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Confirmation and Characterization of the First Case of Acetolactate Synthase (ALS)-Inhibitor—Resistant Wild Buckwheat (*Polygonum convolvulus* L.) in the United States

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Abstract: Wild buckwheat (*Polygonum convolvulus* L.) is a problem weed and ALS-inhibitors (e.g., chlorsulfuron) are commonly used for its management. Recently, a population of wild buckwheat (KSW-R) uncontrolled with ALS-inhibitors was found in a wheat field in Kansas, USA. The objectives of this research were to determine the level and mechanism of resistance to chlorsulfuron and cross resistance to other ALS-inhibitors in the KSW-R population. In response to chlorsulfuron rates ranging from 0 to 16x (x = 18 g ai/ha), the KSW-R wild buckwheat was found >100-fold more resistant compared to a known ALS-inhibitor susceptible (KSW-S) wild buckwheat. Also, >90% of KSW-R plants survived field recommended rates of sulfonylurea but not imidazolinone family of ALS-inhibitors. A portion of the *ALS* gene covering all previously reported mutations known to bestow resistance to ALS-inhibitors was sequenced from both KSW-R and KSW-S plants. The Pro-197-Ser substitution that confers resistance to the sulfonylurea herbicides was found in KSW-R plants. Our results support the evolution of high level of chlorsulfuron resistance as a result of a mutation in the *ALS*-gene in KSW-R buckwheat. This is the first case of resistance to any herbicides in wild buckwheat in the US.

Keywords: chlorsulfuron-resistant buckwheat; herbicide resistance; target-site mutation

1. Introduction

Wild buckwheat (*Polygonum convolvulus* L.) is a summer annual weed, commonly found in small grain crops such as wheat (*Triticum aestivum*) and oats (*Avena sativa*). This weed was introduced into the US from Europe via grain transport and became a problem weed throughout the Great Plains, Northern Plains, Canada's Prairie provinces, and US Midwest. Wild buckwheat is a competitive weed that can cause yield losses of up to 66% in wheat. Apart from competing for nutrients, the vines of wild buckwheat can tangle and climb on the shoots of host plants which interferes in harvesting operations leading to lower yields and poor quality [1,2]. Furthermore, chemical control of wild buckwheat is a challenge in small grain crops, because some auxinic herbicides (e.g., 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenomyacetic acid (MCPA)), widely used for control of broadleaf weeds in cereal crops are not effective in controlling wild buckwheat [3]. Hence, control of wild buckwheat in these crops is largely dependent on the use of acetolactate synthase (ALS)-inhibiting herbicides.

ALS is one of the key enzymes involved in the biosynthesis of branched-chain amino acids (BCAAs) such as valine, leucine, and isoleucine [4]. Inhibition of the ALS enzyme leads to depletion of BCAAs and several secondary effects resulting in plant death [5]. ALS-inhibiting herbicides include five chemical families, viz., sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinyl-thio benzoates (PTB) and sulfonyl-aminocarbonyl-triazolinone (SCT). Since the commercialization of ALS-inhibitors in 1982, they are widely used for effective control of a broad spectrum of weeds compared to other commercial herbicides [6]. Because of extensive use, 165 weed species have evolved resistance to ALS-inhibitor herbicides [7].

Reduced sensitivity to ALS-inhibitors as a result of one or several mutations in the *ALS* gene, the target site of these herbicides is the most common resistance mechanism found in weeds [8]. A total of 29 amino acid substitutions at eight positions on the ALS protein (Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, and Gly-654, positions based on the *Arabidopsis thaliana* sequence Genbank accession NP_190425) that confer resistance to ALS-inhibitors have been reported in many weed species [7]. Enhanced metabolism of ALS-inhibitors by cytochrome P450 monooxygenases (CYPs) activity has also been reported in several ALS-inhibitor-resistant weeds [6], such as waterhemp (*Amaranthus tuberculatus*) [9], Palmer amaranth (*Amaranthus palmeri*) [10], rigid ryegrass (*Lolium rigidum*) [11], late watergrass (*Echinochloa oryzicola*) [12], and blackgrass (*Alopecurus myosuroides*) [13].

