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# Evaluation of Selection Intensity of Acetolactate Synthase-Inhibitor Herbicide Resistance Endowing Asp-376-Glu Mutation in *Sinapis arvensis* Biotype

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**ABSTRACT. In current study we estimated ecological measures of Acetolactate synthase herbicide resistance evolution at lower than recommended dose in *Sinapis arvensis* herbicide resistance (HR) homozygous for Asp-376-Glu mutation and herbicide susceptible (HS) biotypes collected from wheat fields in Fars Province, Iran. At low herbicide doses of mesosulfuron + iodosulfuron plants can survive and display quantitative variation in dose-responses. LD50 and GR50 values represented 5.4 and 3-fold ALS resistant than susceptible biotypes. Seed number production per plant showed 3- fold resistance. Linear combination of survival and fecundity values, showed 1 and 0.74 fitness values for (HR) and (HS) biotypes respectively when exposed to the low-dose of 187 g ha$^{-1}$. With double increasing from 187 to 375 g ha$^{-1}$ herbicides, fitness decreased by 8-fold in (HS) biotype but reduced slightly by 20% in (HR) biotype. The selection intensity of (HR) at 375 and 187 g ha$^{-1}$ were 9 and 1.4 respectively. Overall, it is highlighted that evaluation of plant survival and fecundity in assessing mesosulfuron + iodosulfuron selection intensity in the (HR) and (HS) *Sinapis arvensis* biotypes is a key factor for measuring herbicide resistance evolution.**

**Keywords:** ALS herbicide resistance, fecundity, fitness, selection intensity, *Sinapis arvensis*.

## INTRODUCTION

Weeds are considerable threat to food security (Oerke, 2006). In most agro-ecosystems worldwide, herbicides are the potent technology used for the weeds control (Heap, 2014). Acetohydroxyacid synthase (AHAS) also referred to as acetolactate synthase (ALS), is the first enzyme in biosynthesis of branched-chain, essential amino acids valine, leucine and isoleucine (McCourt & Duggleby 2006). Also, ALS is target site of chemical group of sulfonylurea herbicides. Nearly, less than 10 years after first introduction of ALS inhibitor herbicides in the early 1980s, herbicide resistance to this group of herbicide was reported in *Lactuca serriola* (Mallory *et al.,* 1990).In the case of target-site resistance, at least single point mutations in the target gene reduce herbicide sensitivity (Heap, 2014).

Up to now a total of eight conserved amino acid residue (122, 205, 197, 376, 377, 574, 653 and 654) in the ALS gene have been detected in resistant weed biotypes (Powels & Yu, 2010; Beckie & Tardif, 2012; Tranel *et al.,* 2014). Of these mutations, the Asp-376-Glu mutant plants in the presence of ALS herbicide would be less competitive than other mutations (Li *et al.,* 2013). Surprisingly, unlike heterozygous, homozygous Asp-376-Glu is a weak ALS resistance mutation in some weed populations such as *Raphanus raphanistrum* (Yu *et al.,* 2012).

Herbicide resistance is an evolutionary consequences or selection intensity every time herbicide is used, susceptible individuals are killed and resistant individual survive and reproduce with resistance alleles (Mcgillion & Storrie, 2006).The dynamics of resistance alleles and evolutionary aspects in populations strongly affected by genetic factor (resistance prone, genetic variability, fitness cost, inheritance and ploidy), biological factors (fecundity, population size, seed dispersion and generation numbers) (Delye *et al.,* 2015). Plants severely affected by herbicide but still alive and set seed in some situations, target weeds can be received sublethal herbicide dose, for example, if there is underdosing, oversize weedy target and suboptimal environmental conditions. These factors can be affected on herbicide resistance eco-evolution (Blackshaw *et al.,* 2006). Several studies have established that herbicide selection intensity at low-dose can rapidly lead to herbicide resistance evolution (Neve *et al.,* 2005; Manalil, 2011; Busi *et al.,* 2015; Yu & Powels, 2014 & Busi & Powels, 2011).

Overall, estimation of herbicide selection intensity are lacking in the literature (Beckie and Morrison, 1993). In this study the wild mustard (*Sinapis arvensis*) herbicide resistant (HR) and herbicide susceptible (HS) biotypes were examined. We evaluated low-doses (below recommended dose) ALS herbicide mesosulfuron + iodosulfuron conferred different resistance status, fitness and selection intensity after assessing the survival and fecundity rates of HR and HS *S. arvensis* biotypes collected from fields of wheat in Fars Province, Iran.

