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Tolerance to Some ACCase Inhibitors in Four Common Roegneria (*Roegneria kamoji*) Populations From China

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Common roegneria, a perennial weed in *Roegneria* genera of the tribe Triticeae (family: Poaceae), is widely distributed in China and could not be effectively controlled by acetyl coA carboxylase (ACCase) inhibitors in wheat in some regions. This study aimed to quantify herbicide tolerance level and investigate its mechanisms of ACCase tolerance in common roegneria. Whole-plant dose–response assay indicated that populations ZJJX and HBJZ (collected in wheat fields) exhibited high tolerance [ED_{50}s >16-fold labeled field rate (LFR)] to fenoxaprop-*P*-ethyl and clodinafop-propargyl [aryloxyphenoxypropionate (APPs)] and pinoxaden (phenylpyrazolin). In addition, two populations collected from uncultivated areas showed similar responses to these herbicides. All common roegneria populations were susceptible to haloxyfop-R-methyl and quizalofop-*p*-ethyl (APPs), clethodim, and sethoxydim (cyclohexanediones) (ED_{50}s < 1/2-fold LFR). The responses to ACCase inhibitors of common roegneria were in complete accord with wheat. ACCase sequencing revealed that the APP tolerance was not conferred by known amino acid substitutions. The sensitivity of common roegneria populations to fenoxaprop-*P*-ethyl could not be reduced by metabolic inhibitors malathion and 4-chloro-7-nitro-benzoxadiazole. *In vitro* ACCase enzyme activity assays revealed that the activities of ACCase were increased in ZJJX, ZJHZ populations and wheat after fenoxaprop-*P*-ethyl treatment, which at 72 h after treatment (HAT) was 1.46-, 1.39-, and 1.34-fold higher than that at 0 HAT, respectively. To our knowledge, this study reported for the first time the natural tolerance to ACCase inhibitors in common roegneria. The enhanced ACCase activity suggested that rapid metabolism of the herbicide might play an important role in the tolerance mechanism of this weed. Rotated with other crops (i.e., oilseed rape) to use different herbicides could serve as important tools for managing common roegneria in wheat.

Keywords: acetyl coA carboxylase (ACCase), fenoxaprop-*P*-ethyl, ACCase activity, non-target-site mechanism, tolerance

INTRODUCTION

Common roegneria (*Roegneria kamoji* Ohwi) is a perennial grass belonging to the genus *Roegneria* of the Poaceae family. It is widespread in China, Korea, and Japan (GBIF; http://www.gbif.org). Common roegneria is commonly found in field borders, roadside, hillside, urban green spaces, and vegetable nurseries, but also commonly occurs as a weed in wheat fields (Li, 1998; Xu et al., 2014). Common roegneria first bloomed in April, with seed mature and dispersal occurring in May to June, and it relies on both seed and rhizome to sustain and renew its populations. It is used for conservation of water and soil or used as forage and possesses potentially valuable traits including wheat improvement (Chang et al., 2011; Zhao et al., 2017b). Since 2016, common roegneria has become a predominant and serious weed in two farms and could not be effectively controlled by fenoxaprop-*P*-ethyl or clodinafop-propargyl even over the labeled field rates (LFRs) in China (**Supplementary Figure 1**).

Acetyl coA carboxylase (ACCase, EC 6.4.1.2), catalyzing carboxylation of acetyl-CoA to malonyl-CoA in a multistep reaction, is involved in the first committed step in fatty acid biosynthesis. In plants, two different types of ACCase enzyme were identified, namely, cytoplasmic and plastidic, and the latter contributes up to 80% of the enzyme activity in grasses (Preston and Mallory-Smith, 2001). ACCase-inhibiting herbicides (hereafter referred to as ACCase inhibitors), which inhibit the plastidic ACCase enzyme activity, results in fatty acid depletion, leading to rapid cell death due to membrane dysfunction (Harwood, 1988; Devine, 1997). They have been widely used for grass weeds control in various crops. All ACCase isoforms is composed of three catalytic domains, the biotin carboxyl-carrier, the biotin carboxylase, and the carboxyl transferase (CT) domains. The CT domain is the binding site of widely used ACCase inhibitors (Sasaki et al., 1995; Délye et al., 2005). ACCase inhibitors can be classified into three classes, namely, the aryloxyphenoxypropionates (APPs), cyclohexanediones (CHDs), and phenylpyrazoline (DENs), based on their chemical structures (Wenger et al., 2012). There are many cases of weed populations not effectively controlled by ACCase inhibitors after continuous application due to the evolution of herbicide resistance. To date, biotypes of 48 weed species worldwide and eight throughout China have been documented resistant to ACCase inhibitors (Heap, 2020).

The mechanisms of resistance to ACCase inhibitors may be divided into two major types: target-site resistance (TSR) and non–target-site resistance (NTSR). In many cases, resistance to ACCase inhibitors is due to the presence of an altered target site, typically resulting from a single amino acid substitution in the CT domain that makes the ACCase insensitive to herbicides (Délye et al., 2005; Powles and Yu, 2010). To date, 15 types of amino acid substitutions have been identified at seven different codon positions in the ACCase CT domain: Ile-1781-Val, Leu, Thr, or Gla; Trp1999-Cys, Leu, or Ser; Trp2027-Cys; Ile2041-Asn, Val or Thr; Asn2078-Gly; Cys2088-Arg; and Gly2096-Ala or Ser (Kukorelli et al., 2013; Kaundun, 2014; Guo et al., 2017). Researchers generally describe the amino acid substitutions in the CT domain of ACCase based on the CT domain of blackgrass (*Alopecurus myosuroides* Huds.) accession AJ310767.