Small grain crops such as wheat, oat, and barley (*Hordeum vulgare*) are naturally tolerant to the SU family of ALS-inhibitors [14]. Specifically, SU herbicides chlorsulfuron, triasulfuron, and thifensulfuron are widely used for postemergence control of wild buckwheat in these crops [2]. As a result of repeated selection, the evolution of resistance to ALS-inhibitors was reported in wild buckwheat biotypes in Canada and Australia [7]. ALS-inhibitor-resistant wild buckwheat was first reported in Queensland, Australia for chlorsulfuron in 1993 [7] and later in 2007 in Alberta (Canada), for SU and TP herbicide families [15]. An amino acid substitution, Trp-574-Leu in the *ALS* gene, was found to bestow resistance in the wild buckwheat population from Alberta [15]. Herbicide-resistant wild buckwheat has not been documented in the US; however, recently a population of wild buckwheat (KSW-R) survived chlorsulfuron applications in a wheat field in Marion County in Kansas. The focus of this research was to confirm and characterize the chlorsulfuron resistance in KSW-R wild buckwheat. This research was based on the hypothesis that similar to ALS-inhibitor-resistant wild buckwheat from Canada, one or more mutations in the ALS gene may contribute to chlorsulfuron resistance in KSW-R wild buckwheat. The objectives of this research were to determine the level and mechanism of resistance to chlorsulfuron and cross resistance to other ALS-inhibitors in KSW-R wild buckwheat.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

A wild buckwheat biotype suspected to have evolved resistance to chlorsulfuron was collected from a winter wheat field in Marion County, Kansas in summer 2017. This population was designated as KSW-R and a biotype known to be susceptible to ALS-inhibitors, collected in Kansas (KSW-S) was also used in this study for comparison. The seeds of KSW-R and KSW-S wild buckwheat were planted in plastic trays (25 x 15 x 2.5 cm) with the commercial potting mixture (ProMix Ultimate, Premier Tech Horticulture, Mississauga, ON, Canada) and kept in 4 °C for 3–4 weeks for a cold treatment to break the dormancy and enhance germination. Later, the trays were moved to a greenhouse. Upon germination, at the 2–3 leaf stage, seedlings were transplanted in square pots (6 x 6 x 6 cm) and grown in a greenhouse maintained at 25/20 °C, 15/9 h a day/night photoperiod with a photosynthetic photon flux density of 750 µmol m^{-2} s^{-1} and relative humidity of 60 ± 10 percent. The plants were fertilized (Miracle GRO®All-purpose plant food, Scotts Miracle-Gro, Marysville, OH, USA) as needed. At the 4–5 leaf stage, KSW-R and KSW-S buckwheat plants were treated with chlorsulfuron (Glean® XP, Wilmington, DE, USA) at 36 g ai ha^{-1} (field recommended rate 1x = 18 g ai ha^{-1}) along with 0.25% nonionic surfactant (NIS) using a bench-type sprayer (Research Track Sprayer, Generation

III, De Vries Manufacturing, Hollandale, MN, USA) equipped with a flat-fan nozzle tip (80015LP TeeJet®tip, TeeJet Spraying Systems, Wheaton, IL, USA) delivering 168 L ha^{-1} at 222 kPa in a single pass at 4.8 km h^{-1}. KSW-R plants that survived the treatment with 36 g ai ha^{-1} of chlorsulfuron were transferred to individual pots and allowed to self-pollinate. Upon maturity, seeds were collected from the self-pollinated plants and used in the dose-response and *ALS* gene sequencing experiments.

2.2. Chlorsulfuron Dose-Response Assay

The KSW-R and KSW-S plants at the 3 to 4 leaf stage grown in square pots (6 x 6 x 6 cm) under greenhouse conditions as described above were used for the whole-plant dose-response assay. Six to ten plants of each KSW-S wild buckwheat treated with 0, 4.5, 9, 18 (field recommended rate), 36 g ai ha^{-1} and the KSW-R wild buckwheat were treated with 0, 9, 18, 36, 72, 144, and 288 g ai ha^{-1} of chlorsulfuron along with 0.25% NIS. The herbicide treatment was applied as described above. The above-ground dry biomass was collected at 3 weeks after treatment (WAT) and oven-dried at 60 °C for 72 h, and then the dry weight of biomass was recorded. The dose-response experiment was repeated following the same experimental procedure, herbicide treatments, and growth conditions.

