علـوم محـیـطی سال ششم، شماره اول، پاییز ۱۳۸۷
ENVIRONMENTAL SCIENCES Vol.6, No.1, Autumn 2008
43-52

Seed Bioassay and ACCase Enzyme Assay to Study the Resistance of *Phalaris minor* to Aryloxyphenoxy-propionate (APP) Inhibitors

Javid Gherekhloo[*1], Mohammad Hasan Rashed Mohassel[1], Mehdi Nassiri Mahallati[1], Eskandar Zand[2], Ali Ghanbari[1], Maria D. Osuna[3], Rafael De Prado[4]

1- Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad
2- Department of Weed Research, Tranian Research Institute for Plant Protection, Tehran, Iran
3-Finca La Orden, Departament de Hortofruticultura, Badajoz, Spain.
4-University of Cordoba, Campus of Rabanales, Cordoba, Spain.

Abstract

During 2005 and 2006, fourteen and seven *Phalaris minor* populations were found in the wheat fields of Fars and Golestan Provinces, respectively, that exhibited resistance to fenoxaprop-P ethyl and diclofop methyl belonging to the aryloxyphenoxy propionate herbicides. Seed bioassay was conducted at the weed science laboratory of Ferdwosi University of Mashhad to study the resistance of the populations to the APP herbicides. Petri dish assay showed that the populations are resistant to applied herbicides with different level of resistance. Using the estimated parameters of concentration-response curves, discriminating concentration was determined for diclofop methyl at 8.04 ppm and for fenoxaprop-P-ethyl at 1.05 ppm. A study was also conducted at the agricultural biochemistry laboratory of Cordoba University to investigate the biochemical basis of resistance to ACCase–inhibiting herbicides in the resistant populations. *In vitro* enzyme assays revealed a herbicide-resistant ACCase enzyme in the AR, MR4 and SR3 populations. Extracted ACCase enzyme from the shoots of these populations was highly resistant to both applied herbicides compared with the susceptible population. The results suggest that the mechanism of resistance to APP herbicides in the three most resistant populations (AR, MR4 and SR3) relates to an altered ACCase. In the case of the rest of the resistant populations, other mechanisms including enhanced metabolism, lack of absorption and translocation, and other unknown mechanisms may be involved. These results also confirmed seven populations are cross-resistant to both the herbicides studied.

Keywords: *Phalaris minor*, seed bioassay, ACCase, aryloxyphenoxy propionate and resistance.

زیست‌سنجی بذر و آنزیم ACCase به منظور مطالعهٔ مقاومت فالاریس (*Phalaris minor*) به بازدارنده‌های آریلوکسی فنوکسی پروپیونات(APP)

جاوید قرخلو[*1]، محمدحسن راشد محصل[1]، مهدی نصیری محلاتی[1]، اسکندر زند[2]، علی قنبری[1]، ماریا اسونا[3]، رافائل دپرادو[4]

۱- گروه زراعت ، دانشکده کشاورزی، دانشگاه فردوسی مشهد
۲-بخش تحقیقات علف‌های هرز، موسسه تحقیقات گیاه پزشکی کشور
۳- مرکز تحقیقات کشاورزی باداخوس، اسپانیا
۴-آزمایشگاه شیمی کشاورزی ، پردیس دانشگاه کوردوبای اسپانیا

