PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Assessing Genetic Variation and Spread of *Phalaris minor* Resistant to ACCase Inhibiting Herbicides in Iran

*Avaliação da Variação Genética e Dispersão de **Phalaris minor** Resistente aos Herbicidas Inibidores da ACCase no Irã*

GHEREKHLOO, J.[1]*
ALCÁNTARA-DE LA CRUZ, R.[2]
OSUNA, M.D.[3]
SOHRABI, S.[4]
PRADO, R.[5]

ABSTRACT - Littleseed canarygrass *(Phalaris minor)* is the second most serious and problematic grass weed in wheat fields in Iran, and has developed resistance to ACCase inhibiting herbicides. Inter-simple sequence repeat (ISSR) analysis was used to assess genetic variation between and within ACCase inhibitor-resistant and susceptible *P. minor* populations in Iran and to determine the origin of resistance and its dispersal. Sixteen *P. minor* populations from different regions in Iran were analysed using seven primers. Genetic relationships generated using UPGMA analysis indicated the presence of more than one genotype among the herbicide resistant populations. The results indicated that the high genetic similarity and physical proximity among the resistant *P. minor* populations in the different regions is mainly due to cross pollination, mechanical seed dispersion and local ecological factors. These findings suggested that independent selection as well as movement of resistant seeds had occurred, which could explain the presence and dispersion of ACCase inhibitor-resistance in these populations.

Keywords: dendrogram, herbicide resistance, ISSR, genotype, UPGMA.

*RESUMO - A erva-cabecinha (**Phalaris minor**) é a segunda planta daninha mais problemática nos campos de trigo do Irã, pois tem desenvolvido resistência aos herbicidas inibidores da ACCase. Marcadores moleculares de intersequência repetitiva simples (ISSR) foram utilizados para avaliar a variação genética entre e dentro de populações resistentes e suscetíveis aos inibidores de ACCase de **P. minor** do Irã, a fim de elucidar a origem da resistência e sua dispersão. Dezesseis populações de **P. minor** de diferentes regiões do Irã foram analisadas usando sete primers ISSR. As relações genéticas obtidas a partir da análise UPGMA revelaram a presença de mais de um genótipo entre as populações resistentes aos herbicidas. Os resultados indicaram que a alta similaridade genética e proximidade física entre as populações resistentes de **P. minor** nas diferentes regiões foi devido principalmente à polinização cruzada, à dispersão mecânica de sementes e a fatores ecológicos locais. Assim, infere-se que ocorreu uma seleção independente, assim como a movimentação de sementes resistentes, explicando a ocorrência e dispersão da resistência nessas populações.*

Palavras-chave: dendrograma, resistência a herbicidas, ISSR, genótipo, UPGMA.

*** Corresponding author:**
<gherekhloo@gau.ac.ir>

Received: April 20, 2018
Approved: August 23, 2018

Planta Daninha 2020; v38:e020195618

[1] Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran; [2] Universidade Federal de São Carlos, São Carlos-SP, Brasil; [3] "Finca La Orden", Badajoz, Spain; [4] Ferdowsi University of Mashhad, Mashhad, Iran; [5] University of Cordoba, Cordoba, Spain.

Doi: 10.1590/S0100-83582020380100026

INTRODUCTION

Weed populations evolve and develop herbicide resistance through selection pressures imposed by repeated use of herbicides with the same mode of action over a period of time (Valverde et al., 2000). Herbicide resistance is defined as the inherited ability of a weed population to survive and reproduce following exposure to a herbicide that is normally lethal to the wild type of that species (Hall et al., 1997). This is a worldwide phenomenon that has affected diverse herbicide groups and is one of the main challenges in agriculture today (Abbas et al., 2016; Takano et al., 2017). Currently, 507 resistant (R) biotypes in 261 species including 152 dicotyledons and 109 monocotyledons have developed resistance to herbicides over 93 crops around the world (Heap, 2018). Resistance to acetyl-CoA carboxylase (ACCase) herbicides is documented in at least 48 grass weeds and is particularly problematic in *Lolium, Alopecurus* and *Avena* species (Kaundun, 2014). More than one million ha of wheat fields in Iran have been infested by resistant weeds including *Avena, Phalaris* and *Lolium* species which have developed resistance to ACCase inhibiting herbicides (Gherekhloo et al., 2016).