2.3. Response to Different ALS-Inhibitors

In order to determine the response of KSW-R and KSW-S biotypes to different ALS-inhibitors (SU or IMI), twelve plants of KSW-R and KSW-S wild buckwheat at the 4–5 leaf stage were treated separately with field recommended rates of thifensulfuron, halosulfuron, nicosulfuron, and imazethapyr along with chlorsulfuron (Table 1). Plant survival was assessed at 3 WAT as per [10]. The experiment was repeated following the same growth conditions and methods as above.

Table 1. List of herbicides used in the study.

Herbicide	Trade Name	Chemical Family	Manufacturer	Field Rate
Glean	Chlorsulfuron	Sulfonylurea	DuPont Wilmington, DE http://cropprotection.dupont.com	18 g ai ha^{-1}
Permit	Halosulfuron	Sulfonylurea	Gowan Company, Yuma, AZ www.gowanco.com	36 g ai ha^{-1}
Harmony	Thifensulfuron	Sulfonylurea	DuPont Wilmington, DE http://cropprotection.dupont.com	36 g ai ha^{-1}
Accent	Nicosulfuron	Sulfonylurea	DuPont Wilmington, DE http://cropprotection.dupont.com	36 g ai ha^{-1}
Pursuit	Imazethapyr	Imidazolinone	BASF Corporation, Research Triangle Park, NC, USA	72 g ai ha^{-1}

2.4. ALS Gene Sequencing

To assess if any known mutations in the *ALS* gene of KSW-R wild buckwheat confer resistance to ALS-inhibitors, leaf tissue was collected from 15 KSW-R wild buckwheat plants that survived 288 g ai ha^{-1} of chlorsulfuron along with five non-treated individuals of KSW-S buckwheat. The genomic DNA (gDNA) was extracted using GeneJET™ Plant Genomic DNA Purification Mini Kit (Thermo Scientific™, Waltham, MA, USA) following the manufacturer's instructions. A wild buckwheat *ALS* gene sequence from a transcriptome assembly deposited at National Center for Biotechnology Information (NCBI) GenBank under the accession GIUI00000000 was used to design primers (*Pc*ALS_F: AGGGAGTCACCAACGTGTTC *Pc*ALS_R: TGGTAAAACCATACCCCCAGT; primer used for sequencing *Pc*ALS_F1: CATGCTGTTGAATAACCAGC) to amplify a portion of the *ALS* gene (~1.8 kb in length) that covers all the previously reported mutation sites. Polymerase Chain reaction (PCR) was performed using T100™ Thermal Cycler (Bio-Rad Inc., Hercules, CA, USA); a mixture containing 50–80 ng of gDNA, 0.5 μM of forward primer, reverse primer and Promega™ ready-to-use

PCR master mix with the following PCR conditions were used, initial denaturation 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s and a final extension at 72 °C 7 min. The PCR products were purified using GeneJET™ PCR Purification Kit (Thermo Scientific™, Waltham, MA, USA) and sequenced by Sanger sequencing platform (GENEWIZ Inc., South Plainfield, NJ, USA), and the sequences were aligned using MultAlin multiple sequence alignment tool (INRA, Paris, France). Further, the Sanger sequencing reads were assembled using the one-click assembly option of EGassembler with default parameters [16]; the assembled sequence contig was translated using the Translate tool—ExPASy [17]. The assembled sequences of wild buckwheat populations KSW-R and KSW-S were deposited at the NCBI GenBank.

2.5. Statistical Analysis

The plant biomass accumulation (in grams per plant) data was converted to percent dry aboveground biomass relative to the non-treated control of the respective, wild buckwheat biotype, i.e., KSW-S and KSW-R. The relationship between herbicide rate and biomass accumulation was estimated by non-linear regression analysis using a three-parameter log-logistic model (1). All the analyses were performed [18] using the 'drc' [19] package in R [20]. To assess the fit of data to various regression models, a "Lack-of-fit" test was performed using the "model fit" function of 'drc':

$$Y = \left\{ \frac{d}{1} + \exp\left[b(logx - loge) \right] \right\} \quad (1)$$

In the three-parameter log-logistic model Equation (1), Y is the response variable, d is the upper limit, b is the slope of the curve, and e is the GR_{50} which is the rate required for 50% reduction of plant biomass [21]. Analysis of variance (ANOVA) was performed following Fisher's LSD test used to separate means at $p \leq 0.05$ using the 'agricolae' package in R [22] to estimate the significant differences in percent dry biomass in response to different rates of chlorsulfuron.