چکیده

طی سالهای ۱۳۸۴ و ۱۳۸۵، تعداد ۱۴ و ۷ توده فالاریس در مزارع گندم استانهای فارس و گلستان شناسایی شدند که بترتیب به علف‌کشهای فنوکساپروپ-پی اتیل و دایکلوفوپ متیل مقاومت نشان می‌دادند. به منظور مطالعه مقاومت توده‌های مذکور به علف‌کشهای یاد شده، آزمون زیست‌سنجی بر روی آنها در آزمایشگاه علف‌های هرز دانشگاه فردوسی مشهد انجام شد. آزمون پتری‌دیش نشان داد که توده‌های فالاریس مقاوم دارای درجات مقاومت مختلفی به علف‌کشهای مورد آزمایش بودند. با استفاده از پارامترهای برآورد شده از منحنی‌های غلظت-پاسخ، غلظت تفکیک کننده به میزان ۸/۰۴ و ۱/۰۵ قسمت در میلیون بترتیب برای علف‌کشهای دایکلوفوپ متیل و فنوکساپروپ-پی اتیل تعیین شد. سپس مطالعه‌ای برای بررسی مبنای بیوشیمیایی مقاومت توده‌های مقاوم به بازدارنده‌های آنزیم ACCase، در آزمایشگاه بیوشیمی دانشگاه کوردوبای اسپانیا انجام شد. آزمون زیست‌سنجی بیانگر وجود آنزیم استیل کوآنزیمA مقاوم در توده‌های AR-1، MR4-1 و SR3-1 بود. آنزیم ACCase استخراج شده از اندامهای هوایی این توده‌ها در مقایسه با توده حساس از مقاومت بالایی به هر دو علف‌کش برخوردار بود. با توجه به نتایج حاصل، احتمالا مکانیسم مقاومت به بازدارنده‌های APP در سه توده بسیار مقاوم (AR-1، MR4-1 و SR3-1) بدلیل وجود آنزیم ACCase تغییر یافته در آنها می‌باشد. در مورد بقیه توده‌های مقاوم مورد مطالعه، احتمالا سایر مکانیسم‌های مقاومتی مانند افزایش متابولیسم و یا فقدان جذب و انتقال علف‌کش دخیل هستند. همچنین نتایج بدست آمده از این پژوهش نشان داد که ۷ توده دارای مقاومت عرضی به هر دو علف‌کش مورد مطالعه بودند.

کلمات کلیدی: مقاومت، علف‌کش، منحنی غلظت-پاسخ، مقاومت عرضی و بازدارنده‌های ACCase

* Corresponding author. *E-mail Address:* jghrekhlo@um.ac.ir

Introduction

Acetyl-CoA carboxylase (ACCase, EC 6.4.1.2) is a key enzyme which catalyzes the initial step in fatty acid synthesis, indeed, in most plant cells, the major pathway consuming malonyl-Co A is plastid-localized fatty acid synthesis (Buchanan *et al.*, 2000). There are two isoforms of ACCase in plants: the plastid ACCase which is essential for biosynthesis of primary fatty acids and the cytosolic ACCase that is involved in biosynthesis of long chain fatty acids. Almost all monocots and dicots plants have the two isoforms of ACCase described above. The Poaceae family is an exception, with similar homomeric forms being found in both plastids and cytocol. The homomeric ACCase in the cytosol of almost all plant species and the heteromeric ACCase in the chloroplasts of dicotolydons are insensitive to APP, CHD, and phenylpyrazolin herbicides. This difference in sensitivity to ACCase has been considered to produce a family of herbicides which selectively control grass weeds. Aryloxyphenoxypropionate (APP), cyclohexanedione (CHD), and phenylpyrazolin (DEN) (Hofer *et al.*, 2006) are three chemical classes of herbicides that inhibit ACCase action in Poaceae, thus preventing fatty acid synthesis and reducing production of phospholipids used in membranes (Delye *et al.*., 2002). Chlorosis, necrosis and finally death of plant tissue occur after applying these herbicides (Ball *et al.*, 2007).

The increase in the use of graminicides belonging to the ACCase inhibitors to control of grass weeds in crops, since their introduction in the 1970s and 1980s (Delye, 2005), has led to a parallel increase in the evolution of populations resistant to these herbicides (Tal *et al.*, 2000). During the past decades, there have been an increasing number of reports of graminicide-resistant weeds (De Prado *et al.*, 2000). To date, graminicide resistance has been reported for 35 grass weed species (Heap, 2008).

In 2005 and 2006, 34 suspected little seed canarygrass populations were sampled from wheat fields of Fars and Golestan where these graminicides had been applied for more than two decades. Pre-screening testing and dose-response experiments at whole plant level had showed that 14 and seven populations are resistant to the fenoxaprp-P ethyl and diclofop-methyl, respectively (Gherekhloo *et al.* Unpublished Data). As whole plant assay needs more labor, takes a long time and a lot of space, it is necessary to develop a simple and rapid test to identify whether little seed canary grass is resistant to diclofop-methyl and fenoxaprp-P ethyl and confirm it with the results of greenhouse dose-response assay which is more accurate method. On the other hand, these assays don't give us any information on the mechanism of herbicide resistance of *Phalaris minor* in Iran. In this study, we conducted two different methods: a rapid and simple seed bioassay, and ACCase enzyme assay, in order (1) to evaluate the resistance of the collected populations to APP herbicides using seed bioassay, (2) to determine discriminating concentration for each herbicide and (3) to establish whether the resistance to APP herbicides in R populations is associated with an alteration in the target ACCase enzyme.