Phalaris minor (Retz.) (littleseed canarygrass), an Irano-turanian member of the genus *Phalaris*, is a diploid, predominantly in-breeding, annual grass weed which is widely distributed in Eurasia, South and North America, East and South Africa and the Australian plains (Keshavarzi et al., 2007). It is highly competitive with wheat and it has been reported to be able to cause yield losses of up to 80% in wheat crops followed by losses in rice crops in India (Singh et al., 1999, 2007; Chhokar et al., 2008; Derakhshan et al., 2014). Wheat is cultivated in more than 6.3 million ha (Iran, 2015) in Iran where it has been reported that weeds reduce the wheat yield by 23% (Gherekhloo et al., 2016). *Phalaris minor* has become a major weed in Iranian wheat fields following the green revolution due to revised agronomic practices including the adoption of poorly competitive, short-stature wheat varieties (Gherekhloo et al., 2011; Derakhshan et al., 2014). A survey showed that *P. minor* is the second most-frequent grass weed in Iranian wheat fields, where this weed has been controlled mainly using herbicides. During the last three decades ACCase-inhibiting herbicides have been widely applied in Iran to control grass weeds, including *P. minor*, in both wheat and barley fields of the country. Selective control of *P. minor* with herbicides began with the introduction of benzoylprop-ethyl (Unknown mode of action) and triallate (Inhibition of lipid synthesis) in the 1960s, followed by difenzoquat (Unknown mode of action) and acetyl coenzyme-A carboxylase-inhibiting herbicides in the 1970s, and acetolactate synthase (ALS) inhibiting herbicides in the 1980s (reviewed by Mengistu et al., 2000). However, *P. minor* has remained one of the most damaging weeds in certain parts of Iran, despite widespread annual herbicide use for its control. Previous studies showed that responses to fenoxaprop-P ethyl and diclofop methyl were variable among *P. minor* populations in the provinces of Fars and Golestan (Gherekhloo et al., 2011).

Currently, herbicide resistance studies in weeds focus on physiological and biochemical aspects. Such information can assist in the management of herbicide-resistant weeds in terms of selecting alternative herbicides and in devising resistance minimization strategies (Matthews, 1990). However, the development of herbicide resistance in weeds also requires an understanding of underlying genetic processes (Jasieniuk et al., 1996). Understanding the evolution of weed populations, as revealed by molecular genetic analyses, is of interest to developing and implement effective weed management strategies. The earliest plant genetic diversity studies used either simply inherited morphological marker traits or enzyme polymorphisms. Hence, the number of loci used to determine genetic diversity was usually small (Zietkiewicz et al., 1994), compared with more recent molecular markers based on polymerase chain reactions (PCR) that are widely used because they require small amounts of DNA and are effective and technically simple (Tanya et al., 2011). The most commonly used molecular markers to characterize interspecific hybridization and to identify genetic relationships between species are the random amplified polymorphic DNA (RAPD) (Li and Nelson, 2002), simple sequence repeat (SSR) markers (Qi et al., 2004), and inter-simple sequence repeat (ISSR) (Van der Nest et al., 2000). The length of the SSR is highly variable due to mutations that occur during replication of the repeated nucleotides. The ISSR markers are dominant markers and consist of a di- or tri- nucleotide simple sequence repeat that amplifies the region between two microsatellite repeats (Tanya et al., 2011). The advantage of ISSR over RAPDs is that the primers are longer, allowing for more stringent annealing

temperatures (Wolfe and Liston, 1998). Other advantages of ISSR markers are the high reproducibility, rapidity, simplicity and low cost of the method (Zietkiewicz et al., 1994; Imaizumi et al., 2013). ISSR, in particular, offers great potential to determine intra- and inter-genomic diversity, compared with the use of RAPD and SSR, because they reveal variation within unique regions of the genome at several loci simultaneously. ISSR markers were applied in *P. minor* with the aim of making an initial estimate of the extent of inter- and intra-population genetic variation in *P. minor* in India (McRoberts et al., 2005) and in *Brassica juncea* in China (Huangfu et al., 2009). ISSR markers have been also used to study the effect of herbicides on selection of genetic diversity within and between resistant and susceptible populations to ACCase-inhibiting herbicides of *Alopecurus mysuroides* of northern France (Menchari et al. 2007); *Schoenoplectus juncoides* from Japan (Imaizumi et al., 2013); in thiobencarb resistant and susceptible *Echinochloa oryzoides* populations from California (Osuna et al., 2011), among others.