3. Results and Discussion

3.1. Chlorsulfuron Dose-Response

The results of the dose-response assay confirmed a very high level of resistance to chlorsulfuron in KSW-R wild buckwheat. No significant differences were found between two runs of dose-response ($p < 0.05$). The KSW-R plants survived up to 16x (288 g ai ha^{-1}) of the field rate of chlorsulfuron. However, the KSW-S plants were heavily injured at 4.5, and 9 g ai ha^{-1} and completely killed at 18 g ai ha^{-1} of chlorsulfuron (Figure 1). The percent reduction in the above-ground biomass relative to non-treated control did not show a significant difference up to 72 g ai ha^{-1} ($p < 0.05$) rate of chlorsulfuron in the KSW-R; however, 16 and 25% reduction in biomass was found in 144 and 288 g ai ha^{-1} treated plants, respectively (Figure 2). The GR_{50} of KSW-R (703.74) was exponentially higher than KSW-S (3.96), indicating that the KSW-R population is >100-fold resistant to chlorsulfuron than the KSW-S wild buckwheat (Table 2). The GR_{50} of the KSW-R buckwheat was higher than the highest rate used (288 g ai ha^{-1}) in this study; and the biomass reduction of KSW-R buckwheat was <50% even at the highest rate (Figure 2). Previously, GR_{50} values that are more than the highest rate used in the experiments have been reported in several ALS-inhibitor-resistant weeds such as Palmer amaranth [10], henbit (*Lamium amplexicaule*) [23], and mouse barley (*Hordeum murinum)* [24]. Control of wild buckwheat in small grain crops, especially wheat, is primarily dependent upon ALS-inhibitors [2], and therefore, considerable selection pressure is expected in wheat producing regions. Resistance to ALS-inhibitor herbicides has been previously reported in a wild buckwheat population collected from wheat fields in Alberta (Canada) with 10–20-fold resistance to thifensulfuron/tribenuron and florasulam, respectively [15]. Similarly, a biotype of wild buckwheat from Queensland, Australia, also was found to have evolved resistance to chlorsulfuron [7], although the level of resistance or the mechanism of resistance to chlorsulfuron in the Australian biotype is not yet available.

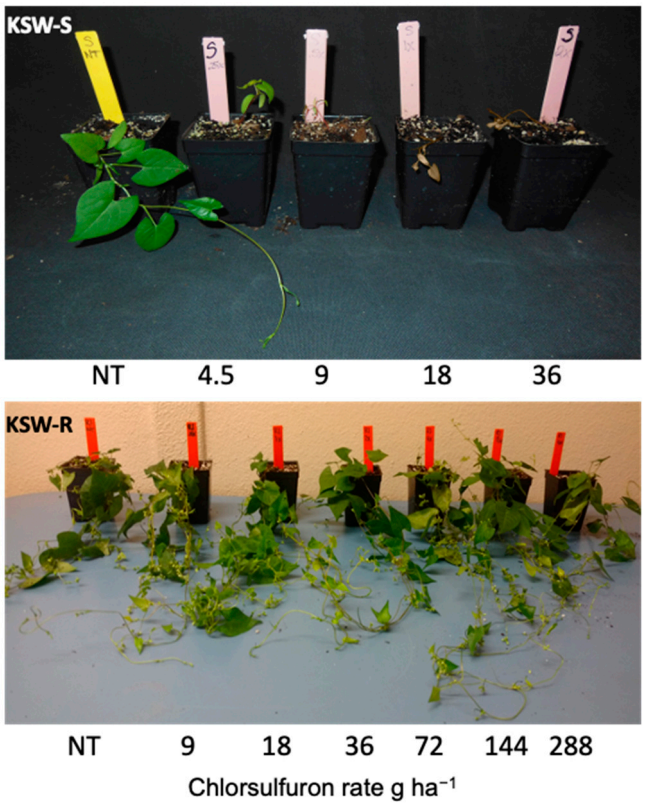

Figure 1. The whole-plant response of KSW-R (resistant) and KSW-S (susceptible) wild buckwheat populations to different rates of chlorsulfuron at 3 weeks after treatment.

