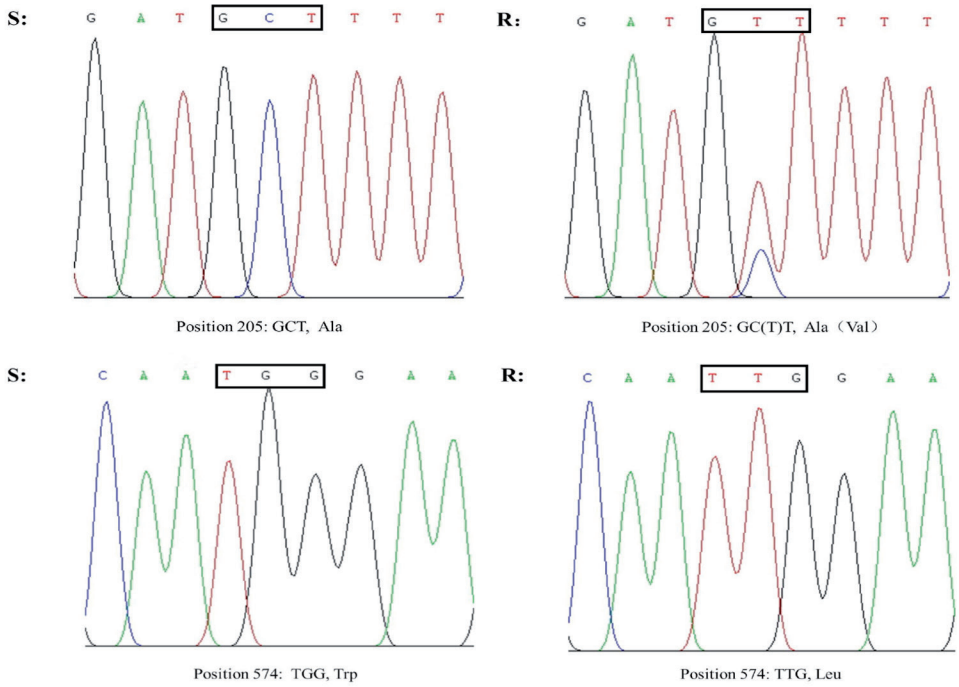
Whole-plant dose-response experiments reconfirmed the susceptibility of the SD population and the resistance of the HLJ population to thifensulfuron-methyl (Figure 2, Table 5) and fomesafen (Figure 3, Table 4). The GR_{50} values to thifensulfuron-methyl for the SD and HLJ populations were

Table 3. Target-site mutations in the acetolactate synthase (ALS) gene conferring herbicide resistance in resistant *Amaranthus retroflexus* populations from Heilongjiang province.

		Amino acid substitutions and times of occurrence	
Population	Phenotype	Ala-205-Val	Trp-574-Leu
HLJ	R	GTT Val-205 (12 times)	TTG Leu-574 (8 times)
SD	S	GCT Ala-205	TGG Trp-574

HLJ: Heilongjiang; SD: Shandong; R: Resistant population; S: susceptible population.

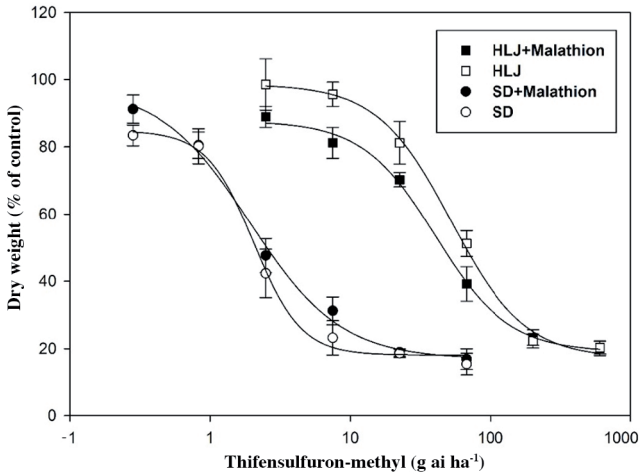
Figure 1. The mutations in ALS gene of the susceptible (S) and resistance (R) redroot pigweed plants.