NTSR involves any mechanisms that minimize the amount of active herbicide ingredient reaching the target site (for example, reduced penetration, altered patterns of translocation, sequestration, and enhanced detoxification of the toxophores). To date, increased metabolic rate in response to herbicides has been considered the main NTSR mechanism (Délye et al., 2011). A variety of enzymes are involved in NTSR, of which cytochrome P450 mixed-function oxidases (CytP450) and glutathione *S*-transferase (GST) and/or glycosyl-transferase have been confirmed to cause enhanced herbicide metabolism in several grass weed species (Cummins et al., 1997; Busi et al., 2011; Ghanizadeh and Harrington, 2017). Several CytP450 inhibitors, such as piperonyl butoxide and malathion, and the GST inhibitor 4-chloro-7-nitro-benzoxadiazole (NBD-Cl), have long been used as indicators of CytP450 or GST involvement in metabolic resistance to ACCase inhibitors (Cummins et al., 2013; Zhao et al., 2017a).

Natural tolerance of some cereal crops, such as barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), is due to a less sensitive ACCase enzyme or a higher rate of metabolic degradation (Cataneo et al., 2013). Previous reported grass weeds resistance to ACCase inhibitors has 99% identity to *A. myosuroides* and 95% identity to *T. aestivum* in the CT domain. As a wild relative of wheat, it is hypothesized that common roegneria may have similar response to ACCase inhibitors.

In this study, two common roegneria populations known to have survived applications of fenoxaprop-*P*-ethyl at its LFRs were collected from winter wheat fields, and two populations were collected from wastelands with no herbicide applied history. We aimed to (1) quantify the tolerance level to various ACCase inhibitors in the four common roegneria populations and (2) characterize the potential mechanisms of tolerance to ACCase inhibitors.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of two populations (HBJZ and ZJJX) that were not effectively controlled by fenoxaprop-*P*-ethyl were collected from wheat fields in Jingzhou City, Hubei Province, and Jiaxing City, Zhejiang Province, in June 2017, respectively (**Table 1**). These fields were wheat-rice rotations during the last 5 years and had been treated with ACCase inhibitors for grass weeds control in the wheat cropping season. Two populations (HNHY and ZJHZ) were collected from uncultivated wastelands that had never been treated with herbicides in Hengyang City, Hunan Province, and Hangzhou City, Zhejiang Province, in June 2017, respectively. The collected seeds were cleaned, dried at room temperature ($20^{\circ}C \pm 5^{\circ}C$) and kept in paper bags until used. A local wheat cultivar (Yangmai 25) was included as a reference material for this study.

The plant seedlings were cultivated as described in Tang et al. (2014) with minor modification. Briefly, seeds of the four common roegneria populations and wheat were germinated

TABLE 1 | Geographic locations of the common roegneria populations used in this study.

Population	Location			Coordinates
	Province	County	Village(Farm)	
ZJJX	Zhejiang	Haiyan, Jiaxing	Huaxing	30°29′N, 120°51′E
ZJHZ	Zhejiang	Fuyang, Hangzhou	Huangtianfan	30°04′N, 119°55′E
HBJZ	Hubei	Jiangling, Jingzhou	Sanhu	30°13′N, 112°33′E
HNHY	Hunan	Hengnan, Hengyang	Daguang	26°53′N, 112°29′E

in 9-cm Petri dishes containing moistened double layers of filter paper (Double Ring #102, Hangzhou Special Paper Industry Co. Ltd., China). Petri dishes were sealed with Parafilm (American National Can, Greenwich, CT) to prevent evaporation and then cultivated in a germination cabinet (GXZ-300C, Dongnan Instrument Manufacture, Ningbo, Zhejiang, China) at fluctuating day/night temperatures (25°C/15°C) and 80% relative humidity. Dishes were inspected daily, and germinated seeds (with 5-mm coleoptile) were transplanted into 9-cm-diameter plastic pots containing a suitable growing medium (1:1:1:2 vegetable garden soil/compost/peat/dolomite) and grown at ∼20°C under natural conditions. There were four uniform seedlings in each pot, and the pots were watered as required.

Tolerance to ACCase Inhibitors Assay

Experiments were conducted in a screenhouse (a 6 × 40-m chamber framed with 2-cm iron mesh and covered overhead with a transparent plastic cover to prevent rain damage) during 2017 and 2018 at the China National Rice Research Institute (CNRRI, 30°04′N, 119°55′E), Hangzhou, Zhejiang Province, China. At the three-leaf growth stage, plants were sprayed with the commercial formulations of ACCase inhibitors in a compressed air laboratory spray tower equipped with a flat fan nozzle (Teejet TP6501E), which delivered 200 L ha^{-1} solution at 0.21 MPa. After herbicide application, the plants were returned to the screenhouse described above. The aboveground parts of the plant were harvested at 21 days after treatment (DAT) for fresh biomass determination. Fresh weight was expressed as a percentage of the untreated control to standardize comparisons between populations. The experiment was conducted with four pots and was conducted twice. A total of seven ACCase inhibitors were applied in this study and the details are given below (**Table 2**). The herbicide treatments were determined based on a preliminary study (data not shown) treated at the LFRs of each herbicide.