We have previously determined the diversity of resistance to ACCase-inhibiting herbicides (specifically aryloxyphenoxypropionate (APP) inhibiting herbicides among *P. minor* populations collected from two Iranian provinces, Golestan in the north and Fars in the south. There, we applied diclofop methyl and fenoxaprop-P ethyl, which are commonly used for selective control of *P. minor* in wheat fields. Thirty-four populations were collected from the two provinces (18 from Golestan and 16 from Fars), which were subjected to a screening test in glasshouse at the recommended doses of diclofop methyl and fenoxaprop-P ethyl herbicides, separately. Results showed that among all the sampled populations, 14 populations had developed resistance to fenoxaprop-P ethyl and 7 populations to both fenoxaprop-P ethyl and diclofop methyl, showing 14 single and cross-HR phenotype combinations among collected populations with different resistance levels (Gherekhloo et al., 2011). The resistance mechanism in two of those populations, which had the highest resistance factor, was a mutation of a single nucleotide substitution (G/C) that resulted in a substitution of Trp 2027 to Cys (AR-population); and an A/G transition that resulted in a substitution of Asp 2078 to Gly (MR4-population) in the ACCase encoding gene (Gherekhloo et al., 2012).

The mutation for herbicide resistance in populations of a weed species is considered to occur independently in separate geographic regions but the spread of the R biotype within a region is generally considered a result of gene flow (Imaizumi et al., 2008, 2013). The objective of this study was to determine the genetic relationships between resistant *P. minor* populations to verify whether gene flow or independent selection were more relevant for the widespread distribution of resistance across Iran. This information may be helpful not only in the management of *P. minor* but also to develop management strategies to reduce the impact of herbicide selection.

MATERIALS AND METHODS

Plant material

Seed samples from *P. minor* populations, selected on the basis of suspected resistance to diclofop methyl (DIC) or fenoxaprop-P ethyl (FEN), were collected from locations throughout two provinces in Iran (Fars and Golestan) during 2005 and 2006, where these ACCase inhibiting herbicides had been applied for several years. As susceptible standard, two populations were collected from Fars and Golestan provinces which had never been treated with the referred herbicides.

DNA extraction

Plants from 17 populations of *P. minor*: two susceptible populations (S), three resistant to all APP herbicides and 12 populations with different level of resistance to one or two APP were used (Table 1). As already noted, these populations had been characterized previously (Gherekhloo et al., 2011). At the four-leaf stage, plants of all populations mentioned in Table 1 were sprayed with commercial formulations at the recommended field dose of DIC (900 g a.i. ha^{-1}) and FEN (67.5 g a.i. ha^{-1}), separately. Leaf tissue material from each individual plant was taken (prior to treatment), immediately frozen in liquid nitrogen and stored at -80 °C until used.

Table 1 - *Phalaris minor* collection used in ISSR genotyping (Adapted from Gherekhloo et al., 2011)

Province	Population	Longitude	Latitude	Fenoxaprop-P ethyl		Diclofop methyl	
				ED50	RF	ED50	RF
Fars	AR	52° 43’	29° 46’	357.56	9.4	2439.6	5.67
	SR3	54° 04’	28° 55’	367.89	9.66	5105	11.87
	MR4	52° 12’	29° 39’	332.01	8.72	2557.6	5.95
	FR2	57° 52’	24° 29’	85.02	2.23	819.62	1.96
	FR3	52° 48’	29° 52’	121.45	3.19	-	-
	FR4	58° 29’	24° 52’	159.42	3.18	831.72	1.93
	FR5	52° 42’	29° 40’	123.83	3.25	-	-
	FR6	52° 45’	29° 36’	145.88	3.83	-	-
	FR8	52° 46’	29° 45’	105.64	2.77	1859.2	4.32
	MR1	52° 52’	29° 50’	117.14	3.08	-	-
	MR2	53° 03’	30° 04’	79.23	2.08	-	-
	ER1	53° 89’	29° 14’	113.56	2.98	-	-
	ER2	54° 14’	29° 01’	104.73	2.75	-	-
	ES	54° 50’	28° 51’	38.08	-	430.01	-
Golestan	GR2-1	54° 08’	36° 55’	65.09	1.73	778.36	1.82
	GR4-1	54° 17’	36° 50’	42.18	1.35	-	-
	GS	54° 38’	37° 01’	37.16	-	428.39	-