DNA sequencing results showing 205GC(T)T for Ala(Val); 574TTG for Leu.
R: Resistant population; S: susceptible population.

2.05 and 52.45 g ai ha^{-1}, respectively. Based on the RI, HLJ population of redroot pigweed was 25.6-fold resistant to thifensulfuron-methyl (Table 5). The GR_{50} values to fomesafen for the SD and HLJ populations were 31.78 and 211.97 g ai ha^{-1}, respectively. Based on the RI, HLJ population of redroot pigweed was 6.7-fold resistant to fomesafen (Table 4).

Figure 2. Dose-response of HLJ (R) and SD (S) to thifensulfuron-methyl and malathion plus thifensulfuron-methyl. The values are expressed as the percentage of the untreated control.

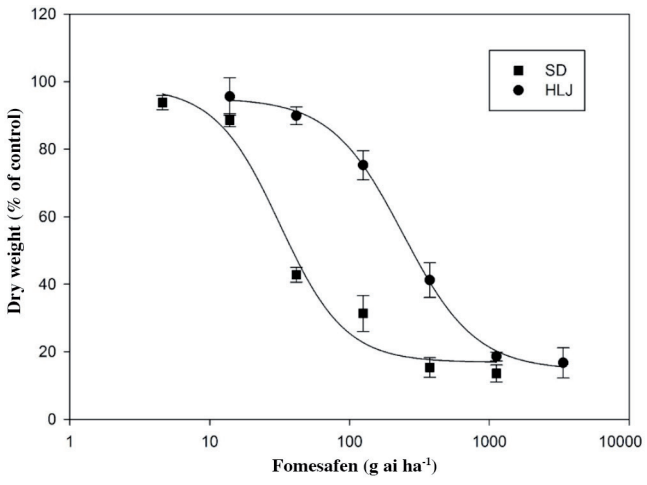


The data represent the mean ± SE of two experiments, each containing three replicates.
HLJ: Heilongjiang; SD: Shandong; R: resistant population; S: susceptible population.

Cross resistance to other herbicides

Three ALS-inhibiting herbicides, one PPO-inhibiting herbicide and one photosystem II (PS II)-inhibiting herbicide were used in this study. The dry weights were recorded at 21 d after treatment. As expected, the S population was completely controlled and the dry weight

Figure 3. Dose-response of HLJ (R) and SD (S) populations to fomesafen. Values are expressed as the percentage of the untreated control.



The data represent the mean ± SE of two experiments, each containing three replicates.
HLJ: Heilongjiang; SD: Shandong; R: resistant population; S: susceptible population.

Table 4. Parameters of the four-parameter log-logistic equation[a] used to calculate the 50% growth reduction (GR_{50}) values of the HLJ (R) and SD (S) populations of redroot pigweed treated with rates of fomesafen. Standard errors are in parentheses.

Population	Regression parameters				GR_{50}[b] (g ai ha^{-1})	RI[c]
	c	d	b	R^2		
S	16.86 (6.12)	98.47 (11.57)	-1.87 (0.95)	0.98	31.78 (9.56)	1
R	14.60 (1.95)	95.17 (1.82)	-1.68 (0.18)	0.99	211.97 (19.2)	6.7