Materials and Methods

Plant materials. Seed samples of small seed canary grass populations were collected from wheat fields in Fars Province, Iran, during 2005 and 2006. One population also was collected from the same region that had never been treated with ACCase inhibiting herbicides.

Concentration-Response Assay in Petri Dishes. Concentration-response experiments were conducted using 9-cm diameter plastic Petri-dishes. Five pre-germinated seeds were placed on two sheets of filter paper (Whatman #1) placed on the bottom of the dish. Each Petri dish was considered as one replication and the experiment was conducted with three replications for each herbicide. Five ml aliquot of aqueous solution of commercial formulation of diclofop methyl was applied at 0, 0.5, 1, 2, 4, 8, 16, 32 and 64 ppm of active ingredient. After applying the herbicide solutions, the Petri dishes were left open for about 30

minutes in the hood to evaporate the solvents. This experiment was also done for a commercial formulation of fenoxaprop-P ethyl at concentrations of 0, 0.1, 0.5, 1, 2, 4, 8, 16 and 32 ppm ai. The Petri dishes were then placed on a regulated growth chamber with 12/12 h photoperiod and a temperature of 25 ^{o}C. Seven days later, shoot lengths were measured and expressed as percentages of the control.

ACCase Extraction. ACCase isoforms were isolated using the protocol of De Prado *et al.*. (2000) with slight modifications. Leaves (6 g fresh weight) of all biotypes were harvested from plants at the 3-4 leaf stage and ground in liquid N_2 with a mortar and then added to 24 mL of extraction buffer [0.1 M Hepes-KOH (pH 7.5), 0.5 M glycerol, 2 mM EDTA, and 0.32 mM phenylmethyl sulfonyl fluoride]. The homogenate was mixed for 10 minutes with a magnetic stirrer and filtered sequentially through four layers of cheesecloth. The crude extract was centrifuged (24000*g*, 20 min). The obtained supernatant was fractionated with ammonium sulfate. The protein fraction including the ACCase enzyme was precipitated from the crude extract at 50% saturation of $(NH4)_2SO_4$. The supernatant was discarded and the pellet was re-suspended in extraction buffer (1 mL), and the re-suspension was applied to a Sephadex G-25 PD-10 column which previously equilibrated with S400 buffer. The desalted protein extract was immediately used for ACCase enzyme activity assays.

ACCase Assays. The enzyme activity was assayed by measuring the ATP-dependent incorporation of $NaH(^{14}C)O_3$ into an acid-stable ^{14}C product. The reaction product was previously shown to be [14C]-malonyl-CoA. Assays were conducted in 7 mL scintillation vials containing 0.1 M Tricine-KOH (pH 8.3), 0.5 M glycerol, 0.05 M KCl,2 mM EDTA, 0.5 mM DTT, 1.5 mM ATP, 5 mM $MgCl_2$, 15 mM $NaH(^{14}C)O_3$ (33 $Ci.mol^{-1}$)[1], 45 µL of enzyme fraction, and 5 mM acetyl-CoA (lithium salt) in a final volume of 0.2 mL. The assay temperature was 34°C. The reaction was stopped after 5 minutes by adding 30 µL of 4N HCl. The acidified reaction medium was dried at 40°C under a stream of air. The dried samples re-dissolved in 0.5 mL of 50% (v/v) ethanol followed by 3 mL liquid scintillation cocktail. Then the acid-stable radioactivity was determined using a Liquid Scintillation Counter. Herbicide concentrations resulting in a 50% inhibition of enzyme activity (I_{50} values) were estimated for each herbicide using obtained concentration response curves.

