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First report of *Vaccaria* sp. from Iran exhibiting cross-resistance to acetolactate synthase inhibitors in wheat fields

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Abstract

The spread of weed resistance to herbicides threatens both Iranian and global agriculture. In 2019, a study was conducted to ascertain the resistance of *Vaccaria* spp. to conventional herbicides used in wheat fields. The study employed a completely randomized design, which included five treatments and five replications. The 25 biotypes were collected from 16 cities across five provinces through a national call. To assess the resistance of these biotypes, herbicide treatments, including iodosulfuron + mesosulfuron (Atlantis), tribenuron-methyl (Granstar), 2,4-D + MCPA (U46 Combi-Fluid), bromoxynil + MCPA (Bromicide MA), and iodosulfuron + mesosulfuron + diflufenican (Othello), were applied at the recommended dose per hectare according to the Moss and Adkins evaluation method. Subsequently, a population distribution map was created using ArcGIS Pro software, categorizing biotypes into three groups: resistant, developed resistant, and sensitive to herbicides. The results indicated that seven biotypes (28%) were resistant to Granstar herbicide, two biotypes (8%) to Atlantis herbicide, and one biotype (4%) was resistant to Othello herbicide. Additionally, three biotypes (12%) were classified in the developed resistant group for Granstar herbicide, one biotype (4%) for Atlantis herbicide, and two biotypes (8%) for Othello herbicide. Notably, based on the evaluation criteria of Adkins and Moss, all collected biotypes were sensitive to the two herbicides, U46 Cambi Floyd and Bromicid MA.

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Introduction

Wheat is one of the most crucial agricultural crops, and plays a significant role in food security. This crop is the most important grain across the world, providing more than 20% of the calories and protein in the human diet, and is the staple food for more than 40% of the world's population (Giraldo et al., 2019). In Iran, wheat is the primary source of protein and calories, supplying 45% of the protein and approximately 55% of the required calories for the country's population (Mohammadi et al., 2017). With 352 reported cases of herbicide resistance, wheat constitutes a significant portion of herbicide-resistant weeds among crop plants (Heap, 2023). The yield reduction of this essential crop due to weeds has been a subject of concern for a long time. On average, weeds globally cause a 34% reduction yield in wheat. *Vaccaria hispanica* L. is an annual plant belonging to the family *Caryophyllaceae* that competes intensely with wheat to obtain nutrients, water, and light. Today, herbicides are widely used as a primary tool for weed control in most parts of the world. Over the past half-century, herbicide-based weed management methods have been predominant in the most developed countries. The development of resistant weeds is an unintended consequence of the repeated and successive application of one or more herbicides from a specific herbicide group (Pacanoski, 2017). The issue of weed resistance to herbicides was first raised as early as 1957. Hence, today it has become a major problem and challenge in weed management and threatens global food security (Pacanoski, 2017). Weed resistance has increased sharply since 1975. Currently, there are 518 distinct herbicide-resistant weed (species * site of action) combinations globally, involving 251 species (146 dicotyledons and 105 monocotyledons) across more than one million fields worldwide (Heap, 2023). Herbicide-resistant weeds have been identified in 90 crops across 66 countries (Heap, 2023). Multiple reports have documented the resistance to ALS-inhibiting herbicides in various countries. To date, 172 cases of weed resistance to ALS-inhibiting herbicides reported (Heap, 2024). Based on this, weed resistance to ALS-inhibiting herbicides in wheat fields has constituted a significant proportion of resistance cases, meriting considerable attention. Numerous researchers have investigated the weed resistance status in agroecosystems (Heap, 2022).