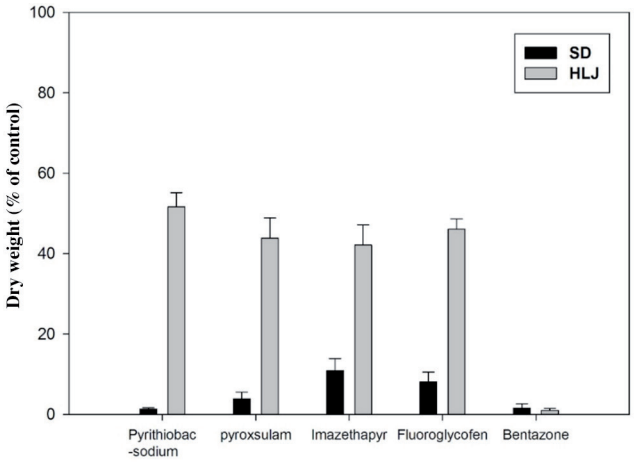
[a] $y = c + (d - c)/\{1+exp[b(logx-logED_{50})]\}$, where y is the percentage of the control, c and d are lower and upper asymptotic limits, b is the slope of the curve through GR_{50}.
[b]Data in the table are expressed as mean ± standard deviation.
[c]RI: Resistance index was calculated as the ratio of GR_{50} values of the resistant (R) and susceptible (S) populations.
HLJ: Heilongjiang; SD: Shandong.

was less than 10% of the control at the recommended field rate of all herbicides (Figure 4). However, the R population manage to survive the ALS inhibitors and PPO inhibiting herbicide treatments. The biomass of plants was relatively low compared with the untreated plants when treated with pyrithiobac-sodium, pyroxsulam and imazethapyr. Still, the biomasses were obviously higher than that of the S population (Figure 4) and the plants survived of R population could produce seeds. We speculate that the level of resistance to ALS inhibitor herbicides will get higher and higher with the overuse of herbicide. No plants survived treatment of PS II-inhibiting herbicide bentazone, and the biomasses of S and R population were similar (Figure 4). Besides, the survival of plants treated with fluoroglycofen of HLJ population reconfirmed that the HLJ population has resistance to PPO inhibitor herbicides.

CYP inhibition by malathion

The GR_{50} value of SD plants treated with thifensulfuron-methyl was similar when applied in mixtures with malathion at 1000 g ai ha^{-1}, however, the GR_{50} value of HLJ plants treated with malathion plus thifensulfuron-methyl was down

Figure 4. Herbicide sensitivity tests of HLJ (R) and SD (S) population following treatments at the recommended rate. The values are expressed as the percentage of the untreated control.



The data represent the mean ± SE of two experiments, each containing three replicates.
HLJ: Heilongjiang; SD: Shandong; R: resistant population; S: susceptible population.

to 40.63 from 52.45 contrast with HLJ plants treated with thifensulfuron-methyl only (Figure 2, Table 5), suggesting that the mechanism of resistance may be associated with both TSR and NTSR.

DISCUSSION

The present study reported that 574 homozygous mutant or 205 heterozygous mutant in ALS gene resulted in high-level thifensulfuron-methyl resistance and cross-resistance to pyrithiobac-sodium, pyroxsulam, imazethapyr. $Trp_{574}Leu$ mutation has been identified in 32 weed species and $Ala_{205}Val$ mutation has been detected in five weed species all over the world (Heap, 2017). Trp_{574} residue was important for defining the shape of the ALS active site channel (McCourt et al., 2006). The shape of the binding

Table 5. Parameters of the four-parameter log-logistic equation[a] used to calculate the 50% growth reduction (GR_{50}) values of the HLJ (R) and SD (S) populations of redroot pigweed with rates of thifensulfuron-methyl and thifensulfuron-methyl + malathion. Standard errors are in parentheses.