Statistical Analysis. Using non-linear regression analysis on the R software-drc add-on package (Anonymous, 2006), the three-parameter log-logistic model (Equation 1) was fitted to data and EC_{50} and I_{50} values were estimated for each population and each herbicide (Ritz and Streibig, 2005).

$$\textbf{(1)}\quad f(x,(b,d,e)) = \frac{d}{1+\exp\{b(\log(x)-\log(e))\}}$$

where *e* is the EC_{50} or I_{50}. The upper limit is *d*. The parameter *b* denotes the relative slope around *e*.

Results and Discussion

The seedling roots were very sensitive to herbicides in both R and S populations. As the seedlings did not have roots at almost all concentrations, it was not considered for this study (data not shown). Tal *et al.* (2000) reported that in seed bioassay, the root of R or S biotypes of *Phalaris minor* and *Lolium rigidum* was more sensitive than the shoot to diclofop methyl and fenoxaprop-P ethyl, respectively, and the effects of both herbicides on root elongation were noticeable even at the lowest concentration. The difference between the shoot length of resistant and susceptible populations was visible after 7 days' incubation. Results of Petri dish assay showed the shoot length of seedlings decreased according to a sigmoidal trend and was fitted to a log-logistic equation (Figure 1). The decreasing shoot length of the S population happened at lower concentrations than that of R populations and this confirmed that the susceptible population is more sensitive to herbicides than resistant populations.

Resistance factors and estimated nonlinear regression parameters for both applied herbicides are given in Table 1.

Regarding fenoxaprop-P-ethyl, estimated EC_{50} was 0.29 ppm for S and for R populations ranged between 0.46 and 133.73 ppm for ER2 and SR3, respectively (Table 1). The resistance factors (RF) for all populations were >1 and these values were statistically significant and confirmed the populations are resistance to applied herbicides. The estimated EC_{50} and RFs showed that the all resistant populations have a level of resistance to fenoxaprop-P-ethyl compared with the susceptible one (SS) (Table 1).

While 2.44 ppm diclofop-methylf inhibited the shoot length of susceptible population by 50%, this amount for R populations was between 3.17 and 356.89 ppm for GR2-1 and SR3 populations, respectively (Table 1).

The discriminating differences in shoot length between R and S were determined from the respective estimated models. The largest difference in shoot length between R (population C) and S (population D) was determined for diclofop methyl at 8.04 ppm and for fenoxaprop-P-ethyl at 1.05 ppm. A discriminating dose is the minimum herbicide concentration that provides the largest vertical difference between the concentration-response curves for the R and S populations and that result in at least an 80% reduction in shoot length of the S populations (Beckie *et al.*, 2000). Once concentration response information has been obtained, it is often possible to use a single (or two or three) discriminating concentration(s) in future screening assays, which allows for many more populations to be tested as fewer Petri dishes per population are needed (Moss, 2008). The discriminating concentration of diclofop methyl for *L. rigidum* was determined at 6.0 mg/l (Tal *et al.* 2000). They also found that the useful discriminating concentrations for *P. minor* and *A. myosuroides* were 8.0 and 0.06 mg/l in response to fenoxaprop-P and clodinafop, respectively. In addition to different weeds studied, the difference between discriminating concentrations (DC) can be explained through different methods of estimating the DC and also the sensitivity rate of susceptible biotypes to applied herbicides.