In Western Australia in 1982, the resistance of annual ryegrass (*Lolium rigidum* L.) to the herbicides chlorosulfuron, diclofop-methyl, fluazifop-butyl, sethoxydim, and tralkoxydim has been confirmed (Llewellyn & Powles, 2001). Letouzé and Gasquez (1999) reported that biotypes of foxtail weed (*Alopecurus myosuroides* Huds.) and ryegrass collected from cereal fields in France were resistant to the herbicides fenoxaprop-P-ethyl and diclofop-methyl. Additionally, the results showed that four wild oat ecotypes collected from Canadian farms indicated resistance to Acetolactate Synthase (ALS) inhibitors and eight ecotypes indicated multiple resistance to ALS and ACCase inhibitor herbicides (Beckie et al., 2012). The occurrence of resistance in wild oat populations to ALS inhibitors from American wheat fields has also been reported (Keith et al., 2015). To date, the resistance of the weed *Vaccaria hispanica* L. to ALS inhibiting herbicides (metsulfuron-methyl, thifensulfuron-methyl, tribenuron-methyl) has been reported solely in Canadian wheat fields as of 2012 (Heap, 2024). For the first time in Iran, multiple resistance of wild oat weed (*Avena* sp.) to ACCase and ALS inhibitor herbicides has been reported in wheat fields of Khuzestan province in 2010 (Heap, 2018). Additionally, a second report of multiple resistance in *Galium aparine* weed to two groups of herbicides (including ACCase inhibitors and pseudo-auxins) from wheat fields was documented in 2017 (Heap, 2018). Over the past two decades, the use of ALS inhibitor herbicides have become a common approach for controlling narrow-leaved weeds in wheat and canola fields (Zand et al., 2019).

Based on the research conducted until 2018, sixteen weed biotypes from nine different species across four provinces in Iran have demonstrated resistance to various herbicides. Among those, seven biotypes are resistant to ACCase inhibitors, and five biotypes are resistant to ALS inhibitors. One biotype exhibits multiple resistance to both ACCase and ALS inhibitors, and another biotype is resistant to PSII inhibitors. Additionally, there is a biotype with multiple resistance that has shown resistance to both auxin-like herbicidal groups (such as 2,4-D and MCPA) and ALS inhibitors (Heap, 2018).

Investigations indicate that over the last two decades, there has been a significant increase in the use of various herbicides to control broadleaf weeds in wheat fields. The extensive use of herbicides in wheat fields and farmers' dissatisfaction with the effectiveness of common herbicides on *Vaccaria* spp. weeds have raised concerns regarding the resistance of this species. Therefore, the primary objective of this study was to determine the resistance status of *Vaccaria* spp. weeds to commonly used herbicides in the country's wheat fields, and the secondary objective was to prepare a distribution map of *Vaccaria* spp. resistant to the aforementioned herbicides.

Materials and Methods

To evaluate the resistance of Vaccaria weed during a national recall in 2017, seeds of suspicious biotypes from five provinces, including Ilam (11 ecotypes), Markazi Province (seven ecotypes), Lorestan (four ecotypes), Kermanshah (two ecotypes), and West Azerbaijan (one ecotype), were collected. In total, 25 suspected biotypes were collected from 25 fields across 15 cities: Hersin (HE), Mahi Dasht (MA), Urmia (OR), Al-Shatter (AL), Borujerd (BR), Salsleh (SE), Tafresh (TA), Khandab (KH), Saveh (SA), Shazand (SH), Farahan (FA), Abdanan (AB), Badra (BA), Dareh Shahr (DA), Malek Shahi (ML), and Ilam (IL) according to the W method. After collection, the seeds were stored at -16 °C until carrying out the screening experiments.

Breaking Seed Dormancy and Seed Germination

To conduct the greenhouse experiments, it was essential to overcome seed dormancy to achieve uniform germination and seedling establishment. To break seed dormancy and stimulate germination of *Vaccaria* spp. weed, seeds were exposed to the alternating temperature technique including low temperatures (5 and 7.5 °C) followed by high temperature (20 °C) (Duddu & Shirtliffe, 2014). Thirty seeds of each biotype were placed in Petri dishes containing Whatman No. 1 filter paper. To prevent contamination and maintain seed viability, the Petri dishes were disinfected with a 1% sodium hypochlorite solution for three minutes and then rinsed with distilled water. Additionally, to minimize evaporation and maintain humidity, the Petri dish lids were sealed with parafilm and maintain in the germinator under a 14/25 °C day/night temperature regime until the germination of seeds.

After the roots have emerged (visible at 2 mm length), the seedlings were transferred to plastic pots containing a mixture of clay, sand, field soil, and rotted manure in a 1:1:1:1 ratio. Twelve healthy seedlings having sound roots, were planted in each pot. The seedlings were covered with a 1-centimeter layer of soft soil to prevent root desiccation. Four uniform seedlings were kept per pot for the screening test.

Screening and Bioassay Tests

The screening test was conducted using a bioassay method in the pot (the whole plant survey) as a completely randomized design including five treatments, with five replications for each biotype, in the Plant Protection Research Institute of Iran. The aim of this test was to test a large number of samples within each biotype to identify resistant plants. Treatments were applied to the five to six-leaf stages of *Vaccaria* sp. plants (Table 1) using a backpack spray equipped with a T-jet flat fan nozzle EVS 8002, capable of delivering 200 liters per hectare at a pressure of 250 kPa.