ED_{50} is the dose mean of herbicide resulting in 50 percent reduction of plant growth in compare with non-treated control. RF or resistance factor is the ratio of the ED_{50} of the resistant population to the ED_{50} of the susceptible population (ED_{50} R/ED_{50}S).

DNA was extracted from those individual plants by using the Qiagen DNA Extraction Kit from 100 mg of frozen leaf material and quantified in agarose gels. In all cases, the DNA was quantified using a NanoDrop, diluted to a final concentration of 10 ng μL^{-1} and used for PCR or stored at -80 °C until used.

ISSR and data analysis

ISSR PCR reactions were conducted separately on 5 plants from each of the 16 populations with at least two replicates per plant. A total of seven ISSR primers from primer set #9 from the University of British Columbia Biotechnology Laboratory (Vancouver, Canada) were selected for DNA amplification that amplified several bands showing polymorphisms (Table 2). DNA amplifications were performed in a reaction mix containing 1 µL (10 ng μL^{-1}) of DNA; 2.5 µL of Buffer 10X; 2 µL of dNTP mix (2.5 mM); 1 µL (µL) primer and 0.2 µL (5U/µL) Taq DNA polymerase per 25 µL reaction mix. PCR amplifications were performed using a Bio-Rad thermocycler with an initial step of denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 15 s; 52 °C for 15 s; 72 °C for 30 s and finally 72 °C for 2 min. PCR products were separated on a 2% agarose gel and stained using ethidium bromide.

Table 2 - Number of loci scored and the proportion of polymorphic loci for each of the seven primers used on 16 populations

Oligo name	Primer	Loci scored	Polymorphic loci (%)
807	AGAGAGAGAGAGAGAGT	7	66.6
808	AGAGAGAGAGAGAGAGC	5	40.0
809	AGAGAGAGAGAGAGAGG	4	75.0
810	GAGAGAGAGAGAGAGAT	6	83.3
811	GAGAGAGAGAGAGAGAC	6	50.0
812	GAGAGAGAGAGAGAGAA	5	60.0
823	TCTCTCTCTCTCTCTCC	8	50.0
Total		41	

Lanes on the gels were compared and the presence or absence of bands scored. Dendrograms were constructed by UPGMA cluster analysis and genetic distances were determined based on Nei's method using TFPGA software (Tools for Population Genetic Analysis) (http://www.marksgeneticsoftware.net/). Principal co-ordinate analysis (PCA) was conducted using GENALEX software (Peakall and Smouse, 2006). This multivariate approach was chosen to complement the cluster analysis information, as cluster analysis is more accurate for closer neighbor relationships and less accurate when representing distances between groups.

RESULTS AND DISCUSSION

Resistance to ACCase inhibitors

Previous studies confirmed the resistance status of 17 populations of *P. minor*: Two susceptible populations, three resistant to all APP herbicides and 12 populations which are resistant to one or two APP (Gherekhloo et al., 2011). Table 1 indicates the geographical localization where seeds of these populations were collected; and the ED_{50} and RF values calculated when the plants were treated with different doses of FEN and DIC.