ACCase Gene Sequencing

The leaf tissues (100 mg) were collected from five individuals of the four common roegneria populations at three- to four-leaf stage, and their genomic DNA was extracted using Plant Genomics DNA secure Plant Kit (DP320, Tiangen Biotech Co. Ltd., Beijing, China) based on the protocol from the manufacturer. The primers (ACCase-F: CTCCTGAATTTCCCA GCGGCAGACAGAT; ACCase-R CCCTTGAGGTTCGAGAA CATTACCCTTT) were designed by Primer Premier 5.0, based on the *ACCase* gene sequence of *A. myosuroides* (AJ310767). The PCR products were detected with 1% agarose gel. The desired bands were extracted by AxyPrep DNA Gel Extraction Kit (Axygene, US) and cloned into a pMD19-T vector (Takara Biotech, China). The recombinant plasmids were transformed into competent *Escherichia coli* JM109 (Takara Biotech, China). The positive clones were sequenced by Shanghai Biosune Biological Technology and Services Co., Ltd. (China). At least three clones of each sample were selected for sequencing, and the DNA sequences were translated into protein and compared with ACCase CT domain of *A. myosuroides* and *T. aestivum* (ACD46685.1) by BioEdit software.

Synergistic Effect of Malathion and NBD-Cl

In 2017 and 2018, seeds of two common roegneria populations (ZJJX from wheat field and ZJHZ from uncultivated area) and wheat were planted as described above in plastic pots. Half of the seedlings at three-leaf growth stage were treated with malathion at 1,000 g ha^{-1}, 30 min before herbicide application. Fenoxaprop-*P*-ethyl was sprayed and followed the same doses as described in **Table 2**. For GST inhibitor experiments, three-leaf growth stage seedlings were treated with 80 g ha^{-1} NBD-Cl plus 0.125% (vol/vol) non-ionic surfactant 48 h before fenoxaprop-*P*-ethyl applied at doses same as in **Table 2**. The aboveground plant material was harvested at 21 DAT, and the fresh weights were measured and expressed as a percent of the control group.

In vitro Assay of ACCase and GST Activity to Fenoxaprop-*P*-ethyl

Seedlings of two common roegneria populations (ZJJX and ZJHZ) and wheat were cultivated as described above. At three-leaf growth stage, the seedlings were sprayed with fenoxaprop-*P*-ethyl at 57 g a.i. ha^{-1} and fresh leaf tissues were collected at 24, 48, and 72 h after treatment (HAT). The untreated samples were also collected as control. To test the ACCase activity variation between the four common roegneria populations and wheat after herbicide treatment, 2 g leaf tissues were homogenized with liquid nitrogen and suspended with extract buffer [100 mmol/L Tris-HCl, (pH 8.3), 300 mmol/L glycerinum, 5 mmol/L DTT, 2 mmol/L EDTA, 0.5 mmol/L PMSF, and 0.01% (vol/vol) Triton X-100] and centrifuged at 1,500 g for 10 min at 4°C. The protein concentration in the enzyme extracts were measured by Bradford method (Kruger, 2002). The ACCase activity assay was performed with the Plant ACCase ELISA Kit (Meimian, Jiangsu, China) using the manufacturer's protocol. The activity unit (U/min/mg leaf protein) of ACCase was defined as the amount of the enzyme that catalyzes the conversion of μmol of substrate per minute per mg protein. To determine whether the GST is involved in the tolerance of common roegneria, 0.2 g leaf tissues were homogenized with liquid nitrogen and suspended with 0.8 mL extraction buffer (1× phosphate-buffered saline, pH 7.4). The homogenates were stirred for 10 min on ice and centrifuged at 10,000 *g* for 5 min at 4°C. The supernatant was immediately used for GST activity and protein concentration assay. The protein concentration in the enzyme extracts were measured

TABLE 2 | Detailed information of herbicides, labeled field rates, and treated doses in the whole-plant dose–response studies.

Herbicide	Group	Formulation and commercial name	Supplier	Labeled field rate (g a.i. ha^{-1})	Treated dose (× labeled field rate)
Fenoxaprop-*P*-ethyl	APP	69 g L^{-1} EW, Puma	Bayer, Hangzhou, China	57	1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×, 8×, 16×
Clodinafop-propargyl	APP	15% WP, Topik	Syngenta, Shanghai, China	45	1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×, 8×, 16×
Haloxyfop-R-methyl	APP	108 g L^{-1} EC, Verdict	Dow AgroSciences, Beijing, China	40.5	1/128×, 1/64×, 1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×
Quizalofop-p-ethyl	APP	10% EC, Jingai	Mefront Agricultural Chemicals, Wenzhou, China	45	1/128×,1/64×, 1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×
Clethodim	CHD	24% EC, Kuowang	Sciencreat Chemicals, Shenyang, China	72	1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×
Sethoxydim	CHD	12.5% EC, Nabujing	Soda, Tianjin, China	168.8	1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×
Pinoxaden	PPZ	5% EC, Axial	Syngenta, Shanghai, China	45	1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, 4×, 8×

by Bradford method (Kruger, 2002). The GST activity assay was performed with the GSH–(ST) Detection Kit (Jiancheng, China). The activity unit (U/min/mg leaf protein) of GST was defined as the amount of the enzyme that catalyzes the conversion of μmol of substrate per minute per mg protein. Each treatment had five replications, and the whole experiment was conducted twice.