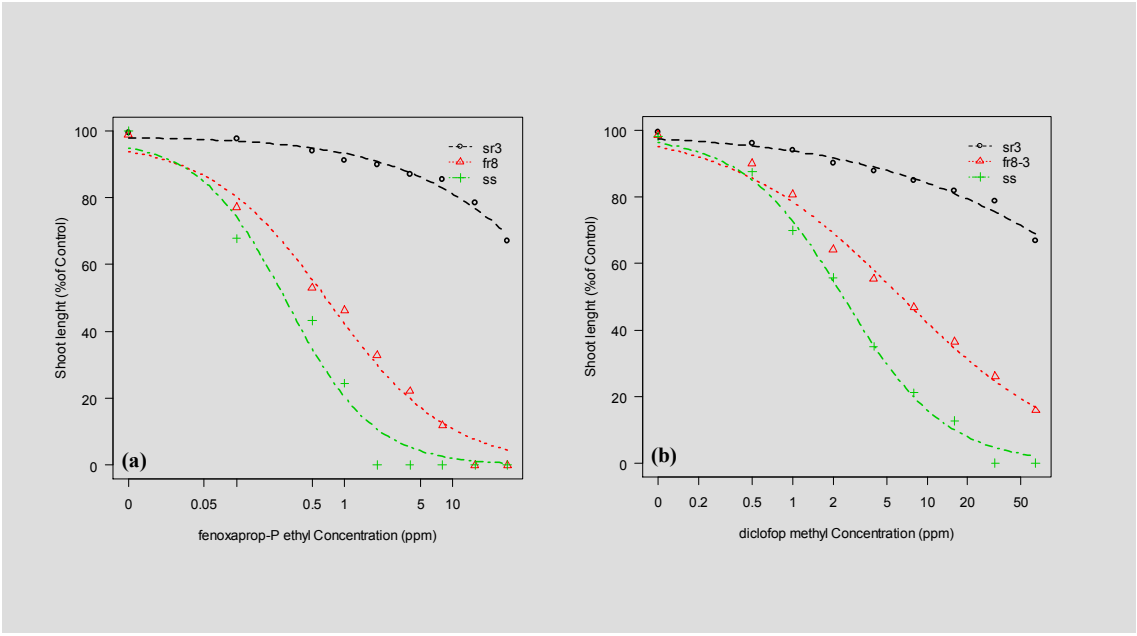

Figure 1 - Response of shoot length of the two resistant (SR3 and FR8) and the susceptible (SS) populations to different concentrations of fenoxaprop-P ethyl (a) and diclofop methyl (b).

Table 1 - Estimated nonlinear regression parameters for the *Phalaris minor* populations in response to different herbicide concentrations.

	Population	(d)	(b)	EC_{50}(e)	P-value of Lack of Fit	RF
fenoxaprop-P ethyl	AR	101.40(2.59)	0.39(0.05)	50.91(12.94)	0.09	173.92 hs
	SR3	98.22(2.32)	0.60(0.15)	133.73(55.93)	0.06	456.99 *
	MR4	99.46(2.43)	0.47(0.06)	51.10(11.45)	0.12	174.56 hs
	FR2	98.58(2.98)	0.85(0.06)	0.81(0.10)	0.10	2.76 **
	FR3	98.01(2.91)	1.08(0.10)	0.59(0.06)	0.06	2.05 **
	FR4	96.84(3.01)	0.66(0.05)	0.97(0.15)	0. 11	3.32 **
	FR5	94.95(3.22)	1.05(0.12)	0.79(0.10)	0.07	2.69 **
	FR6	95.24(3.59)	1.09(0.11)	0.52(0.08)	0.07	1.77 *
	FR8	96.88(3.08)	0.79(0.06)	0.73(0.10)	0.06	2.49 **
	MR1	99.79(2.85)	0.79(0.06)	0.85(0.10)	0.09	2.89 **
	MR2	98.53(2.73)	0.94(0.07)	1.10(0.11)	0.07	3.77 **
	ER1	96.28(3.13)	0.78(0.06)	0.49(0.07)	0.08	1.69 *
	ER2	99.17(2.97)	0.99(0.08)	0.46(0.05)	0.08	1.56 *
	GR2-1	98.92(2.89)	0.87(0.06)	0.48(0.06)	0.07	1.63 *
	SS	97.36(2.89)	1.08(0.08)	0.29(0.04)	0.09	-
diclofop methyl	AR	98.50(1.95)	0.49(0.06)	168.62(34.38)	0.06	69.04 hs
	SR3	99.26(1.89)	0.47(0.08)	356.89(117.69)	0.11	146.13 hs
	MR4	98.28(2.39)	0.51(0.09)	187.17(51.12)	0.57	76.63 hs
	FR2	99.49(2.25)	0.91(0.05)	3.31(0.27)	0.06	1.37 *
	FR4	102.26(3.01)	0.71(0.05)	4.59(0.59)	0.07	1.88 *
	FR8	100.36(2.22)	0.69(0.04)	6.31(0.63)	0.06	2.58 hs
	GR2-1	98.63(2.37)	1.09(0.06)	3.17(0.25)	0.08	1.30 *
	SS	98.54(1.87)	1.16(0.05)	2.44(0.14)	0.16	-

