Weed Turf. Sci. 3(2):110~113
http://dx.doi.org/10.5660/WTS.2014.3.2.110 Print ISSN 2287-7924, Online ISSN 2288-3312

Research Article Weed & Turfgrass Science

Weed & Turfgrass Science was renamed from formerly both Korean Journal of Weed Science from Volume 32(3), 2012, Korean Journal of Turfgrass Science from Volume 25(1), 2011 and Asian Journal of Turfgrass Science from Volume 26(2), 2012 which were launched by The Korean Society of Weed Science and The Turfgrass Society of Korea found in 1981 and 1987, respectively.

Identification of Herbicide-Resistant Barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) Biotypes in Korea

Ok Jae Won[1‡], Jeung Joo Lee[2‡], Min Yong Eom[1], Su Jeoung Suh[1], Su Hyuk Park[1], Ki Seon Hwang[1], Jong Yeong Pyon[3]*, and Kee Woong Park[1]*

[1]*Department of Crop Science, Chungnam National University, Daejeon 305-764, Korea*
[2]*Department of Applied Biology, IALS, Gyeongsang National University, Jinju 660-701, Korea*
[3]*ReSEAT Program, Korea Institute of Science and Technology Information, Daejeon 305-806, Korea*

ABSTRACT. The continuous use of acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase) inhibitors has led to the selection of herbicide resistant barnyardgrass populations in direct-seeded rice fields of Korea. This study was conducted to identify herbicide resistant barnyardgrass biotypes and to determine the cross- and multiple-resistance of them. 25% of the population collected from Taeahn was partially resistant to ACCase inhibitors and 22% collected from Kimjae were partially resistant to ALS inhibitors. However, 8.2% of the population from both sites was resistant to ALS and ACCase inhibitors. Resistance to sulfonylurea herbicide, flazasulfuron was identified from two barnyardgrass accessions collected from both Taeahn and Kimjae. One barnyardgrass accession from both sites was resistant to ACCase inhibitor, sethoxydim. The cross-resistance to ALS inhibitors was identified at one barnyardgrass accession from Taeahn and at two accessions from Kimjae. Further, cross-resistance to ACCase inhibitors was also identified at barnyardgrass accessions from Taeahn and Kimjae. Multiple-resistance to flazasulfuron and sethoxydim was determined at four barnyardgrass accessions from Taeahn and at six accessions from Kimjae. Therefore, the herbicide mixture and sequences within a growing season or the herbicide rotation with different modes of actions across growing seasons are recommended to control herbicide-resistant barnyardgrass in infested fields.

Key words: Acetolactate synthase (ALS), Acetyl coenzyme A carboxylase (ACCase), Barnyardgrass, Herbicide-resistance

Received on May 29, 2014; Revised on June 3, 2014; Accepted on June 9, 2014
***Corresponding author:** Phone) +82-42-821-5726, Fax) +82-42-822-2631; E-mail) parkkw@cnu.ac.kr
Phone) +82-42- 869-0682, Fax) +82-42-869-0699, E-mail) jypyon@cnu.ac.kr
‡Author Contributions: These authors contributed equally to the paper.

Introduction

The use of herbicides in crop production revolutionized weed control and played a major role in increasing agricultural productivity and efficiency. Herbicide-resistant biotypes of a weed species is the population of plants that is not controlled by the normal application rate of an herbicide. Heavy reliance on a few herbicides resulted in the evolution of herbicide resistance in crop fields. The continuous use of the same herbicide or herbicides acting on the same target site leads to the selection of herbicide resistant weed populations (Maxwell and Mortimer, 1994). Today, resistant populations against many different classes of herbicides can be found throughout the world. In Korea, herbicide resistance selected with acetolactate synthase (ALS) inhibitors was first reported in *Monochoria korsakowii* in 1998 (Park et al., 1999). Since then, 12 weed species have developed resistance to ALS inhibitors (Heap, 2012; Park et al., 2010).

Barnyardgrass (*Echinochloa crus-galli* var. *crus-galli* L.) is one of the most troublesome weed species in rice and upland crop fields, causing serious yield losses and therefore, requiring its control in Korea. ALS inhibiting herbicides were widely used for sedge and broadleaf weed control, and acetyl CoA carboxylase (ACCase) herbicides were applied to control grass weed species. Herbicides inhibiting ALS or ACCase enzymes have been successfully used to control barnyardgrass in rice fields of Korea. However, as the inhibitors of ALS and ACCase have been used widely and continuously, herbicide resistance has evolved rapidly. The repeated use of ALS or ACCase inhibiting herbicides in rice fields has resulted in biotypes of resistant barnyardgrass in 2009 (Im, 2009).