Treatment	Population	Regression parameters[a]					
		c	d	b	R^2	GR_{50}[b] (g a.i. ha^{-1})	RI[c]
Thifensulfuron-methyl	S	18.09 (1.99)	84.77 (3.39)	-2.58 (0.70)	0.99	2.05 (0.21)	1
	R	17.17 (3.14)	98.59 (2.56)	-1.65 (0.26)	0.99	52.45 (5.30)	25.6
Thifensulfuron-methyl + malathion	S	16.88 (3.38)	97.81 (7.19)	-1.35 (0.33)	0.99	1.94 (0.38)	1
	R	19.06 (2.48)	87.71 (2.41)	-1.67 (0.26)	0.99	40.63 (4.29)	20.9

[a] $y = c + (d - c)/\{1+exp[b(logx-logED_{50})]\}$, where y is the percentage of the control, c and d are lower and upper asymptotic limits, b is the slope of the curve through GR_{50}.
[b]Data in the table are expressed as mean ± standard deviation.
[c]RI: Resistance index was calculated as the ratio of GR_{50} values of the resistant (R) and susceptible (S) populations.
HLJ: Heilongjiang; SD: Shandong.

site was changed by its mutation, which leading to losing several interactions with herbicides. Moreover, Trp_{574} is important for anchoring SU and IMI herbicides to ALS. Substitution of both Trp_{574} and Ala_{205} can confer a broad spectrum of resistance (Powles and Yu, 2010); however, substitutions of Ala_{205} conferred much lower resistance levels than Trp_{574} (Whaley et al., 2007). And it is no surprise that the HLJ population have resistance to many other ALS inhibitor herbicides.

Fomesafen caused severe injury symptoms on SD population plants with 2 d after treatment and all SD population plant died at 1, 3, 9 times the recommended dose of fomesafen with 14 d after treatment. At 1/3 times the recommended dose, regrowth occurred in a few plants within 7 d after treatments. However, fomesafen slightly injured HLJ population plants within 2 d after treatments. Within 7 d after treatment, the injured leaves recovered and resumed normal growth. In cross resistance study, the survival of HLJ population plants treated with fluoroglycofen reconfirmed that it has resistance to PPO inhibitor herbicides. Based on greenhouse data, the HLJ population produces moderate resistance (RI = 6.7) to fomesafen. Although resistance to PPO-inhibiting herbicides has been evolved slowly, it may be expected to occur in weedy species with large population under continuous and strong selection pressure (Salas et al., 2016). In Northeast China, famers have continuously used fomesafen in soybean fields. And growers tend to increase the application dose of herbicides to achieve rapid and good control effects, which greatly increases selection pressure. Waterhemp (*Amaranthus tuberculatus*) was the first weed to evolve resistance to PPO-inhibiting herbicides in 2001 (Shoup et al., 2003). Former researches showed that a unique target-site amino acid deletion (Gly_{210}) and Arg_{98}Leu substitution in *PPX2* gene confer PPO resistance in waterhemp (Lee et al., 2008) and common ragweed (*Ambrosia artemisiifolia*) (Rousonelos et al., 2012). Further research will be focused on the potential mechanism and progression of resistance to fomesafen.

In recent years, multiple resistance in some weeds has become serious threat and is reducing control product alternatives for famers in China (Bi et al., 2016). In cross resistance experiment, bentazone, PS II-inhibiting herbicide, still had a good control effect on HLJ population and SD population of redroot pigweed. Therefore, bentazone may perform an important role in managing ALS and/or PPO resistant weed in China. Nonetheless, people had better apply bentazone in combination with other herbicides (such as bentazone + fomesafen) to delay or prevent resistance.

In our study, the GR_{50} value of SD plants treated with thifensulfuron-methyl was similar when applied in mixtures with malathion. However, treatment with malathion decreased the GR_{50} value of thifensulfuron-methyl by 22% in HLJ population, suggesting that the mechanism of resistance may be associated with both TSR and NTSR. NTSR is complex, consisting of reduced penetration, sequestration, impaired translocation and enhanced metabolism of herbicides (Délye, 2013). There are few reports about NTSR to ALS inhibitors in broadleaf weeds. However, Délye have shown that different individuals of *Papaver rhoeas* with same genotypes to ALS exhibit different levels of herbicide resistance, which results from the NTSR (Délye et al., 2011). This strongly indicates that the significance of NTSR to ALS-inhibiting herbicides may be underestimated in broadleaf weeds, possibly because NTSR is very often linked with TSR (Délye et al., 2011; Ahmad-Hamdani et al., 2012; Kaundun et al., 2013).