## MATERIALS AND METHODS

*A. Plant materials*

In this article, the researcher investigated the selection intensity effects of ALS resistant and susceptible *S. arvensis* biotype.

Seeds of ALS resistant and susceptible (referred to as HR and HS hereafter, respectively), *S. arvensis* biotypes were collected in fields of wheat located in Fars Province, Iran (Table 1). The seeds were planted in plastic pots which were already filled with a mixture of peat, perlite and sands (1:1:1; v/v/v); later on the seedling which came into leaf (3-4 leaves) were treated with mesosulfuron-methyl (10 g/l) + iodosulfuron-methyl-sodium (2g/l)with recommended dose at 1500 g ha$^{-1}$ (Atlantis,40 OD, Bayer, Crop Science, Germany), using a moving-boom with 8004 Tee-Jet nozzles sprayer delivering herbicide at a rate of 400L water ha$^{-1}$ at a pressure of 200 KPa. The pots were kept outside in growing season. The plants were allowed to cross-pollinate naturally, and individuals homozygous for the resistance mutation Asp-376-Glu were selected.

**Table 1: Geographical origins of *Sinapis arvensis* biotypes used in this research.**

| Biotype | Location | Co-ordinate | Year |
|---|---|---|---|
| MHS3(HS) | Nourabad. M | Lat. 29.97 $^\circ N$<br>Long. 51.83 $^\circ E$ | 2011 |
| RHR4(HR) | Firoozabad | Lat. 28.81 $^\circ N$<br>Long. 52.55 $^\circ E$ | 2012 |

In order to prevent pollen contamination, each population was isolated in separate greenhouse to produce generation. No evidence of cross-pollination between resistant and susceptible populations were observed by re-sequencing analysis. Finally, HS biotype (MHS3) and HR biotype (RHR4) were derived from seeds produced by homozygous HS and HR plants (Accession numbers: KP661599 and KP661600). The two HR and HS biotypes were arranged with a range of phenotypic variability for biotype comparison.

**Whole plant dose-response, survival, growth and fecundity**

Experiments were conducted outdoors during 2013 to determine the level of mesosulfuron + iodosulfuron resistance in the HR *S. arvensis* biotype. Sowing the seeds were conducted as described above. At the 3-4 leaf stage, seedling from HS and HR biotypes were treated with mesosulfuron + iodosulfuron at commercial rates of 0, 98, 187, 375, 750, 1500 and 3000 g ha$^{-1}$.

Pots arranged in a randomized complete block design with four replications. Mesosulfuron + iodosulfuron effects on plant survival, vegetative growth and seed production were determined. Whereas survival and vegetative growth were assessed 4 weeks after treatment, the seed number determined at the end of the growth period. To minimize the effect of different plant densities on reproductive traits, surviving plants at each mesosulfuron + iodosulfuron dose were thinned to 4 individual plants in each pots.

*B. Statistical analysis*

Analysis was carried out to estimate mesosulfuron + iodosulfuron resistance parameters (LD50, GR50 and SY50) for the HS and HR *S. arvensis* biotypes when exposed to increasing rates of mesosulfuron + iodosulfuron. The observed plant survival, vegetative biomass and fecundity data were fitted to three-parameter log-logistic model

$$y = \frac{a}{1 + (\frac{x}{x_0})^b}$$

Where $a$ is the upper limit, $b$ is the slope of the curve, $x$, is constant and $x_0$ is herbicide dose that reduces fresh weight by 50% (Sigma Plot 12.0 Software; Systat, Software, Inc, San Jose, CA).Also, fitness and selection intensity parameters were quantified using method of Goh *et al* (2015).

## RESULTS AND DISCUSSION

As expected, at the recommended mesosulfuron + iodosulfuron dose (1500 g ha$^{-1}$) the HR and HS biotypes represented 66 and 0% plant survival and also 33 and 0% plant dry weight, respectively (Fig. 1,2). The population parameters LD50 (50% population lethal dose), GR50 (growth reduction by 50%) and SY50 (50% reduction in seed number per plant) values were highly different between HR and HS biotypes under double increasing dose (below recommended dose) of mesosulfuron + iodosulfuron (Fig. 1 to 3).