Table 1. Characteristics of the treatments used in the experiment

Common name	Formulation Trade Name	Group[b]	MOA	Field rate (g a.i. ha^{-1})	Company Name	Registration year in Iran
Iodosulfuron+Mesosulfuron +Mefenpyr diethyl	Atlantis/OD1.2%	2	ALS	1/5 Liter	Bayer	2008
Iodosulfuron+Mesosulfuron + diflufenican+Mefenpyr diethyl	Othello/OD 6%	2+12	ALS / synthesis of cartenoids	1/6 Liter	Bayer	2014
Teribenuron methyl	Granstar/DF75%	2	ALS	25 grams	Ron Plank and DuPont	1990
2,4-D+MCPA	U46Combi Floid/SL 67.5%	4	Synthetic auxins	1/5 Liter	Nufarm	1968
Bromoxynil+MCPA	Bromicide MA/ EC40%	6+4	PSII and Synthetic auxins	1/5 Liter	Nufarm	2002

a FT: OD, water dispersible, DF, dry flowable; SL, soluble (liquid) concentrate; and EC, emulsifiable concentrate.
bWSSA, Weed Science Society of America and HRAC, Herbicide Resistance Action Committee.
cMOA: PSII, photosystem II inhibitor; ALS, acetolactate synthase;
SA, synthetic auxins, and CB, Carotenoid biosynthesis inhibitors inhibitor.

Data Collection

Four weeks after the application of treatments, the number of living plants in each pot was counted and recorded. To determine the fresh weight, the plants from each pot were removed from the soil surface and weighed using a digital scale with an accuracy of 0.01 grams. The collected samples were then dried for 48 hours in an oven at 75°C, and the percentage of dry weight of each biotype was calculated relative to the control. To assess the resistance status of the studied biotypes, two methods were utilized according to Adkins et al. (1997) and Moss et al. (2007). Based on the evaluation method of Adkins et al. (1997), after four weeks, a biotype is classified as resistant if both the dry weight percentage and survival rate exceed 80% compared to the control. Biotypes are classified as probably resistant (PR) and resistant (R) if both indicators are at least 50% and more than 50%, respectively. If both indicators are below 50%, the biotype is classified as sensitive (S) to the herbicide.

According to the methodology of Moss et al. (2007), biotypes are classified based on their percentage of weight loss as follows: 0-36% as resistant (RRR), 36-72% as probably resistant (RR), 72-81% as suspected resistant (R?), and 81-100% as sensitive (S) to the herbicide. Furthermore, by integrating the Adkins et al. (1997) and Moss et al. (2007) methods, biotypes were classified as resistant or possibly resistant according to Moss and Adkins evaluations, were grouped as resistant biotypes. Suspected biotypes of resistance were classified in the resistance development group (D).

Preparation of a Distribution Map

To prepare a distribution map of the herbicide-resistant weeds, the coordinates of each biotype was recorded by the GIS device. Finally, the distribution map of the resistant and sensitive biotypes is generated using ArcGIS Pro software based on the results of the screening tests.

Results

According to the integration of the Adkins et al. (1997) and Moss et al. (2007) methods, the results indicated that seven biotypes (28%) to the Granstar herbicide, 2 ecotypes (8%) to the Atlantis herbicide, and one ecotype (4%) to the Othello herbicide were classified in the resistant group (Table 2, Fig. 1). Additionally, the research revealed that three biotypes (12%) related to the Granstar herbicide, one biotype (4%) to the Atlantis herbicide, and two biotypes (8%) to the Othello herbicide were included in the resistant development group (Table 2, Fig. 1).

Moreover, the research showed that three biotypes (12%) were to Granstar herbicide, one biotype (4%) was related to Atlantis herbicide, and two biotypes (8%) were to Othello herbicide, all of which were classified in the resistant development group (Table 2 and Fig. 1). Fortunately, based on the assessment by Adkins et al. (1997) and Moss et al. (2007) methods, all the collected ecotypes were categorized in the sensitive group for the herbicides U46 Cambiloid and Bromycide MA (due to the sensitivity of all the examined samples, the data table was not displayed).

