CROSS RESISTANCE TO ALS-INHIBITING HERBICIDES IN *Sorghum halepense*

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INTRODUCTION

Sorghum halepense (L.) Pers is one of the most common and problematic perennial weeds in areas of crop production worldwide (ANDÚJAR et al., 2013). It is reproduced by seed and rhizomes. This weed is considered one of the three most problematic in corn weed in Mexico, as it can cause a large reduction in grain yield and quality .

Chemical control of this species is becoming more difficult because it presents cross and multiple resistance to a wide range of selective and non- selective herbicides. This means that there is a need for a selective post-emergence herbicide for controlling the weed effectively.

Abuse of ALS inhibiting herbicides for control of *S. halepense* has favored the emergence of resistant biotypes to these herbicides .

The objective of this study is to determine the level of resistance to the 5 classes of ALS inhibitors in a population of *S. halepense* in maize harvested agricultural area of Veracruz.

MATERIALS AND METHODS

Herbicides.

Enzyme assays were performed with herbicides pure (> 98%) (bensulfuron, byspiribac, chlorsulfuron, florasulam, flucarbazone, imazamox, iodosulfuron, metsulfuron, nicosulfuron, rimsulfuron and tribenuron) supplied by different companies.

Plant material

The seeds of the two biotypes of S. halepense used (S and R) were obtained from corn fields in the area of Mexico (Veracruz, Mexico). All seeds were disinfected with 1 mM $CaCl_2$ and planted in pots of 2 L of substrate (2:1 soil: peat) with 1 plant per pot. Growing conditions were: day/night temperature of 28/20 ºC and a photoperiod of 12 h light, while the relative humidity was maintaining constant in 80%.

ALS activity

The ALS activity was measured as the formation of product acetolactate, after its conversion to acetoin by decarboxylation in the presence of acid (RAY, 1984). The plant material used for these tests was obtained from plants of biotypes R and S of S. halepense. The extraction of the enzyme and the enzymatic activity studies were conducted on young leaves following the methodology described by CALHA et al. (2007). Activity assays were performed on enzyme extracts incubated with bensulfuron, byspiribac, chlorsulfuron, florasulam, flucarbazone, imazamox, iodosulfuron, metsulfuron, nicosulfuron, rimsulfuron and tribenuron. The results were expressed as the concentration needed to reduce ALS herbicide activity by 50 % (I_{50}) relative to the control without herbicide. The assay was performed 2 times, with 3 replications each experiment. Data were pooled and fitted to a nonlinear regression model (STREIBIG et al., 1993; SEEFELDT et al., 1995). The parameters for each biotype and herbicide were estimated with a confidence interval of 95%, using the statistical analysis program Sigma Plot 11.0 (RUIZ- SANTAELLA et al., 2006).

RESULTS

ALS activity

The in vitro tests show that the resistant biotype has values higher respect to the susceptible to the different families of ALS inhibiting herbicides (Table 1). The resistance factor (RF) of R-*S. halepense* biotype is 1.89 times more resistant to flucarbazone (sulfonyl -aminocarbonyl- triazolinone), 3.53 times more resistant to byspiribac (pirimidilbenzoate), 11.06 times more resistant to imazamox (imidazolinone), and between 1.15 and 44.68 times more resistant to the different sulfunilureas that the sensitive biotype. Regarding florasulam (triazolopyrimidine) does not present any resistance even seen otherwise (Table 1) occurs. Therefore the established order of R biotype resistance to the different families of herbicides is:

sulfonylureas > imidazolinone > pirimidilbenzoates > sulfonyl -aminocarbonyl-triazolinones >>>>>>>>>>>triazolpyrimidines

Table 1. I_{50} values for ALS herbicides from different chemical families of ALS inhibitors in *S. halepense* R and S.

HERBICIDA	BIOTIPO	I_{50} (µM)	FR
Florasulam	R	4.86	------
	S	5.14	
Flucarbazone	R	2.23	1.89
	S	1.18	
Byspiribac	R	22.80	3.53
	S	6.45	
Imazamox	R	5004.91	11.06
	S	452.26	
Rimsulfuron	R	15.46	18.40
	S	0.84	

A parallel experiment with different herbicides of the sulfunilureas family (which has greater resistance factor) also shows cross-resistance with resistance by: methsulfuron> rimsulfuron> iodosulfuron> bensulfuron> chlorsulfuron> nicosulfuron as can be seen in the table 2.

Table 2. I_{50} values for different ALS herbicides of the chemical family of sulfunilureas for *S. halepense* in R and S

HERBICIDE	BIOTYPE	I_{50} (µM)	FR
Methsulfuron	R	41.55	44.68
	S	0.93	
Rimsulfuron	R	15.46	18.40
	S	0.84	
Iodosulfuron	R	4.28	12.23
	S	0.35	
Bensulfuron	R	83.97	9.71
	S	8.65	
Chlorsulfuron	R	60.28	8.34
	S	7.23	
Nicosulfuron	R	15.03	4.91
	S	3.06	
Tribenuron	R	23.81	1.15
	S	20.74	

DISCUSSION

The results show that *Sorghum halepense* named as resistant is highly resistant to different chemical families ALS enzyme inhibitors, primarily to sulfunilureas, in which also presents cross-resistant to them. Moreover, respect to the family of triazolopyrimidines it do not present resistance, so this family of herbicides would be a suitable candidate to control this biotype. The fact that this presents resistance to some families and not others makes us direct our future research to study the amino acid sequence in the ALS of the material used in this work, in order to determine whether we are dealing with different sequences and / or binding proteins also different.

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