Statistical Analysis

All experiments were conducted using a completely randomized design with four replications. The data from the repeated runs were subjected to analysis of variance using general linear model procedure in SPSS software, version 13.0 (SPSS, Chicago, IL). No statistically significant ($P > 0.05$) trial by treatment interaction was revealed, so the data of the repeated experiments were pooled for analysis. Data of the plant fresh biomass were analyzed by fitting with a log-logistic function (Equation 1) (Seefeldt et al., 1995) using Origin 8.0 (OriginLab Corp. Northampton, MA). The function fitted was:

$$y = C + \frac{D - C}{1 + (x/ED_{50})^b}$$

where C is the lower response limit, D is the upper response limit, x is the herbicide application dose, b is the slope of the curve through ED_{50}, and y is the response at the herbicide dose x. In this study, ED_{50} is the effective dose of herbicide resulting in 50% growth inhibition. As no susceptible populations were detected in a preliminary experiment, the tolerance index (TI) was calculated or estimated by the ratio of ED_{50} between the common roegneria populations and the LFR of the herbicide.

Data obtained from GST activity assay were subjected to analysis of variance by using SPSS software, version 13.0 (SPSS, Chicago, IL). Mean comparison was performed using Fisher protected least significant difference (LSD) test, where the overall differences were significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Whole-Plant Dose–Response to ACCase Inhibitors

As expected, all the plants of ZJJX and HBJZ collected from wheat fields survived the fenoxaprop-*P*-ethyl treatments at 21 DAT during both experimental runs (2017 and 2018). Spot yellowing was observed on the spear leaves of these two populations of the 4- to 16-fold LFR treatments at 10 DAT; however, the symptoms disappeared gradually, and no visual phytotoxicity symptoms were observed after 15 DAT. However, populations of ZJHZ and HNHY, which were collected from areas without herbicide application history, showed the same response pattern to fenoxaprop-*P*-ethyl. Results of the whole-plant dose–response assay showed a high level of tolerance in all the four populations, with ED_{50}s >16-fold LFR to fenoxaprop-*P*-ethyl (**Table 3**). A similar response trend was observed in wheat, which survival or fresh weight biomass was not affected at 4-fold LFR of fenoxaprop-*P*-ethyl. The TIs of the common roegneria populations and wheat were >16. The ED_{50} of a susceptible *Alopecurus japonicas* population collected from the same field of HBJZ tested at the same period was 5.2 g a.i. ha^{-1}, which was 1/10-fold LFR (data not shown). From this point of view, the common roegneria populations were highly tolerant to fenoxaprop-*P*-ethyl. Similarly, all the common roegneria populations were highly tolerant to clodinafop-propargyl (TI > 16) and pinoxaden (TI > 8) in both experimental runs (**Table 3**).

The ED_{50} values of the four common roegneria populations and wheat to the other two APP herbicides haloxyfop-R-methyl and quizalofop-p-ethyl, were <12 and <8 g a.i.$^{-1}$, respectively (**Table 3**). Based on the TI values (<0.3 and <0.2), common roegneria populations were deemed susceptible to haloxyfop-R-methyl and quizalofop-p-ethyl. The herbicide doses required for 95% fresh weight reduction were 2- and 4-fold LFR for CHD herbicides clethodim and sethoxydim, respectively. The ED_{50} values of the four common roegneria populations and wheat to CHD herbicides clethodim and sethoxydim were <50

TABLE 3 | Dose–response parameters of log-logistic function (Eqn. 1[a] used to fit the plant fresh weight data (as a percent of the control) for common roegneria populations treated with different ACCase inhibitors.