Kermanshah Province

Two suspected biotypes of herbicide resistance were collected from Harsin and Mahi Dasht counties. According to the evaluation of Adkins et al. (1997) and Moss et al. (2007) methods, the proportion of the MA1 biotype relative to Atlantis herbicide was classified in the resistant group, while its response to four other herbicides including Othello, Granstar, U46 CombiFloid, and Bromicide MA was classified in the sensitive group.

West Azerbaijan and Lorestan Provinces

One biotype of OR1 was collected from West Azarbaijan Province and three biotypes of AL1, BR1, and SE1 were collected from Lorestan province. Fortunately, the screening results according to Adkins et al. (1997) and Moss et al. (2007) methods indicated the sensitivity of all four biotypes to all five herbicides U46 CombiFloid, Atlantis, Bromicide MA, Granstar, and Othello. As mentioned, bromicide-MA herbicide is a mixture of two groups of auxin herbicides and photosystem II inhibitors (Table 1).

Markazi Province

The screening results in this region indicated that the biotypes TA1, KH1, and SH1, according to Adkins et al. (1997) and Moss et al. (2007) methods, fell into the susceptible group to all five herbicides including U 46 Combi Fluid, Atlantis, Bromicide MA, Granstar, and Othello. However, the FA1 biotype was classified as resistant to the herbicide Atlantis and susceptible to the other herbicides. Additionally, the SA1 and SA2 biotypes were classified as the resistant and the developed resistance to Granstar, respectively, while both biotypes were placed in the susceptible group for the herbicides U 46 Combi Fluid, Atlantis, Bromicide MA, and Othello. Based on the results, it can be stated that resistance to the herbicide Atello has started in the FA2 biotype in Fars Province.

According to the assessment by Adkins et al. (1997) and Moss et al. (2007) methods, all biotypes were categorized as susceptible to two herbicides, U 46 Kombi Fluid and Bromicide MA (data not shown). Additionally, biotypes BA3, AB2, and IL3 were classified as susceptible to three herbicides- Atlantis, Othello, and Granstar- according to Adkins and Maas's evaluation. However, based on Adkins et al. (1997) and Moss et al. (2007) methods, the AB1 biotype exhibited evolved resistance to Othello, BA1 and BA2 biotypes to Granstar, and the DA1 biotype to Atlantis. Furthermore, biotypes DA1, DA2, IL1, and IL2 were deemed potentially resistant to Granstar as Adkins et al. (1997) and Moss et al. (2007) methods, while the ML1 biotype was considered potentially resistant to both Othello and Granstar. It is recommended to avoid the use of the herbicides Othello and Granstar in the specified field to prevent the development of cross-resistance.

Table 2. The status of resistance in the *Vaccaria* sp. biotypes collected from different provines to the herbicide Atlantis, Othello and Granstar in wheat using Adkins et al. (1997) and Moss et al. (2007) evulation methods