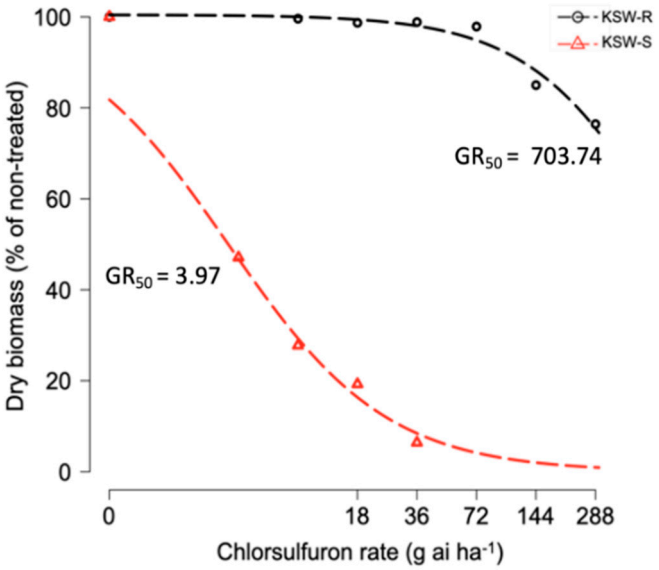

Figure 2. Chlorsulfuron dose-response curves obtained by three parameter log-logistic regression. $Y = \left\{\frac{d}{1} + \exp[b(logx - loge)]\right\}$ analysis of above-ground dry biomass of KSW-R (resistant) and KSW-S (susceptible) wild buckwheat at 3 weeks after treatment; GR_{50} herbicide rate required for 50% growth reduction.

Table 2. Regression parameters describing the response of wild buckwheat populations KSW-R (resistant) and KSW-S (susceptible) to chlorsulfuron; herbicide rate required for 50% growth reduction (GR_{50}), slope of the curve (b), upper limit (d) and, standard error (SE).

Genotype	GR_{50} (SE)	b (SE)	d (SE)
KSW-R	703.74 (333.11)	1.24 (0.55)	100.46 (3.0)
KSW-S	3.97 (0.79)	1.06 (0.21)	100.01 (4.80)

3.2. Response to Different ALS-Inhibitors

In addition to chlorsulfuron, KSW-R buckwheat survived field recommended rates of other SU herbicides, e.g., thifensulfuron, halosulfuron, and nicosulfuron, but did not survive the application of imazethapyr (IMI herbicide). The KSW-S plant did not survive the applications of the above herbicides (Table 3). Cross-resistance to different ALS-inhibitors has been previously reported to be associated with altered *ALS* gene or due to CYP based metabolism of ALS-inhibitors in weed species [25,26]. Cross-resistance endowed by altered *ALS* gene depends on type of amino acid substitutions and weed species. The single nucleotide polymorphisms in the *ALS* gene resulting in common amino acid substitution at the Pro-197 position confers a high level of resistance primarily to SU herbicides in many weed species [27,28]. Nonetheless, other amino acid substitution at the same position, for example, Pro-197-Ser bestows cross-resistance to SCTs in addition to SUs [29], while Pro-197-Leu substitution provides high or moderate level of cross-resistance to herbicides in different families of ALS-inhibitors [27]. Amino acid substitutions at Ala-122 or Ser-653 confer resistance to both IMI and SU herbicides, while Asp-376-Glu substitution provides resistance to all five chemical families and substitutions at Trp-574 confer resistance to IMIs, SUs, and TPs [27,30].

Table 3. Response of the KSW-R (resistant) and KSW-S (susceptible) wild buckwheat treated with different ALS inhibitors at their field rates at 3 weeks after treatment.

Trade Name	Field Rate	% Survival [a]	
		KSW-R	KSW-S
Chlorsulfuron	18 g ai ha^{-1}	100%	0%
Halosulfuron	36 g ai ha^{-1}	100%	0%
Thifensulfuron	36 g ai ha^{-1}	90%	0%
Nicosulfuron	36 g ai ha^{-1}	90%	0%
Imazethapyr	72 g ai ha^{-1}	0%	0%

[a] A total of 12 plants were treated and the number of plants that survived was expressed as the percent (%) survival.