Additionally, *P. minor* populations showed that the resistance mechanism in the AR and MR4 populations was mediated by an altered and insensitive form of the ACCase enzyme. Thus, comparison of the nucleotide sequence of the B region of this enzyme from the AR population differed from the sensible population (ES) by a single nucleotide change (G/C), resulting in the substitution of the amino acid Trp 2027 to Cys in the resistant population. This substitution endowed resistance to all APP inhibiting herbicides (Liu et al., 2007; Hochberg et al., 2009; Gherekhloo et al., 2012). Moreover, sequences alignment also revealed that plants from the resistant MR4 population had an Asp-2078 to Gly substitution caused by an A/G transition causing resistance to all chemical groups of ACCase inhibiting herbicides Both single nucleotide changes described above were absent in the ES population (Gherekhloo et al., 2012).

ISSR genotyping

Five individuals from each of the seventeen populations were genotyped. The ISSR analysis produced 41 polymorphic ISSR loci using all seven primers among the individuals from the two provinces in Iran. The populations exhibited more than four polymorphic loci. The polymorphic percentage ranged from 40 to 83.3% (Table 2).

Genetic relationships among populations

Results obtained from the ISSR PCR amplification were used to establish the relationship between *P. minor* populations used in this study. The UPGMA cluster analysis based on Nei's unbiased genetic distance showed that all 16 *P. minor* populations were grouped into two main clusters (Figure 1). All the populations from the Golestan region clustered together. This cluster included a susceptible population (GS) from the Fars region. The populations from Fars region formed another cluster comprised of two subclusters. The second subcluster included the AR and MR4 populations, which presented a mutation in the *ACCase* encoding gene. The FR8 and SR3 populations were separated individually from the other populations from Fars (Figure 1).

In the PCA analysis, the two components explained 57% of the variation in the estimates of genetic similarity. Three distinct groups were revealed by the first two principal coordinates (Figure 2). Group 1 included all the populations from the Fars region. Group 2 was formed by all the populations from the Golestan region. Group 3 only included a susceptible population from the Fars region.

The genetic relationships between *P. minor* populations inferred from the ISSR banding patterns suggest that variation between samples increased with greater geographic distance. The frequency of the mutations varies according to the weed species and region and is due to the local herbicide selection pressure applied (*Délye* et al., 2010; Kaundun, 2014). There was some evidence from the sequencing data that distinct mutation in ACCase gene (MR4 and AR

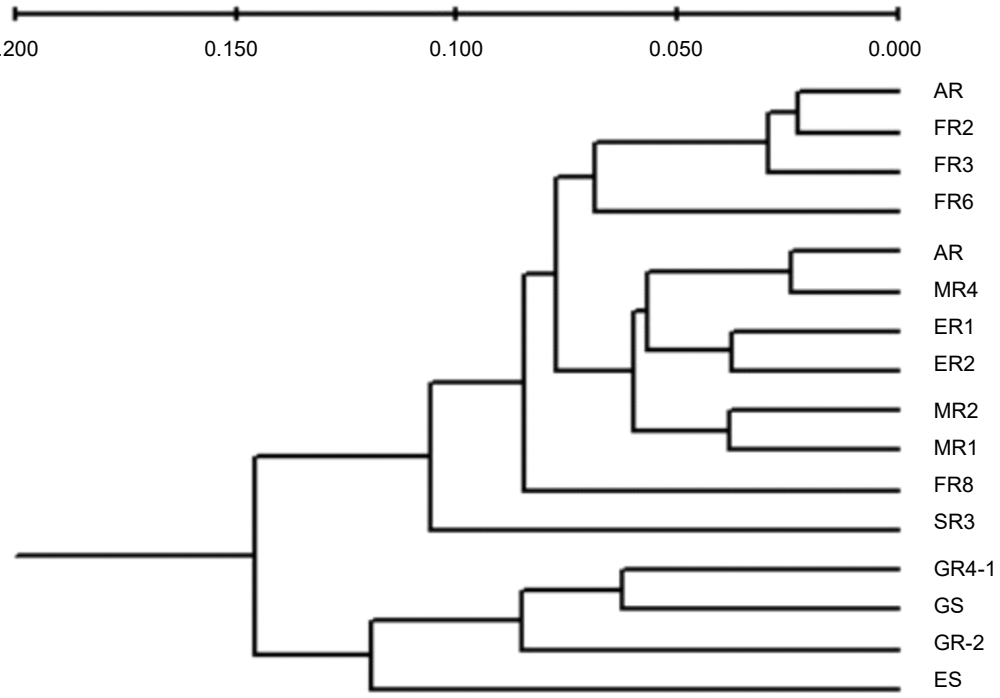

Figure 1 - Unpaired group arithmetic mean (UPGMA) dendrogram resulting from cluster analysis using Nei´s unbiased genetic distance.