Province	Biotype	Iodosulfuron+Mesosulfuron+Mefenpyr diethyl (Atlantis)						Iodosulfuron+Mesosulfuron+ diflufenican+Mefenpyr diethyl (Othello)						Teribenuron methyl (Granstar)					
		percentage compared to the control			Resistance rating system		Status of Resistance**	percentage compared to the control			Resistance rating system		Status of Resistance**	percentage compared to the control			Resistance rating system *		Status of Resistance**
	Cow cockle	survival	Dry weight	Wet weight loss	Adkins	Moss		survival	Dry weight	Wet weight loss	Adkins	Moss		survival	Dry weight	Wet weight loss	Adkins	ماس Moss	
Kermanshah	HE1	00.00	27.50	95.65	S	S	S	00.00	31.00	98.00	S	S	S	88.21	75.32	65.41	RP	RR	R
	MA1	72.00	76.65	44.00	RP	RR	R	00.00	24.65	94.32	S	S	S	00.00	21.30	94.31	S	S	S
West Azarbaijan	OR1	00.00	15.78	95.41	S	S	S	15.32	32.10	95.00	S	S	S	00.00	31.00	97.65	S	S	S
Lorestan	AL1	00.00	26.41	94.25	S	S	S	00.00	20.00	94.00	S	S	S	13.32	31.21	96.74	S	S	S
	AL2	10.41	21.42	90.34	S	S	S	00.00	15.32	99.41	S	S	S	00.00	31.00	95.96	S	S	S
	BR1	00.00	19.21	92.35	S	S	S	12.31	28.00	98.00	S	S	S	17.62	23.00	98.35	S	S	S
	SE1	00.00	21.31	97.12	S	S	S	00.00	19.00	98.00	S	S	S	21.60	34.52	91.65	S	S	S
Markazi	TA1	08.32	19.50	94.75	S	S	S	00.00	22.00	97.89	S	S	S	00.00	30.00	90.64	S	S	S
	KH1	00.00	27.52	99.52	S	S	S	05.32	21.42	99.32	S	S	S	07.62	21.00	94.00	S	S	S
	SA1	07.54	29.41	98.12	S	S	S	00.00	31.41	100.00	S	S	S	83.00	76.65	44.00	RP	RR	R
	SA2	00.00	24.41	98.45	S	S	S	00.00	31.00	100.00	S	S	S	00.00	21.00	75.32.	S	R?	D
	SH1	00.00	25.32	94.75	S	S	S	03.21	21.00	98.00	S	S	S	00.00	24.85	98.32	S	S	S
	FA1	70.00	68.41	39.00	RP	RR	R	00.00	25.30	96.00	S	S	S	00.00	31.00	97.62	S	S	S
	FA2	00.00	23.52	94.26	S	S	S	00.00	28.00	74.85	S	R?	D	14.32	28.50	96.34	S	S	S
Ilam	AB1	00.00	27.65	99.41	S	S	S	00.00	16.20	75.00	S	R?	D	00.00	35.52	99.32	S	S	S
	AB2	00.00	19.45	94.65	S	S	S	00.00	24.12	96.20	S	S	S	00.00	34.00	98.00	S	S	S
	BA1	09.45	28.35	96.32	S	S	S	13.41	28.00	97.00	S	S	S	10.20	31.00	79.12	S	R?	D
	BA2	00.00	24.65	91.36	S	S	S	00.00	24.12	98.23	S	S	S	00.00	21.00	76.50	S	R?	D
	BA3	00.00	29.52	98.42	S	S	S	00.00	19.45	96.45	S	S	S	00.00	38.21	95.00	S	S	S
	DA1	06.23	26.65	97.65	S	R?	D	00.00	20.00	100.00	S	S	S	91.00	72.30	60.24	RP	RR	R

	DA2	00.00	34.12	97.42	S	S	S	07.22	21.00	98.00	S	S	S	92.41	70.68	65.48	RP	RR	R
	ML1	00.00	18.45	97.25	S	S	S	80.42	68.20	40.12	RP	RR	R	91.21	70.65	68.65	RP	RR	R
	IL1	00.00	28.12	97.24	S	S	S	00.00	24.58	95.65	S	S	S	91.31	75.65	65.24	RP	RR	R
	IL2	11.32	27.65	98.61	S	S	S	05.98	30.25	97.65	S	S	S	91.54	72.32	56.87	RP	RR	R
	IL3	00.00	30.00	97.65	S	S	S	00.00	30.00	98.32	S	S	S	00.00	19.32	99.68	S	S	S

*According to the Adkins et al. (1997) method, the biotypes dry weight and survival compared to the control are more than 80 and 50% (R), at least 50 and more than 50% (PR), respectively, and if both are less than 50% (S). **According to the Moss et al. (2007) method, the biotypes fresh weight reduction compared to the control are between zero-36 (RRR). Between 36 to 72 (RR), between 72 to 81 (R?) and between 81 to 100% (S).