(): Standard error in parenthesis, *: significant at 0.05, **: significant at 0.01, hs: high significant

RF: Resistant Factor, b: Hill slope, d: Upper limit, c: Lower limit

Although direct comparisons among the Petri dish and greenhouse assays are not possible because of different conditions, results of the Petri dish assay were in agreement with whole plant assay (data not shown) and showed similar trends. This means that the results of the Petri dish assay are reliable and can be trusted. Seed bioassay has already been applied to screen resistance within populations. Heap and Knight (1986) and Gill (1990) used rapid bioassay for screening diclofop methyl resistance in *L. rigidum* populations. Murray *et al.* (1996) have developed this assay to identify resistant biotypes of *A. fatua* to ACCase inhibiting herbicides. Letouze and Gasquez (1999) reported a reliable rapid test for screening APP resistant biotypes of *Alopecucurs myosuroides* and *Lolium* spp. Tal *et al.* (2000) described a seed bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. Petri dish bioassay is a simple and rapid and inexpensive method that takes up less space than the whole-plant assay in a greenhouse and is very useful for screening a large number of suspected resistant populations.

ACCase Sensitivity Assay

Results showed the ACCase enzymes of the AR, SR3 and MR4 populations were 37-, 72-, 14-fold less sensitive to inhibition by fenoxaprop-P ethyl than ACCase of the S population, with I_{50} values of 2268.8, 4449.7, 869.7 and 61.4 µM, respectively (Table 2). A similar insensitivity of ACCase from the three populations was observed to diclofop methyl. I_{50} values for diclofop methyl were 3999.3, 10379, 4448.15 and 127.75 μM^2 for the AR, MR4, SR3 and S populations, respectively.

Table2 - Estimated I_{50} (µM) and calculated RF for ACCase enzyme of the little seed canary grass populations in response to fenoxaprp-Pethy and diclofop-methyl.

Herbicide / Population	fenoxaprop-P ethyl		diclofop ethyl	
	I_{50}	RF	I_{50}	RF
AR	2268.8 (149.61)	37.19 *	3999.3 (59.98)	31.30 *
SR3	4449.7 (138.5)	72.47 *	10379.1 (461.94)	81.25 *
MR4	869.7 (127.52)	14.60 *	4448.15 (141.48)	34.82 *
FR2	73.85(9.94)	1.20 ns	141.37(20.38)	1.11 ns
FR3	64.29(11.79)	1.05 ns	-	-
FR4	69.58(10.64)	1.13 ns	138.94(19.46)	1.09 ns
FR5	71.36(14.71)	1.16 ns	-	-
FR6	66.82(15.20)	1.09 ns	-	-
FR8	82.29(31.27)	1.34 ns	167.62(23.09)	1.31 ns
MR1	77.62(13.29)	1.26 ns	-	-
MR2	80.12(21.11)	1.30 ns	-	-
ER1	68.90(17.32)	1.12 ns	-	-
ER2	72.13(14.03)	1.17 ns	-	-
GR2-1	60.78(12.65)	0.99 ns	129.16(28.51)	1.01 ns
SS	61.4 (15.78)	-	127.75 (31.29)	-

RF: Resistant Factor, *: significant at 0.05, (): Standard error in parenthesis

Although the whole-plant assay (Gherekhloo *et al.,* unpublished data) and seedling bioassay showed clearly that the other populations (FR2, FR3, FR4, FR5, FR6, FR8, MR1, MR2, ER1, ER2 and GR2-1) are resistant to fenoxaprop-P ethyl and FR2, FR4, FR8 and GR2-1 are also resistant to diclofop methyl, the ACCase assay could not confirm it, resulting in RF ~ 1 (Table 2). It seems that the resistant populations have developed different patterns of resistance to the APP herbicides.