Herbicide	Population	Regression parameters				ED_{50} (g a.i. ha^{-1}) (SEM)[b]	TI[c]
		C (SEM)	*D* (SEM)	*b* (SEM)	r^2		
Fenoxaprop-*P*-ethyl[d]	ZJJX	72.6% at 912 g a.i. ha^{-1}				>912	>16
	HBJZ	80.7% at 912 g a.i. ha^{-1}				>912	>16
	ZJHZ	72.7% at 912 g a.i. ha^{-1}				>912	>16
	HNHY	70.6% at 912 g a.i. ha^{-1}				>912	>16
	wheat	79.2 % at 912 g a.i. ha^{-1}				>912	>16
Clodinafop-propargyl	ZJJX	75.4% at 720 g a.i. ha^{-1}				>720	>16
	HBJZ	85.8% at 720 g a.i. ha^{-1}				>720	>16
	ZJHZ	77.3% at 720 g a.i. ha^{-1}				>720	>16
	HNHY	78.8% at 720 g a.i. ha^{-1}				>720	>16
	wheat	83.3 % at 720 g a.i. ha^{-1}				>720	>16
Haloxyfop-R-methyl	ZJJX	2.80 (1.40)	87.96 (2.31)	1.24 (0.12)	0.9794	8.31 (0.74)	0.21
	HBJZ	3.80 (2.00)	90.66 (2.02)	1.15 (0.11)	0.9785	6.73 (0.62)	0.17
	ZJHZ	2.89 (1.67)	100.14 (2.61)	2.30 (0.21)	0.9955	11.92 (1.08)	0.29
	HNHY	2.80 (1.80)	88.47 (3.03)	1.50 (0.15)	0.9834	6.41 (0.57)	0.16
	wheat	1.74 (1.11)	99.65 (2.93)	2.87 (0.48)	0.9970	10.46 (0.53)	0.26
Quizalofop-p-ethyl	ZJJX	4.32 (0.51)	95.01 (3.41)	2.60 (0.25)	0.9988	6.41 (0.34)	0.14
	HBJZ	4.67 (0.56)	94.30 (1.26)	1.97 (0.08)	0.9996	6.79 (0.24)	0.15
	ZJHZ	7.02 (1.97)	96.62 (8.46)	2.44 (0.69)	0.9840	7.42 (1.33)	0.16
	HNHY	2.07 (1.14)	99.38 (2.03)	1.84 (0.11)	0.9993	6.33 (0.29)	0.14
	wheat	4.93 (3.11)	99.49 (3.25)	2.33 (0.35)	0.9820	7.71 (0.59)	0.17
Clethodim	ZJJX	5.75 (3.06)	97.35 (2.11)	1.73 (0.23)	0.9991	15.13 (1.21)	0.21
	HBJZ	3.99 (2.33)	99.56 (5.39)	3.63 (1.09)	0.9697	30.94 (2.71)	0.43
	ZJHZ	2.56 (1.48)	93.27 (3.26)	4.19 (0.49)	0.9983	20.92 (0.99)	0.29
	HNHY	1.38 (0.79)	98.87 (4.92)	2.49 (0.35)	0.9940	12.02 (1.04)	0.17
	wheat	8.28 (3.07)	99.59 (3.40)	1.87 (0.24)	0.9789	46.04 (3.17)	0.64
Sethoxydim	ZJJX	7.98 (1.99)	98.17 (2.33)	2.18 (0.13)	0.9961	116.34 (5.1)	0.69
	HBJZ	8.60 (0.40)	99.22 (5.16)	2.85 (0.28)	0.9986	137.62 (8.18)	0.82
	ZJHZ	3.05 (1.54)	99.71 (2.32)	2.00 (0.08)	0.9980	126.13 (2.83)	0.75
	HNHY	4.85 (2.50)	100.31 (1.03)	2.29 (0.06)	0.9994	123.76 (1.98)	0.73
	wheat	3.96 (2.85)	99.73 (3.85)	1.80 (0.07)	0.9969	145.13 (3.76)	0.86
Pinoxaden	ZJJX	70.7 % at 360 g a.i. ha^{-1}				>360	>8
	HBJZ	63.1 % at 360 g a.i. ha^{-1}				>360	>8
	ZJHZ	60.0 % at 360 g a.i. ha^{-1}				>360	>8
	HNHY	69.9% at 360 g a.i. ha^{-1}				>360	>8
	wheat	65.5 % at 360 g a.i. ha^{-1}				>360	>8

[a] *Eqn. 1.* $y = C + (D - C)/[1 + (x/ED_{50})b]$, *C is the lower limit, D is the upper limit, x is the herbicide application dose, b is the slope of the curve at* ED_{50}, *and y is the growth response (as a percent of the untreated control).*

[b] ED_{50} *refers to the herbicide rates required to reduce aboveground biomass by 50%.*

[c] *TI, tolerance index. TI was calculated as the ratio between the* ED_{50} *value of the common roegneria populations and the field-recommended rate.*

[d] *Values could not be estimated by the function due to the insufficient covering of the dose range, fresh weight (% of control) at the highest treated dose, and estimated* ED_{50} *and TI were listed.*

and <150 g a.i.$^{-1}$, respectively. Based on the corresponding TI values (<0.7 and <0.9), common roegneria populations were susceptible to CHD herbicides. This contrasts with TSR mechanisms in ACCase resistance that confer broader resistance to ACCase herbicides, such as APP specific (mutations at codon positions 2,027, 2,041, and 2,096) and APP/CHD specific (mutations at codon positions 1,781 and 2,078) previously reported in *A. myosuroides* (Kaundun, 2014).

ACCase Gene Sequencing

The fragments of ACCase gene CT domain were amplified from four common roegneria populations. The predicted open reading frame (ORF) of ACCase CT domain in common roegneria was found by ORF finder, and these fragments covered all the reported resistance-related substitutions. After BLAST analysis of the ACCase CT domain of common roegneria (GenBank accession MT877224) in NCBI database, we found that ACCase