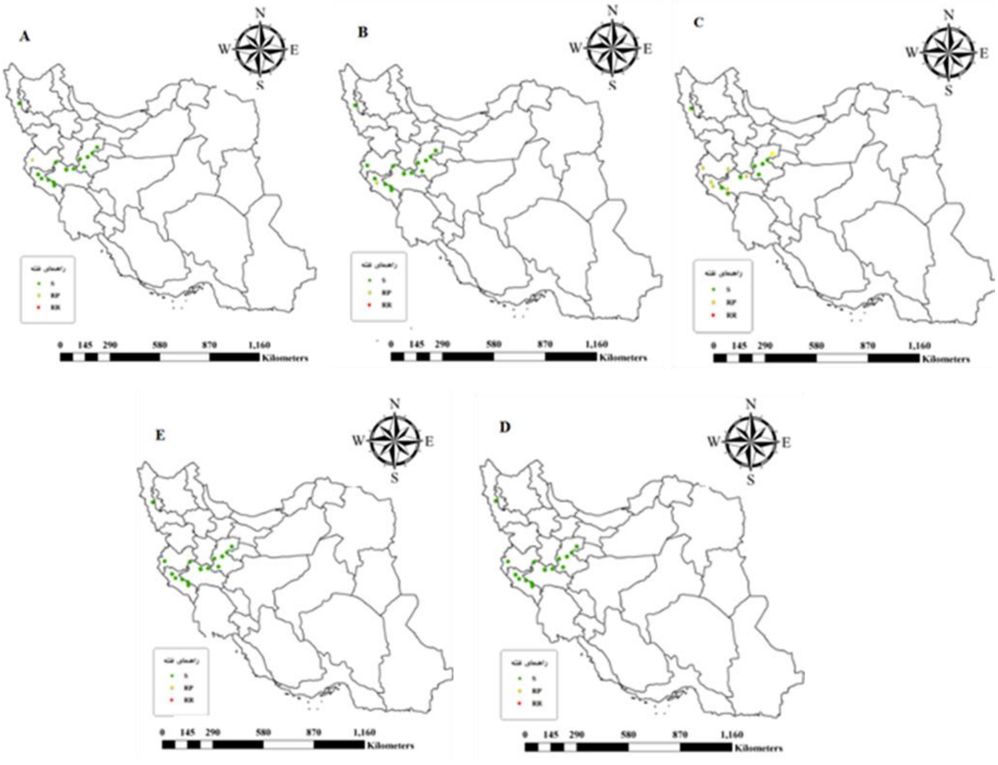

Fig. 1. Mapping the distribution of herbicide-resistant *Vaccaria* sp. following five herbicides, A) mesosulfuron + iodosulfuron (MI), B) mesosulfuron + iodosulfuron + diflufenican (MD), C) tribenuron-methyl (TM), D) 24D+ MCPA (DM) and D) bromoxynil + MCPA (BM), according to integration of the Adkins et al. (1997) and Moss et al. (2007) assessment.

Discussion

In a similar study conducted in Iran using Adkins et al. (1997) and Moss et al. (2007) methods, 18 and 3 biotypes of wild oat (Avena sterilis subsp. ludoviciana) from wheat fields in Khuzestan province were found to be resistant to Iodosulfuron+Mesosulfuron+Mefenpyr diethyl (Atlantis) and Iodosulfuron+Mesosulfuron+ diflufenican+Mefenpyr diethyl (Othello) herbicides, respectively (Joumi et al., 2022). ALS-inhibiting herbicides are highly popular among farmers. The significant usage of ALS-inhibiting herbicides is attributed to factors such as low application rates, favorable environmental characteristics, low toxicity to animals, broad selectivity in agricultural plants, as well as high efficacy (Aghajani et al., 2009). However, due to the presence of primary resistance genes, the failure of ALS-inhibiting herbicides to bind to the catalytic site of the target enzyme, and the potential for diverse mutations in different regions of the ALS gene, resulting in amino acid substitutions at various positions (Tranel & Wright, 2002). Therefore, there is substantial resistance pressure on these herbicides. Consequently, their continuous use accelerates the development of resistance.

ALS-inhibiting herbicides are among those with a high risk of resistance development. Resistance to these herbicides was reported five years after the application of chlorosulfuron, in wild lettuce (Lactuca serriola) (Mallory-Smith et al., 1990) and kochia (Kochia scoparia) (Primiani et al., 1990). In Iran, the first report of ALS inhibitor resistance in wild mustard (Sinapis arvensis L.) from wheat fields in Golestan Province was reported in 2009 (Heap, 2022). This occurred shortly after the registration and usage of this group of herbicides in Iran. The frequent use of ALS inhibitors and the lack of rotation with other herbicide groups are identified as the primary reasons of the occurrence of resistance.

Regarding the reason for less resistance in these auxin herbicides, it has been pointed out that auxin herbicides are usually foliar-applied and easily degradable and have less selective pressures for the evolution of weed resistance (Cobb & Reade, 2011). Thus, the reduced adoption by farmers and the lower resistance risk of this herbicide group compared to ALS inhibitors have delayed the development of resistance in weeds to this herbicide group. Implementing appropriate planning (especially herbicide rotation and mixing) and continuous field monitoring to assess the effectiveness of applied management strategies are crucial to preventing the early onset of resistance (Moss et al., 2007). Otherwise, the occurrence of resistance is inevitable. For example, in Serbia, a small country with less than three million hectares of agricultural land, weed resistance has been confirmed over the past 15 years. Specifically, Amaranthus retroflexus, Setaria viridis, and Abutilon theophrasti have developed resistance to photosystem II (PSII) inhibitors. Additionally, Amaranthus retroflexus, Echinochloa crus-galli, Datura stramonium, Chenopodium album, and Sorghum halepense have shown resistance to ALS inhibitors (Malidža et al., 2014; Bozic et al., 2015).