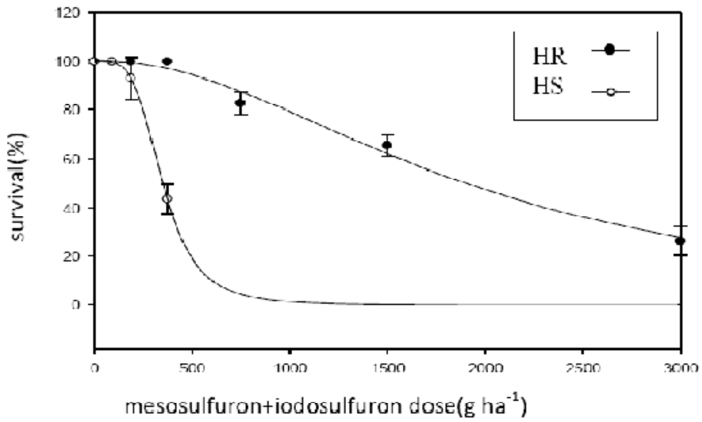


**Fig. 1.** Dose-response curve based on LD50 of mesosulfuron + iodosulfuron resistant (HR) and susceptible (HS) *S. arvensis* biotypes. Each point is the mean of four replications. Vertical bar is the standard error of the mean.

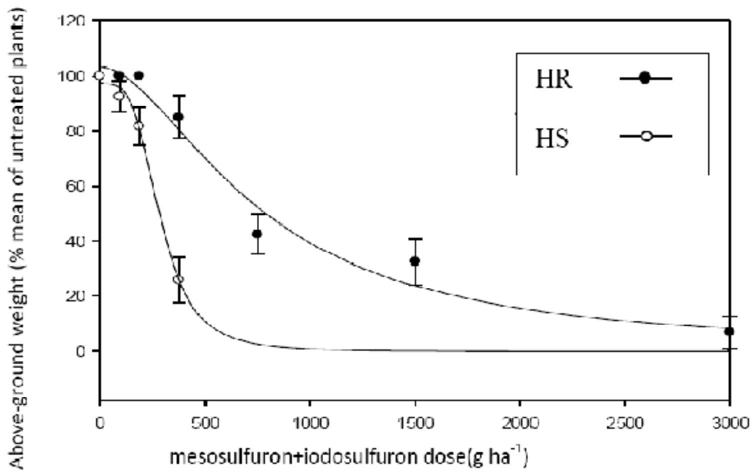


**Fig. 2.** Dose-response curve based on GR50 of mesosulfuron + iodosulfuron resistant (HR) and susceptible (HS) *S. arvensis* biotypes. Each point is the mean of four replications. Vertical bar is the standard error of the mean.

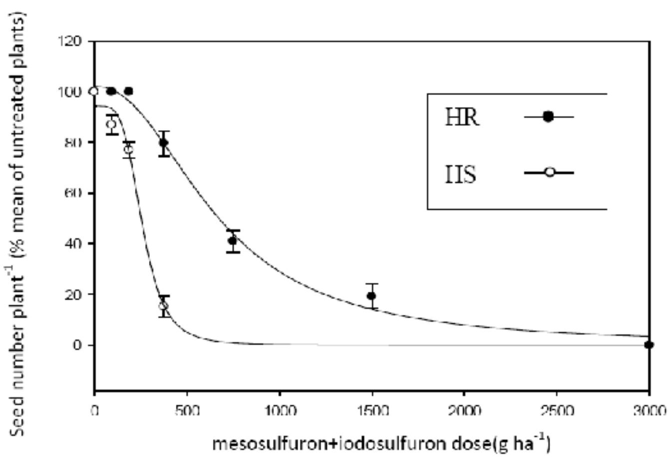


**Fig. 3.** Dose-response curve based on individual seed number per plant (SY50) of mesosulfuron + iodosulfuron resistant (HR) and susceptible (HS) *S. arvensis* biotypes. Each point is the mean of four replications. Vertical bar is the standard error of the mean.

**Table 2: Equation Parameters of the log-logistic model used to estimate the $LD_{50}$, $GR_{50}$ and $SY_{50}$. Resistance index (RI) of mesosulfuron + iodosulfuron was calculated for herbicide resistant (HR) and susceptible (HS) *S. arvensis* biotypes.**

| Biotype | a | b | $R^2$ | $LD_{50}$ | RI |
|---|---|---|---|---|---|
| HR | 100.4(0.45) | 2.07(0.32) | 0.98 | 1896(98.30) | 5.41 |
| HS | 100.2(3.32) | 4.07(0.44) | 0.99 | 350(94.53) | |
| **Biotype** | **a** | **b** | **$R^2$** | **$GR_{50}$** | **RI** |
| HR | 103.4(7.85) | 1.78(0.65) | 0.96 | 762(90.5) | 3 |
| HS | 97.23(4.2) | 3.76(0.4) | 0.98 | 288(71.32) | |
| **Biotype** | **a** | **b** | **$R^2$** | **$SY_{50}$** | **RI** |
| HR | 102(5.65) | 2.22(0.33) | 0.99 | 660(87.32) | 3 |
| HS | 94.4(3.11) | 4.37(0.67) | 0.95 | 260(28.55) | |

Values in parentheses are standard errors of the mean.