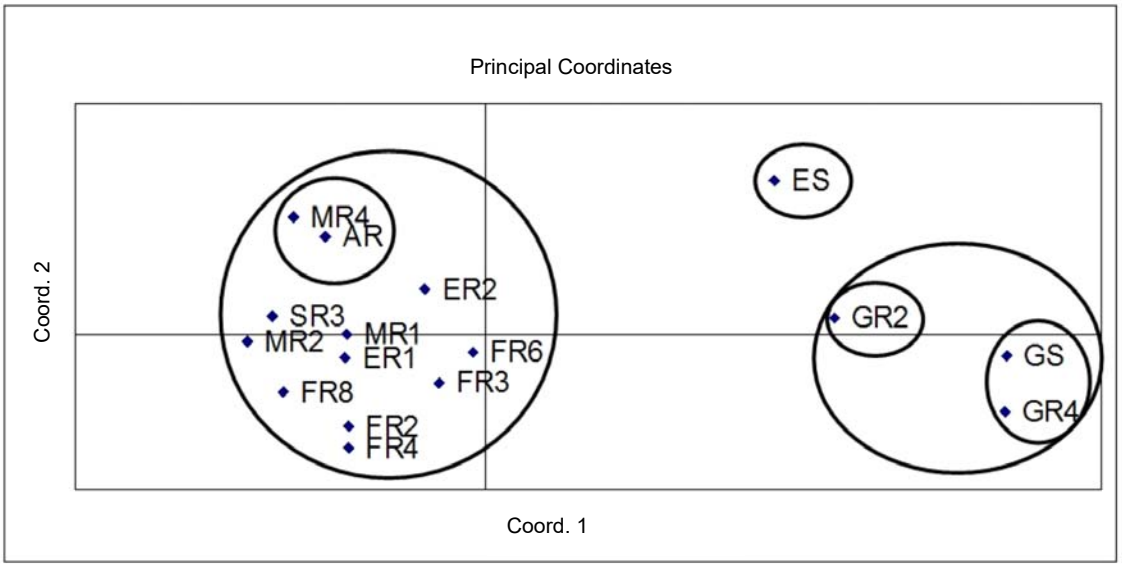

Figure 2 - Principal coordinate analysis of 16 *P. minor* populations with the first and second principal co-ordinate derived from Nei´s genetic distance matrix.

populations) clustered according to a geographical basis and that at least two different mutations conferring herbicide resistance occurred close together. Some ACCase mutations are intrinsically more prevalent (Sammons et al., 2007). For example, the I-1781-L mutation in *A. mysuroides* is predominant in the United Kingdom and France, while the G-2096-A mutation predominates in Germany (Ruiz-Santaella and Laber, 2011). *Lolium* spp. populations from the United Kingdom and Australia showed a predominance of the D2078G and I2041N mutations, respectively (Alarcón-Reverte et al., 2013; Malone et al., 2014)

The remaining resistant populations from the Fars region clustered together. In these populations, resistance to ACCase inhibiting herbicides is not target site related (data not shown).

The genetic relationships inferred by ISSR genotypes combined with the resistance mechanism data indicate that ACCase inhibitor resistance has evolved independently through selection pressures. The effect of resistance selection on genetic diversity between populations depends on the breeding system, and the size of the genome region whose diversity is reduced by selection depending on the recombination frequency (Imaizumi et al., 2013).

Herbicide resistance can spread across a cropping region as a consequence of the gene flow via pollen or mechanical seed dispersal (Maxwell et al., 1990), and by independent evolution within weed populations, which is generally due to local selection for existing mutations rather than *de novo* mutation events (Burgos et al., 2013). This explains why resistant individuals with similar genotypes were identified (mainly in Fars area), which were probably from the same progenitor and had spread by via seed movement in the area.