3.3. Molecular Basis of ALS-Inhibitor Resistance in KSW-R Wild Buckwheat

A high level of resistance to chlorsulfuron in KSW-R wild buckwheat (Table 2) indicates the possible presence of one or more previously reported mutations in the *ALS* gene. A portion of the *ALS* gene from 15 KSW-R and 5 KSW-S wild buckwheat individuals was sequenced. The amplified region includes all eight previously reported amino acid positions, i.e., Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, and Gly-654 [27]. A single nucleotide polymorphism (C/T)CC resulting in amino acid substitution at position 197 from proline (CCC) to serine (TCC) was found in all the KSW-R but not in KSW-S wild buckwheat plants (Figure 3). These results suggest that alteration in the *ALS* contributes to chlorsulfuron resistance in KSW-R wild buckwheat. Mutations in the *ALS* gene resulting in amino acid substitution is the most common mechanism of resistance to ALS-inhibitors in weed species [6]. All the 15 KSW-R buckwheat plants sequenced were homozygous for the Pro-197-Ser mutation. A high level of resistance to chlorsulfuron conferred by Pro-197-Ser substitution was previously reported in Palmer amaranth [10] and wild radish (*Raphanus raphanistrum)* [31]. However, in ALS-inhibitor-resistant wild buckwheat from Canada, an amino acid substitution, $Trp_{574}Leu$ in the *ALS* gene was found and this mutation also was shown to confer resistance to SU and TP herbicides [15].

Although a wild buckwheat population resistant to chlorsulfuron was reported from Queensland (Australia), the mechanism of resistance has not been identified.

Position	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207
Amino acid	P	L	V	A	I	T	G	Q	V	P/S	R	R	M	I	G	T	D	A	F	Q
KSW-S_1	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	CCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-S_2	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	CCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-S_3	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	CCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-S_4	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	CCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-S_5	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	CCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_1	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_2	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_3	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_4	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_5	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_6	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_7	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_8	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_9	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_10	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_11	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_12	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_13	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_14	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG
KSW-R_15	CCC	CTC	GTT	GCC	ATC	ACC	GGC	CAG	GTC	TCC	CGC	CGC	ATG	ATC	GGC	ACT	GAC	GCC	TTC	CAG

Figure 3. Nucleotide sequence alignment and analysis of a portion of the *ALS* gene sequence from KSW-R (15 resistant plants) and KSW-S (5 susceptible plants) wild buckwheat. Nucleotide/amino acid numbering refers to the *Arabidopsis thaliana ALS* gene sequence.

Since the Pro-197-Ser substitution is specific for resistance to SU and SCT herbicides in most weed species [27], the KSW-R wild buckwheat was found resistant to only SUs but not the IMI herbicides (Table 3). The evolution of target-site resistance leading to cross-resistance within the single group of herbicides essentially depends on the ALS protein structure and the amino acid domains on the protein. The ALS protein in higher plants generally will have five highly conserved domains, namely, A, B, C, D, and E [31]. The domain A located in the N-terminal end consists of 13 amino acids including the Pro-197 [32]. Any mutations in domain A largely confer resistance to SU and/or TP, but moderate or no resistance to IMIs [27]. The SU herbicides interact with more amino acid residues compared to IMIs due to the difference in binding pockets making, SU resistance most common to several substitutions [29].

The coexistence of both target and non-target site resistance mechanisms such as altered *ALS* gene and enhanced metabolism of ALS-inhibitors in the same weed species has been reported [10,33,34]. Although altered *ALS* gene is known to confer a high level of resistance [28], enhanced metabolism also can bestow a high level of resistance in some weed species [10,35,36]. The presence of enhanced metabolism or other non-target site mechanisms such as reduced absorption or translocation also need to be tested in the KSW-R wild buckwheat to rule out contribution of non-target resistance mechanism to ALS-inhibitor resistance in this weed.

4. Conclusions

In conclusion, weed control in small grain crops such as wheat, oats, and barley are highly dependent on use of ALS-inhibitors. However, due to selection, several summer annual weeds, including wild buckwheat evolved resistance to these herbicides, which is a challenge for effective and efficient weed management. The evolution of resistance to SU herbicides in KSW-R is the first case of herbicide resistance in wild buckwheat in the US. Weed management strategies such as reducing the selection pressure by rotating herbicides with different modes of action, developing effective pre-emergence programs, use of weed-free crop seeds s and other integrated management techniques along with improved stewardship need to be followed to reduce further spread and evolution of weed resistance.

Author Contributions: M.J. conceived research hypothesis, methodology, led and supervised the research; B.A.P. and A.F. conducted the research and collected data; M.L. assisted with sequence analysis; D.E.P. identified and provided KSW-R and KSW-S wild buckwheat seed and P.V.V.P. co-supervised and partially supported funding of B.A.P. All authors have read and agreed to the published version of the manuscript.

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