CONCLUSION

Based on our results in this research, it can be concluded that Trp_{574}Leu and Ala_{205}Val amino acid substitution within the acetolactate synthase (ALS) gene of Heilongjiang (HLJ) population may be the main mechanism of thifensulfuron-methyl in redroot pigweed. Also, non-target-site resistance (NTSR) maybe contribute to thifensulfuron-methyl resistance in HLJ population. The HLJ population produces high resistance to thifensulfuron-methyl and moderate resistance to fomesafen. Meanwhile, the HLJ population has cross-resistance to pyrithiobac-sodium, pyroxsulam, imazethapyr and fluoroglycofen but no resistance to bentazone.

ACKNOWLEDGEMENTS

This research was supported by the Special Fund for Agroscientific Research in the Public Interest (201303031). The authors thank all the workers for assistance in conducting this research.

REFERENCES

Ahmad-Hamdani, M.S., Yu, Q., Han, H., Cawthray, G.R., Wang, S.F., and Powles, S.B. 2012. Herbicide resistance endowed by enhanced rates of herbicide metabolism in wild oat (*Avena* spp.) Weed Science 61:55-62.

Amini, R., Alizadeh, H., and Yousefi, A. 2014. Interference between red kidneybean (*Phaseolus vulgaris* L.) cultivars and redroot pigweed (*Amaranthus retroflexus* L.) European Journal Agronomy 60:13-21.

Beale, S.I., and Weinstein, J.D. 1990. Tetrapyrrole metabolism in photosynthetic organisms. McGraw-Hill, New York, USA.

Beckie, H.J., and Tardif, F.J. 2012. Herbicide cross resistance in weeds. Crop Protection 35:15-28.

Bensch, C.N., Horak, M.J., and Peterson, D. 2003. Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Science 51:37-43.

Bi, Y.L., Liu, W.T., Guo, W.L., Li, L.X., Yuan, G.H., Du, L., et al. 2016. Molecular basis of multiple resistance to ACCase- and ALS-inhibiting herbicides in *Alopecurus japonicus* from China. Pesticide Biochemistry and Physiology 126:22-27. doi:10.1016/j. pestbp.2015.07.002.

Chen, J., Huang, Z., and Zhang, C. 2015. Molecular basis of resistance to imazethapyr in redroot pigweed (*Amaranthus retroflexus* L.) populations from China. Pesticide Biochemistry and Physiology 124:43-47.

Corbett, C.A.L., and Tardif, F.J. 2006. Detection of resistance to acetolactate synthase inhibitors in weeds with emphasis on DNA-based techniques: a review. Pest Management Science 62:584-597.

Délye, C. 2013. Unravelling the genetic bases of non-target-site based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Management Science 69:176-187.

Délye, C., Pernin, F., and Scarabel, L. 2011. Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase (ALS) in corn poppy (*Papaver rhoeas* L.) Plant Science 180:333-342.

Duggleby, R.G., McCourt, J.A., and Guddat, L.W. 2008. Structure and mechanism of inhibition of plant acetohydroxyacid synthase. Plant Physiology and Biochemistry 46:309-324.

Fernández-Moreno, P.T., Bastida, F., and Prado, R.D. 2017. Evidence, mechanism and alternative chemical seedbank-level control of glyphosate resistance of a rigid ryegrass (*Lolium rigidum*) biotype from Southern Spain. Frontiers in Plant Science 8:450. doi:10.3389/fpls.2017.00450.

Ghanizadeh, H., Lorzadeh, S., and Aryannia, N. 2014. Effect of weed interference on *Zea mays*: growth analysis. Weed Biology and Management 14:133-137.

Guo, W.L., Lv, L.L., Zhang, L.L., Li, Q., Wu, C.X., Lu, X.T., et al. 2016. Herbicides cross resistance of a multiple resistant short-awn foxtail (*Alopecurus aequalis* Sobol.) population in wheat field. Chilean Journal of Agriculture Research 76:163-169.