At present, many weeds have evolved resistance to APP herbicides such as fluazifop-P, fenoxaprop-P, diclofop, and quizalofop (Cocker *et al.*. 1999; Devine 1997; Leach *et al.*. 1995; De Prado *et al.*. 1999; Volenberg and Stoltenberg 2002, Yang *et al.* 2007). In many studies it has been concluded that resistance to ACCase is due to one (or more) mutation(s) in the ACCase gene resulting in an altered target site in resistant biotypes that is less sensitive to APP herbicides. Molecular study on the DNA of these resistant biotypes has showed different mutations which are responsible for different levels of resistance to various APP herbicides (Brown *et al.*, 2002; Delye *et al.*, 2005; Tosapon *et al.*, 2006). However cases of metabolism based of resistance have been reported (Devine, 1997). In this study we found 14 biotypes are resistant to fenoxaprop-P ethyl and 7 biotypes are cross resistant to diclofop methyl. However, enzyme assay revealed the resistance of FR2, FR3, FR4, FR5, FR6, FR8, MR1, MR2, ER1, ER2 and GR2-1 populations to fenoxaprop-P ethyl. Moreover, the cross-resistance of FR2, FR4, FR8 and GR2-1 populations to diclofop methyl are not due to altered in ACCase enzyme and may be due to other mechanisms including enhanced metabolism, lack of absorption and translocation, and other unknown mechanisms are involved (Cocker *et al.*. 1999). I_{50} values obtained from ACCase enzyme assay confirmed that the resistance of AR, MR4 and SR3 is target-site-based and these populations have an altered acetyl Co A carboxylase which is less sensitive than that of the susceptible one to applied herbicides. The resistance in *Avena fatua* L. (Christoffers and Messersmith, 1999) and *Setaria viridis* (Zhang and Devine, 2000; De Prado *et al.*., 2004), *L. rigidum* (Zagnitko *et al.*, 2001), and *Alopecurus myosuroides* (Brown *et al.*, 2002), has been shown to be associated with a mutation in the ACCase gene, resulting in an isoleucine to leucine substitution in the ACCase. Future studies concerning graminicide resistance need to focus on cloning the ACCase gene and identifying the sequence encoding the graminicide-binding region in R and S biotypes. However, the high level of EC_{50} of the most three resistant populations (AR, MR4 and SR3) suggests that the resistance in these populations is may not just be because of a single mechanism and multiple mechanisms are probably involved. We are going to elucidate the other resistance mechanisms by biochemical and molecular approaches in further studies.

Acknowledgments

The authors wish to acknowledge the financial support afforded by Ferdowsi University of Mashhad and Cordoba University of Spain for the implementation of this project. The authors also thank the Plant Protection Research Institutes of the Agricultural Research Centers of Golestan and Fars Provinces and weed researchers who collected and sent samples to the Ferdowsi University of Mashhad for conducting this study.

Note

1- Curio per Mole
2- Micro Mole

References

Anonymous (2006). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org.

Ball, D. A., Frost, S. M. and L. H. Bennett (2007). ACCase-inhibitor herbicide resistance in downy

brome (*Bromus tectorum*) in Oregon. *Weed Sci.*, 55: 91–94.

Beckie, H., Heap, I. M. Smeda, J. and L. M. Hall. (2000). Screening for herbicide resistance in weeds. *Weed Technol.*, 14: 428-445.

Brown, A. C., Moss, S. R. Wilson, Z. A. and L. M. Field (2002). An isoleucine to leucine substitution in the ACCase of *Alopecurus myosuroides* (black-grass) is associated with resistance to the herbicide sethoxydim. *Pestic. Biochem. Physiol.*, 72:160–168.

Buchanan, B. B., Gruissem, W. and R. L. Jones. (2000). *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiology, Rockville, Maryland, Courier Companies, Inc. 1367 pp.

Christoffers, M. J. and C. G. Messersmith, 1999. Molecular analysis of acetyl-CoA carboxylase genes from herbicide-resistant and susceptible wild oats. *Proc. N. Weed Sci. Soc.*, 54: 113.

Cocker, K. M., Moss, S. R. and J. O. D. Coleman. (1999). Multiple mechanisms of resistance to fenoxaprop-P-ethyl in United Kingdom and other European populations of herbicide-resistant Alopecurus myosuroides (black-grass). *Pestic. Biochem. Physiol.* 65:169-180.

De Prado, J. L., De Prado, R.and R. H. Shimabukuro. (1999). The effect of diclofop on membrane potential, ethylene induction, and herbicide phytotoxicity in resistant and susceptible biotypes of grasses. *Pestic. Biochem. Physiol*, 63:1–14.