Genetic differentiation between susceptible *P. minor* populations (ES and GS) can also occur without herbicide selection, due to local ecological and management factors, such as tillage practices, crop and soil type, fertilizers, etc. (Imaizumi et al., 2013; Nybom and Bartish, 2000).

In conclusion, these findings indicate that the high genetic similarity and physiological proximity between *P. minor* populations resistant to ACCase-inhibiting herbicides in the different regions studied in this research was mainly due to cross pollination, mechanical seed dispersion and local ecological factors.

ACKNOWLEDGEMENTS

The authors thanks to Dr. Phillip Villani (The University of Melbourne) for the correction of the English language of this paper.

REFERENCES

Abbas T, Nadeem MA, Tanveer A, Ahmad R. Evaluation of Fenoxaprop-P-Ethyl Resistant Littleseed Canarygrass (*Phalaris minor*) in Pakistan. Planta Daninha. 2016;34(4):833-8.

Alarcón-Reverte R, Shanley S, Kaundun S, Karp A, Moss SR. ASNaPshot assay for the rapid and simple detection of known point mutations conferring resistance to ACCase-inhibiting herbicides in *Lolium* spp. Weed Res. 2013;53:12-20.

Iran. Ministry of Agriculture – Agricultural statistics Jihad. Available at: http://www.maj.ir/Portal/File/ShowFile.aspx?ID=6f66d3e3-0884-4823-b12d-6319a2edad84.(Accessed: 31 August 2015).

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK, et al. Confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci. 2013;61:4-20.

Chhokar RS, Singh S, Sharma RK. Herbicides for control of isoproturon-resistant littleseed canarygrass (*Phalaris minor*) in wheat. Crop Prot. 2008;27:719-26.

Délye C, Michel S, Berard A, Chauvel B, Brunel D, Guillemin JP, et al. Geographical variation in resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides* Huds. (black-grass). New Phytol. 2010;186:1005-17.

Derakhshan A, Gherekhloo J, Vidal RA, De Prado R. Quantitative description of the germination of Littleseed canarygrass (*Phalaris minor*) in response to temperature. Weed Sci. 2014;62:250-7.

Gherekhloo J, Rashed-Mohassel MH, Nassiri Mahallati MN, Zand E, Ghanbari A, Osuna MD, et al. Confirmed resistance to aryloxyphenoxypropionate herbicides in *Phalaris minor* populations in Iran. Weed Biol Manag. 2011;11:29-37.

Gherekhloo J, Osuna MD, De Prado R. Biochemical & molecular basis of resistance to ACCase-inhibiting herbicides in Iranian *Phalaris minor* populations. Weed Res. 2012;52:367-2.

Gherekhloo, J, Oveisi M, Zand E, De Prado R. A review of herbicide resistance in Iran. Weed Sci. 2016;64(4):551-561.

Hall LM, Moss SR, Powles SB. Mechanisms of resistance to aryloxyphenoxypropionate herbicides in two resistant biotypes of *Alopecurus myosuroides* (black-grass): herbicide metabolisms a cross-resistance mechanism. Pest Biochem Physiol. 1997;57:87-98.

Heap I. The International Survey of Herbicide Resistant Weeds. Available at: http://www.weedscience.com. 2018.

Hochberg O, Sibony M, Rubin B. The response of ACCase-resistant *Phalaris paradoxa* populations involves two different target site mutations. Weed Res. 2009;49:37-46.

Huangfu C, Song X, Qiang S. ISSR variation within and among wild *Brassica juncea* populations: implication for herbicide resistance evolution. Genet Res Crop Ev. 2009;56:913-24

Imaizumi T, Kataoka Y, Ogata S, Uchino A. Genetic diversity within and between sulfonylurea resistant and susceptible populations of *Schoenoplectus juncoides* in Japan. Weed Res. 2013;53:290-8.

Imaizumi T, Wang GX, Tominaga T. Genetic diversity of sulfonylurea-resistant and –susceptible *Monochoria vaginalis* (Burm. f.) Kunth populations in Japan. Weed Res. 2008;48:187-96.

Jasieniuk M, Brule-Babel AL, Morrison IM. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 1996;44:176-93.

Kaundun SS. Resistance to acetyl-CoA carboxylase-inhibiting herbicides. Pest Manag Sci. 2014;70:1405-17.