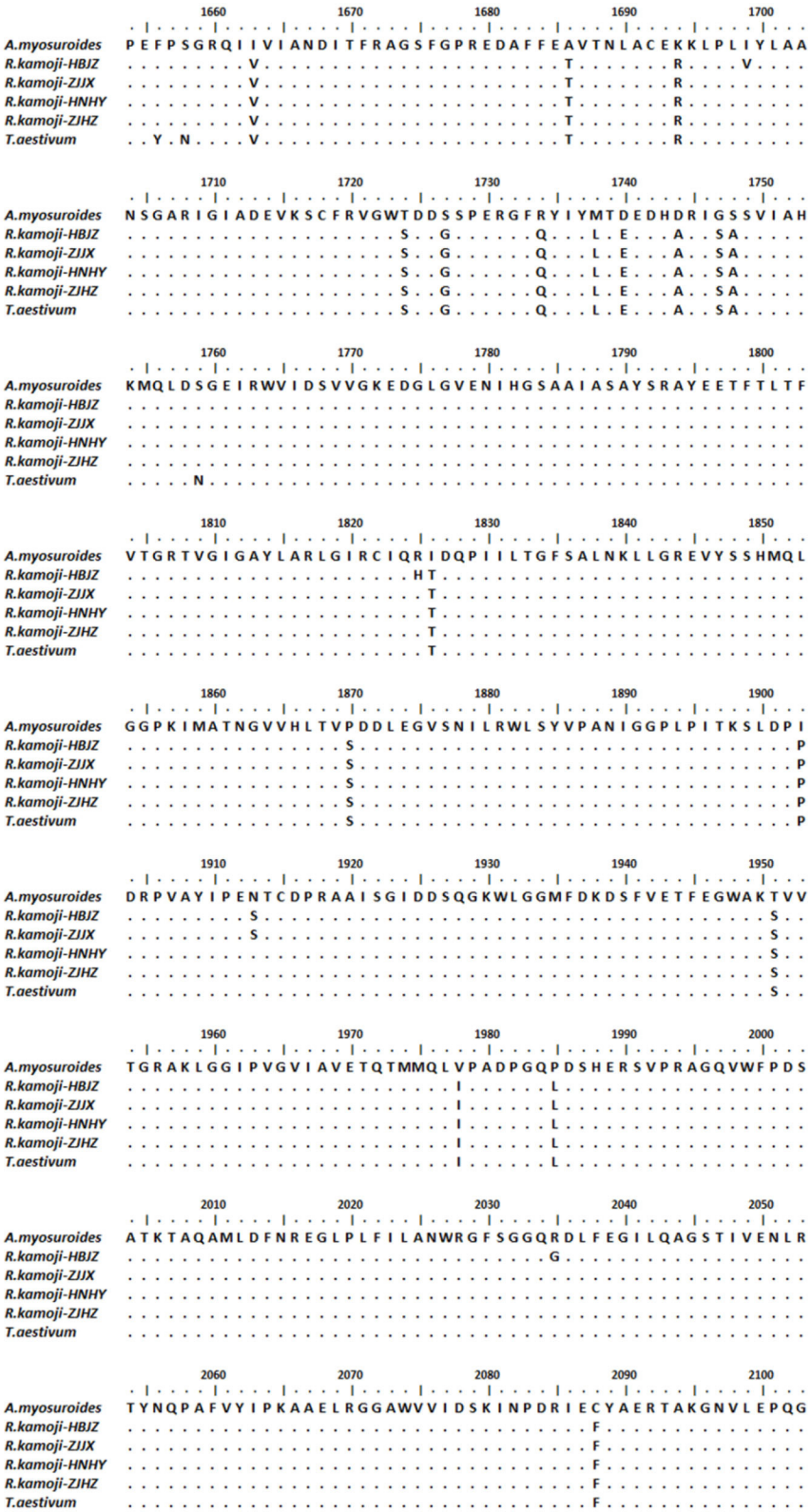

FIGURE 1 | Sequence alignment of a highly conserved region of ACCase gene from four common roegneria populations and blackgrass (*Alopecurus myosuroides* Huds., accession number: AJ310767).

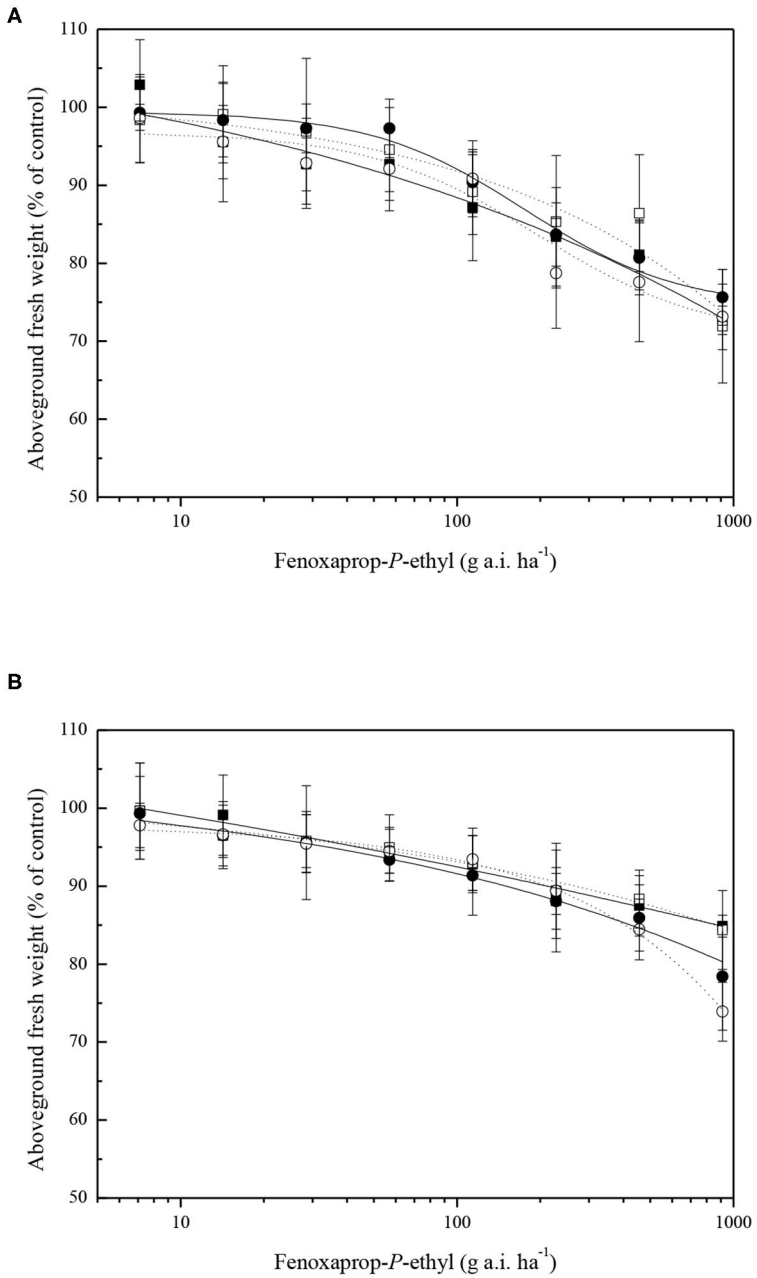

FIGURE 2 | Dose–response curves for fresh weight reduction of two common roegneria populations (ZJJX, ■ or □) and (ZJHZ, • or ○) treated with a range of fenoxaprop-*P*-ethyl doses plus (filled symbols) or minus (open symbols) 1,000 g ha^{-1} of malathion **(A)** and 80 g ha^{-1} NBD-Cl **(B)**. Vertical bars represent mean ± SE.