Heap, I. 2017. The international survey of herbicide resistant weeds. Available at http://www.weedscience.org (accessed October 2017).

Iwakami, S., Uchino, A., and Kataoka, Y. 2014. Cytochrome P450 genes induced by bispyribac-sodium treatment in a multiple-herbicide-resistant biotype of *Echinochloa phyllopogon*. Pest Management Science 70:549-558.

Jacobs, J.M., and Jacobs, N.J. 1993. Porphyrin accumulation and export by isolated barley (*Hordeum vulgare*) plastids (effect of diphenyl ether herbicides). Plant Physiology 101:1181-1187.

Karimmojeni, H., Bazrafshan, A.H., Majidi, M.M., Torabian, S., and Rashidi, B. 2014. Effect of maternal nitrogen and drought stress on seed dormancy and germinability of *Amaranthus retroflexus*. Plant Species Biology 29:e1-e8.

Kaundun, S.S., Bailly, G.C., Dale, R.P., Hutchings, S.J., and McIndoe, E. 2013. A novel W1999S mutation and non-target site resistance impact on Acetyl-CoA carboxylase inhibiting herbicides to varying degrees in a UK *Lolium multiflorum* population. PLOS ONE 8:e58012.

Lee, R.M., Hager, A.G., and Tranel, P.J. 2008. Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Science 56:371-375.

Lee, H.J., Lee, S.B., Chung, J.S., Han, S.U., Han, O., Guh, J.O., et al. 2000. Transgenic rice plants expressing a *Bacillus subtilis* protoporphyrinogen oxidase gene are resistant to diphenyl ether herbicide oxyfluorfen. Plant and Cell Physiology 41:743-749.

McCourt, J.A., Pang, S.S., and King-Scott, J. 2006. Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. Proceedings of the National Academy of Sciences of the United States of America 103:569-573.

Owen, M.J., Goggin, D., and Powles, S.B. 2012. Non-target-site based resistance to ALS-inhibiting herbicides in six *Bromus rigidus* populations from Western Australian cropping fields. Pest Management Science 68:1077-1082.

Petit, C., Bay, G., Pernin, F., and Délye, C. 2010. Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. Pest Management Science 66:168-177.

Powles, S.B., and Yu, Q. 2010. Evolution in action: plants resistant to herbicides. Annual Review of Plant Biology 61:317-347.

Preston, C., Tardif, F.J., and Christopher, J.T. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochemistry and Physiology 54:123-134.

Rousonelos, S.L., Lee, R.M., Moreira, M.S., VanGessel, M.J., and Tranel, P.J. 2012. Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS- and PPO-inhibiting herbicides. Weed Science 60:335-344.

Salas, R.A., Burgos, N.R., Tranel, P., Singh, S., Glasgow, L., Scott, R.C., et al. 2016. Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Management Science 72:864-869.

Sheibany, K., Meybodi, M.A.B., and Atri, A. 2009. Competitive effects of redroot pigweed (*Amaranthus retroflexus*) on the growth indices and yield of corn. Weed Biology and Management 9:152-159.

Shoup, D.E., Alkhatib, K., and Peterson, D.E. 2003. Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Science 51:145-150.

Tranel, P.J., Wright, T.R., and Heap, I.M. 2015. Mutations in herbicide-resistant weeds to ALS inhibitors. Available at http://www.weedscience.com (accessed October 2015).

Whaley, C.M., Wilson, H.P., and Westwood, J.H. 2007. A new mutation in plant ALS confers resistance to five classes of ALS-inhibiting herbicides. Weed Science 55:83-90.

Yu, Q., Han, H., and Cawthray, G.R. 2013. Enhanced rates of herbicide metabolism in low herbicide dose selected resistant *Lolium rigidum*. Plant Cell and Environment 36:818-827.