Keshavarzi M, Khaksar M, Seifali M. Systematic study of annual *Phalaris minor* Retz. (Poaceae) in Iran. Pak J Biol Sci. 2007;10:1336-42.

Li Z, Nelson RL. RAPD marker diversity among cultivated and wild soybean accessions from four Chinese provinces. Crop Sci. 2002;42:1737-44.

Liu WJ, Harrison DK, Chalupska D, Gornicki P, O'Donnell CC, Adkins SW, et al. Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. Proc Nat Acad Sci *USA*. 2007;104:3627-32.

Malone JM, Boutsalis P, Baker J, Preston C. Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in *Lolium rigidum* across grain cropping areas of South Australia. Weed Res. 2014;54:78-86.

Matthews JM, Holtum JAM, Liljegren DR, Furness B, Powles SB. Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*). 1. Properties of the herbicide target enzymes acetyl-coenzyme A carboxylase & acetolactate synthase. Plant Physiol. 1990;94:1180-6.

Maxwell BD, Roush ML, Radosevich SR. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technol. 1990;4:2-13.

Mcroberts N, Sinclair W, Mcpherson A, Frankea C, Saharanr P, Malik K, et al. An assessment of genetic diversity within and between populations of *Phalaris minor* using ISSR markers. Weed Res. 2005;45:431-9.

Menchari Y, Delye C, Corre VLE. Genetic variation and population structure in black-grass (*Alopecurus myosuroides* Huds.), a successful, herbicide resistant, annual grass weed of winter cereal fields. Molec Ecol. 2007;16:3161-72.

Mengistu LW, Muller-Warrant GW, Barker RE. Genetic diversity of *Poa annua* in western Oregon grass seed crops. Theor Appl Genet. 2000;101:70-9.

Nybom H, Bartish I. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol Evol Syst. 2000;3:93-114.

Osuna MD, Okada M, Ahmad R, Fischer AJ, Jasienuik M. Genetic diversity and spread of thiobencarb resistant early watergrass (*Echinochloa oryzoides*) in California. Weed Sci. 2011;59:195-201.

Peakall R, Smouse PE. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molec Ecol Notes. 2006;6:288-95.

Ruiz-Santaella J, Laber B. Mapping of herbicide resistant weeds in Great Britain, Germany and France. In: 2nd Workshop of EWRS Weed Mapping Working Group 22–23 Sep. 2011. Jokioinen, Finland: 2011..

Sammons DR, Heering DC, Dinicola N, Glick H, Elmore GA. Sustainability and stewardship of glyphosate and glyphosate-resistant crops. Weed Technol. 2007;21:347-54.

Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, et al. An integrated genetic map and a new set of simple sequence repeat markers for pearl millet *Pennisetum glaucum*. Theor Appl Genet. 2004;109:1485-93.

Singh S, Kirkwood RC, Marshall G. Biology and control of *Phalaris minor* Retz. (littleseed canarygrass) in wheat. Crop Prot. 1999;18:1-16.

Singh S. Role of management practices on control of isoproturon-resistant littleseed Canarygrass (*Phalaris minor*) in India. Weed Technol. 2007;21:339-46.

Takano HK, Oliveira JR, Constantin J, Braz GBP, Gheno EA. Goosegrass resistant to glyphosate in Brazil. Planta Daninha, 2017;35:e017163071.

Tanya P, Taeprayoon P, Hadkam Y, Srinives P. Genetic diversity among jatropha and jatropha-related species based on ISSR markers. Plant Molec Biol Rep. 2011;29:252-64.

Valverde BE, Riches CR, Caseley JC. Prevention and management of herbicide-resistant weeds in rice: Experiences from Central America with *Echinochloa colona*. Costa Rica: Cámara de Insumos Agropecuarios; 2000. 123p.

Van der Nest MA, Steenkamp ET, Wingfield BD, Wingfield MJ. Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR). Plant Breed. 2000;119:433-6.

Wolfe AD, Liston A. Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis PS, Soltis DE, Doyle JJ, editors. Molecular Systematics of Plants II. DNA sequencing. New York: Chapman Hall; 1998. p.43-86.

Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20:176-83.