protein of common roegneria has 99% identity to wheat and 95% identity to *A. myosuroides* (**Figure 1**). Using BioEdit to compare the amino acid sequence of four common roegneria populations, *A. myosuroides*, and wheat, the results showed that some amino acids of common roegneria are different from wheat, but none of them were related to the reported resistant mutations. These results indicated that the tolerance to ACCase inhibitors in common roegneria populations may not be caused by TSR, but likely by NTSR.

The resistance pattern to ACCase inhibitors, caused both by TSR or NTSR mechanism, has proven to show special resistance to a class of ACCase inhibitors or resistant to all ACCase inhibitors. *Polypogon fugax* and *Lolium rigidum*, for instance, segregating the Ile-2041-Asn mutation, are only cross-resistant to APPs (Liu et al., 2007; Tang et al., 2014). Several *L. rigidum* populations were resistant to ACCase inhibitors by the enhancement of herbicide metabolism (Holtum et al., 1991; Neve and Powles, 2005; Manalil et al., 2012; Yu and Powles, 2014). In

this study, the common roegneria populations were determined only highly tolerant to APP herbicides applied in wheat and were susceptible to APP herbicides applied in other crops. In addition, no susceptible populations of common roegneria were identified; the tolerance pattern of this species is in complete accord with wheat and different to the reported resistant weed species.

Synergistic Effect of Malathion and NBD-Cl

Common roegneria plant responses to fenoxaprop-*P*-ethyl with or without malathion pretreatment were determined. Pretreatment with malathion showed no enhancement of the phytotoxicity of fenoxaprop-*P*-ethyl when sprayed on ZJJX and ZJHZ plants, respectively (**Figure 2A**). The ED_{50} values for fenoxaprop-*P*-ethyl treatments of ZJJX and ZJHZ plants were similar and all >16× LFR. Similarly, NBD-Cl did not enhance the herbicidal activity of fenoxaprop-*P*-ethyl when applied to ZJJX and ZJHZ populations (**Figure 2B**).

Pretreatment with malathion before herbicide application has long been used to detect whether CytP450 was involved in NTSR mechanism. Malathion can reverse the resistance to herbicides in some resistant weed species (Christopher et al., 1994; Feng et al., 2016; Zhao et al., 2017a). NBD-Cl, derivatives of which target GST in tumor cells, has proven enhanced the herbicidal activity of fenoxaprop-*P*-ethyl, clodinafop-propargyl and chlorotoluron (Ricci et al., 2005; Turella et al., 2006; Cummins et al., 2013). Instead, we propose that CytP450 or GST may be involved in the metabolic detoxification of fenoxaprop-*P*-ethyl in common roegneria. However, both malathion and NBD-Cl did not reverse the tolerance of common roegneria to fenoxaprop-*P*-ethyl. The results of the current study suggest that CytP450 or GST might play a different role in the ACCase inhibitors tolerance in common roegneria plants.

In vitro Assay of GST and ACCase Activities to Fenoxaprop-*P*-Ethyl

The results showed that in wheat plants without fenoxaprop-*P*-ethyl treatment, the GST activity 17.08 was U/min/mg leaf protein^{-1}, which was 4.50 and 3.53 times higher than that in ZJHZ and ZJJX common roegneria plants, respectively (**Figure 3**). After fenoxaprop-*P*-ethyl treatment, GST activity was significantly decreased in wheat at 24 HAT, but subsequently increased and reached its peak at 72 HAT. However, the activity of GST was not significantly affected in both ZJJX and ZJHZ populations with fenoxaprop-*P*-ethyl treatment, suggesting GST activity was more sensitive to fenoxaprop-*P*-ethyl in wheat than common roegneria. It is reported that relatively higher glutathione transferase activities in wheat and barley are responsible for resistance to fenoxaprop-*P*-ethyl (Romano et al., 1993; Cummins et al., 1997). In this study, GST activity in common roegneria populations is lower than that in wheat, but the GST activity was not affected with herbicide treatment, suggesting that GST may play a different role in the detoxification mechanism of fenoxaprop-*P*-ethyl in common roegneria as compared to wheat.

The ACCase activities of common roegneria populations were similar to wheat before fenoxaprop-*P*-ethyl spraying (**Figure 4**). The ACCase activities of the ZJJX and ZJHZ populations after fenoxaprop-*P*-ethyl treatment were 1.46- and 1.39-fold higher at 72 HAT than that in 0 HAT, respectively. The ACCase activity of wheat showed similar trend with the common roegneria populations after fenoxaprop-*P*-ethyl treatment. It indicated that ACCase activities of common roegneria and wheat were not inhibited after the application of fenoxaprop-*P*-ethyl; instead, the activity of the target enzymes was increased, which may have been caused by rapid metabolism of the herbicide.