The LD50 index ratio of HR biotype was 5.4-fold greater than HS biotype (Fig. 1; Table 2). The mesosulfuron+ iodosulfuron dose of 762 g $ha^{-1}$ caused GR50 in the HR biotype, whilst, the same GR50 for HS biotype required 288g $ha^{-1}$ herbicide (Fig. 2; Table 2). According to GR50, the HR biotype was detected to be 3 fold more resistant than HS biotype (Table 2). The mesosulfuron + iodosulfuron dose required to SY50 was found to be 3-fold more than HS biotype (Table 2). Totally, based on RI indices, LD50 value was higher than SY50 and GR50.

Plant survival and seed number of HR and HS biotypes at two low doses of mesosulfuron+ iodosulfuron were quantified in (Fig. 3; 3). At the 187g $ha^{-1}$ mesosulfuron + iodosulfuron, the survival rates in the HR and HS biotypes were 100 and 93% respectively (Table 3).

Increasing amount of herbicide dose to 375g ha-1 reduced survival rate to 43% in HS biotype without any reduction in HR survival rate (Table 3). The results revealed that in both low doses of herbicide, fecundity rate decreased, but at 375 g $ha^{-1}$ herbicide, seed number produced by HR biotype was 4-fold upper than HS biotype (Table 3). At 375 g ha-1 herbicide rate, HR biotypes showed 9-fold selection intensity compared with HS biotype. Whereas, mesosulfuron + iodosulfuron selection intensity based on survival at 375 g $ha^{-1}$ was 2-fold. When the mesosulfuron+ iodosulfuron dose was doubled from 187 to 375g $ha^{-1}$, the fitness of HS biotype decreased by 88% (from 0.74 to 0.09), whilst, fitness of HR biotype descended by a 20% from 1 to 0.8 (Table 3). In HR compare with HS biotype, herbicide doses, based on LD50, approximately 5-fold mesosulfuron + iodosulfuron resistance index was found. Plant fitness is an ecological key factor for determination of plant number and fecundity in the next generations (Busi & Powles, 2009; Manalil, *et al.,* 2011 & Neve *et al.,* 2009).

**Table 3: Estimated fitness and selection intensity (SI) for mesosulfuron + iodosulfuron resistant (HR) and susceptible (HS) *S. arvensis* biotypes based on survival rate and fecundity (seed number $plant^{-1}$) under two lower recommendation doses at 187 and 375 g $ha^{-1}$.**

| Mesosulfuron + iodosulfuron dose(g $ha^{-1}$) | Biotypes | Survival | Fecundity | Fitness | Mesosulfuron + iodosulfuron selection intensity |
|---|---|---|---|---|---|
| | HR | 1 | 1 | 1 | 1.4 |
| 187 | HS | 0.93 | 0.8 | 0.74 | |
| | HR | 1 | 0.8 | 0.8 | 9 |
| 375 | HS | 0.43 | 0.2 | 0.09 | |

Data ranged from (0 to 1) represented (0 to 100%) of each parameters.

Selection intensity is another important factor for predicting the dynamics of herbicide resistance alleles in agricultural systems. It is noted that for accurate estimation of plant fitness for assessing the selection intensity, it should be conjunction with crop competition (Goh *et al.,* 2015). Therefore, resistance evolution in HR biotype is undoubtedly due enable survivors possessing genetically endowed traits at low rates of mesosulfuron + iodosulfuron in Asp- 376-Glu resistant biotype.

Our study emphasis that in weak ALS resistance mechanisms such as homozygous Asp- 376-Glu versus heterozygous, under recommended field dose (1500 g $ha^{-1}$) treatment, plant fitness was highly reduced, unlike reported by Goh *et al.* (2015).

## CONCLUSION

The practical implication of this study is that mesosulfuron + iodosulfuron should be used at rates that cause very high target weed mortality.

Thus minimizing the accumulation of weak resistance gene traits (homozygous Asp- 376-Glu) in target weed populations, especially, in *S. arvensis* as a cross-pollinated weed species. However, when used in conjunction with other management strategies, it can be quite effective in reducing fecundity, survival and finally fitness of Asp- 376-Glu herbicide resistant weeds. The estimation of plant survival and seed number is an ecological factor for determination of herbicide resistance evolution.

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