De Prado, R., Gonzalez-Gutierrez, J., Menendez, J., Gasquez, J. Gronwald, J. W. and R. Gimenez-Espinosa. 2000. Resistance to acetyl-CoA carboxylase inhibiting herbicides in *Lolium multiflorum*. *Weed Sci.*, 48: 311-318.

De Prado, R., Osuna, M. D. and A. J. Fischer (2004). Resistance to ACCase inhibitor herbicides in a green foxtail (*Setaria viridis*) biotype in Europe. *Weed Sci.*, 52: 506–512.

Delye, C. 2005. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Sci.*, 53: 728-746.

Delye, C., Wang, T. and H. Darmency. (2002). An isoleucine–leucine substitution in chloroplastic acetyl-CoA carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. *Planta*, 214: 421-427.

Devine, M. D. (1997). Mechanisms of resistance to acetyl coenzyme A carboxylase: a review. *Pestic. Sci.*, 51:259–264.

Gill, G. S. (1990). Evaluation of a Petri dish assay for screening diclofop methyl resistance in partially resistant populations of annual ryegrass, Lolium rigidum. In: *Proc. 9th Australian Weeds Conference*, Adelaide, South Australia, pp.220-223.

Heap, I. M., Murray, B.G. Loeppky, H. A. and I. N. Morrison. (1993). Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*). *Weed Sci*, 41:232-238.

Heap, I., and R. Knight.(1986). The occurrence of herbicide cross-resistance in a annual ryegrass, *Lolium rigidum*, resistant to diclofop methyl. A. J. Agric. Res. 37: 149-156.

Heap,I.M. International Survey of Herbicide Resistant Weeds. Available (008). at: http://www.weedscience.org/summary/MOASummary.asp. Accessed 6/May/2008.

Hofer, U., Muehlebach, M., Hole, S. and A. Zoschke (2006). Pinoxaden-for broad spectrum grass weed management in cereal crops. *J. Plant Dis. Prot*, 20: 989–995.

Leach, G. E., Devine, M. D., Kirkwood, R. C. and G. Marshall (1995). Target enzyme-based resistance to acetyl-coenzyme A carboxylase inhibitors in *Eleusine indica*. *Pestic. Biochem. Physiol*, 51:129–136.

Letouze, A. and J. Gasquez (1999). A rapid reliable test for screening aryloxyphenoxypropionic acid resistance within Alopecurus myosuroides and *Lolium* spp. populations. *Weed Res.* 39:37–48.

Moss, S. R. (2005). Detecting herbiside resitance. http://www.hracglobal.com/Publications/DetectingHerbicideResistance/tabid/229/Default.aspx, Accessed 6/May/2008.

Ritz, C. and J. C. Streibig (2005). Bioassay analysis using. *J. Statistical Software*, 12: 1-22.

Tal, T., Kotoula-Syka, E.and B. Rubin (2000). Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. *Crop Protect.*, 19: 467-472.

Tosapon, P., Parnuwat, M. and U. Kenji (2006). The role of altered acetyl-CoA carboxylase in conferring resistance to fenoxaprop-P-ethyl in Chinese sprangletop (*Leptochloa chinensis* (L.) Nees). *Pest Manage. Sci.*, 62:1109–1115.

Volenberg, D. and D. Stoltenberg (2002). Altered acetyl-coenzyme A carboxylase confers resistance to clethodim, fluazifop and sethoxydim. *Setaria faberi* and *Digitaria sanguinalis Weed Res*, 42: 342-350.

Yang, C., Dong, L., Li, J. and S. R. Moss (2007). Identification of Japanese foxtail (Alopecurus japonicus) resistant to Haloxyfop using three different assay techniques. *Weed Sci.*, 55:537–540.

Zagnitko, O., Jelenska, J., Tevzadze, G., Haselkorn, R. and P. Gornicki (2001). An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanodione inhibitors. *Proc. Natl Acad. Sci. USA*, 98: 6617-6622.

Zhang, X. and M. D. Devine (2000). A point mutation in the plastidic ACCase gene conferring resistance to sethoxydim in green foxtail (*Setaria viridis*). *Weed Sci. Soc. Am. Abstr*, 40: 81.