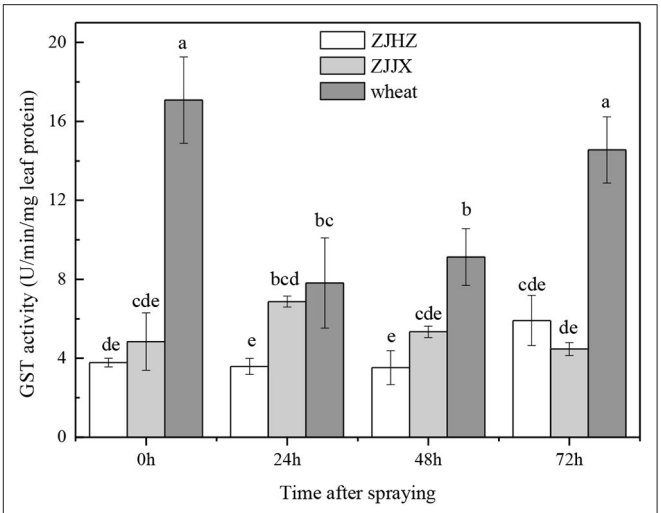

FIGURE 3 | GST activity in wheat and common roegneria populations ZJJX and ZJHZ at 0, 24, 48, and 72 h after fenoxaprop-*P*-ethyl treated. Bars are mean ± SE (n = 10). Bars designated by the same lowercase letter are not different according to Fisher protected LSD at $P \leq 0.05$.

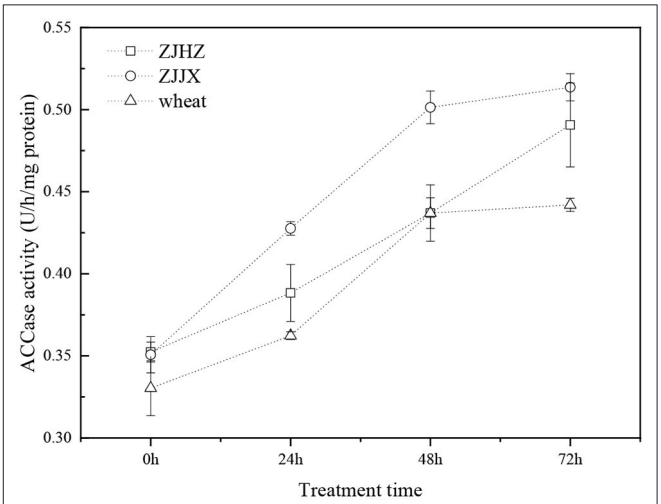

FIGURE 4 | ACCase activity in wheat and common roegneria populations ZJJX and ZJHZ at 0, 24, 48, and 72 h after fenoxaprop-*P*-ethyl treated. Data are the means ± SE from five replicates of two experiments (n = 10).

General Tolerance to ACCase Inhibitors

It has been reported in many weed species that resistance to APP herbicides is caused by the reduction in ACCase sensitivity.

For example, insensitive ACCases are responsible for resistance to ACCase inhibitors in *L. rigidum* (Cocker et al., 1999), *Avena fatua* (Seefeldt et al., 1996), *Avena sterilis* (Shukla et al., 1997), and *Echinochloa crus-galli* (Huan et al., 2013). However, in these cases, the presence of insensitive ACCases was related to mutations in the ACCase gene. Moreover, certain ACCase inhibitors can be used selectively in cereals because of the addition of safeners (e.g., mefenpyr-diethyl and clonquintocet-mexyl), which result in enhanced metabolization and detoxification of the herbicidal ingredients (Cataneo et al., 2013; Taylor et al., 2013). In this study, we determined that there were no reported mutations in the ACCase gene, and the ACCase activity was increased by the application of fenoxaprop-*P*-ethyl; therefore, it suggests that the increase in ACCase activity might have caused the tolerance.

Chang et al. (2011) investigated the leaf anatomical characteristics of six *Roegneria* species and indicated that anatomical structure was similar to *Triticeae* species. The phylogenic relationships between *Roegneria* and *Triticeae* species was analyzed, and results indicated that common roegneria was closely related to *Triticeae* (Zhang et al., 2014). This might explain the similar responses to ACCase inhibitors between common roegneria and wheat. In our recent study, the effects of flooding on seed survival were compared under field conditions, and results showed that seed germination and perennial rhizome regenerative ability decreased significantly after 60 days under flooded paddy soil than in dryland soil (data not shown). It is likely that the water-saving cropping practices (i.e., direct-seeding rice or no-tilling after wheat cropping in wheat-rice rotation system) adopted recently in the middle and lower regions of the Yangtze River might have accelerated the survival and spread of common roegneria.

In summary, common roegneria was tolerant to ACCase inhibitors applied in wheat, which have the potential to spread and develop into a serious weed in wheat or cereal crops based on chemical control of grass weeds. In addition, common roegneria is a perennial weed, which could reproduce both by seeds and rhizome and makes it difficult to eradicate. Rotating to other winter crops (i.e., oilseed rape) with rice, to allow the use of different herbicides, or adopting transplant rice after winter crops to ensure a relatively longer flooding period may help manage this weed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

WT and XY designed the research study and wrote the manuscript. WT, XY, JC, and LX performed the experimental work. WT carried out the data analysis. YL provided helpful suggestions during the experiments and preparation of the manuscript. All authors discussed the results of the study and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2020.587651/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.