Crop rotation, tillage, and the use of fallow land followed by the application of general herbicides in areas where multiple resistance has occurred can somewhat mitigate this issue. Another approach for managing herbicide-resistant weeds is to study and compare the biology and ecology of sensitive versus resistant populations. This knowledge can be leveraged in managing resistant populations and should be a priority in these areas (Keshtkar et al., 2019).

Recent studies stated that cross-resistance in wild mustard (in wheat fields of Golestan Province) to the herbicide Granstar has been reported (Gherekhloo et al., 2018). Furthermore, similar investigations have shown that cross-resistance in weed species to all sulfonylureas, imidazolinones, and triazolopyrimidines is very high (Riar et al., 2015; Thompson et al., 1997; Deng et al., 2017). To prevent the occurrence of cross-resistance in Vaccaria, the repeated application of this herbicide group should be reviewed. In this regard, the use of herbicides with different modes of action can be considered to reduce the selection pressure of herbicides (Axhgar et al., 2021). If the selective pressure of the herbicide is alleviated, and if the fitness of resistant plants is lower than that of susceptible plants, the susceptible plants will eventually replace the resistant ones over time (Axhgar et al., 2021). However, if this is not the case, the frequency of resistant plants in the population is unlikely to decrease. In such cases, for long-term management of resistant plants, both reducing the intensity of selection and incorporating other management strategies should be applied. In the results, it is recommended to perform target and non-target resistance for each biotype that has been confirmed to be resistant to each herbicide. It is also recommended to assess the degree of resistance with different doses of the herbicides to which resistance was confirmed in this experiment. Considering the importance of wheat cultivation for the country, it is also recommended to collect more biotypes from all over the country and test their resistance to the herbicides commonly used in that region.

Conclusion

Based on the obtained results, it can be stated that resistance in Vaccaria sp. to the herbicides Teribenuron methyl (Granstar), Iodosulfuron + Mesosulfuron + Mefenpyr diethyl (Atlantis), and Iodosulfuron + Mesosulfuron + diflufenican + Mefenpyr diethyl (Othello) Acetolactate Synthase (ALS) inhibitors has occurred in Iran. This is the first report for Vaccaria sp. resistance occurrence to ALS-inhibiting herbicides in the country. Additionally, cross-resistance has been observed in Kermanshah province (biotype MA1) to the herbicides Atlantis and Granstar and in Ilam Province (biotype ML1) to the herbicides Othello and Granstar. Since cross-resistance to ALS inhibitors is very common, these results were not unexpected, as previous reports indicated cross-resistance in ryegrass (*Lolium* sp.), wild oat (*Avena fatua*), and Reed canary grass (Phalaris sp.) in Iran to ALS inhibitors. The results indicate that resistance to ALS inhibitors has developed in the provinces of Kermanshah, Markazi, and Ilam. Considering the high efficacy of the herbicides U 46 Combi Fluid and Bromicide MA on the weed species *Vaccaria* and the susceptibility of this species to these herbicides, it is recommended that Bromicide MA and U 46 Combi Difluid herbicides can be applied in rotation for the control of *Vaccaria* in wheat fields, particularly in the provinces of Kermanshah, Markazi, and Ilam. It is also recommended to avoid the repeated use of one or several ALS inhibitor herbicides in a single field. To delay resistance, other weed management practices should be employed. These practices include crop rotation, the use of fallow methods followed by mechanical tools, and the application of broad-spectrum herbicides such as glyphosate to eliminate emerged weeds. To be most effective, these strategies should be implemented in a systematic manner and be reviewed every few years. Additionally, because of limitations of weed control methods in wheat cultivation, the primary approach for weed management in this strategic crop relies on herbicides. Therefore, considering the conditions in Iran, implementing herbicide rotation and restricting the use of herbicides for which resistance has been documented, along with addressing sanitary issues such as quarantine compliance and the use of certified seeds, represent the proper practical and reasonable approaches

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Conflict of interest

No potential conflict of interest was reported by the author(s).

CRediT author statement

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