In the Sacramento Valley of California, however, herbicide resistance in *Echinochloa phyllopogon* has been found, and the resistant population is resistant to multiple herbicides with different modes of action and different chemical groups, such as bispyribac-sodium, fenoxaprop, molinate and thiobencarb (Fischer et al., 2000). Recent findings have shown that the resistant population also exhibits resistance to bensulfuron-methyl, penoxsulam and cyhalofop-butyl (Yasuor et al., 2009; Ruiz-Santaella et al., 2006; Osuna et al., 2002).

Hence, this study was conducted to identify barnyardgrass populations resistant to ALS and/or ACCase inhibitors in direct-seeded rice fields in Korea and to determine the cross-resistance and multiple-resistance of barnyardgrass biotypes.

Materials and Methods

Seed source of barnyardgrass

A road survey of barnyardgrass plants was conducted in direct-seeded rice fields during October 2012 in Korea. Mature seeds of barnyardgrass, before rice harvest, were collected from 10 plants, each from 15 sites (155 accessions) at five locations (Kimjae, Taeahn, Daejeon, Gongju and Yesan) where barnyardgrass had failed to be controlled in the rice paddy fields (Figure 1). Barnyardgrass seeds were immersed in 4ºC water for 30 days in order to remove the germination inhibitors of seeds.

Fig. 1. Monitoring of herbicide resistant barnyardgrass biotypes infested in the rice fields in Kimjae, Korea in 2012.

Whole-plant herbicide resistance assay

In separate experiments, 155 *Echinochloa* accessions were screened in order to identify the possible resistant accessions to flazasulfuron (sulfonylurea herbicide), imazaquin (imidazolinone herbicide), sethoxydim (cyclohexanedione) and fluazifop-p-butyl (aryloxyphenoxypropanoate). The selected resistant barnyardgrass accessions were further evaluated for cross- or multiple-resistance to flazasulfuron, imazaquin and sethoxydim and fluazifop-p-butyl. All whole-plant trials were carried out in plastic pots (10 cm diameter) filled with potting mix. Plants were grown in a growth chamber at temperature of 30/25ºC and 16/8 h day/night. Two ALS inhibiting herbicides (sulfonylurea, SU; imidazolinone, IMI) and two ACCase inhibiting herbicides (cyclohexanedione, CHD; aryloxyphenoxypropanoate, AOPP) were used to determine the cross- and multiple-resistance patterns of the barnyardgrass populations.

After sowing the barnyardgrass seeds in pots, flazasulfuron (SU) and imazaquin (IMI) were applied at a rate of 90 and 180 g ai ha^{-1}, respectively, and sethoxydim (CHD) and fluazifop-p-butyl (AOPP) were applied at a rate of 175 and 300 g ai ha^{-1}, respectively, at two weeks after the emergence of barnyardgrass. Herbicide resistance of barnyardgrass seedlings was rated visually at two weeks after treatment and was assessed on a S, r, and R scale where S = death or necrosis of all plant tissues; r = visible symptoms with possible reduction in biomass; R = very mild symptoms or no visible injury.

Results and Discussion

It was determined that among 155 barnyardgrass accessions collected from 15 sites at five locations, 42 barnyardgrass accessions were resistant to at least one of ALS or ACCase inhibiting herbicides (Table 1 and 2). The assay results indicated that 25% of the population collected from both Taeahn and Kimjae were partially resistant to ACCase inhibitors, and 22% collected from Kimjae were partially

Table 1. Number of barnyardgrass accessions resistant to ALS and / or ACCase inhibitors.

Sites	No. of accessions	ALS inhibitors			ACCase inhibitors			ALS/ACCase
		SU[†]	IMI	SU/IMI	CHD	AOPP	CHD/AOPP	
Taeahn	63	6	1	1	14	3	2	4
Kimjae	59	12	6	5	13	3	1	6
Other area	33	3	0	0	0	0	0	0
Total	155	21	7	6	27	6	3	10

[†]SU: flazasulfuron; IMI: imazaquin; CHD: sethoxydim; AOPP: fluazifop-p-butyl.

Table 2. Barnyardgrass biotypes resistant to ALS and /or ACCase inhibitors collected from the Mid-west regions of Korea in 2012 (42 accessions out of 155 accessions).

Sites	Accession No.	Herbicides			
		SU[†]	IMI	CHD	AOPP
Taeahn	T1-03	S[††]	S	r	S
	T2-01	S	S	R	r
	T2-04	S	S	r	S
	T3-01	S	S	r	S
	T3-02	S	S	r	r
	T3-06	R	S	r	S
	T3-10	S	S	r	S
	T3-13	S	S	r	S
	T4-01	S	S	S	r
	T4-06	S	S	r	S
	T5-02	S	S	r	S
	T5-04	S	S	r	S
	T5-05	R	S	r	S
	T5-08	r	r	S	S
	T5-12	r	S	S	S
	T5-15	r	S	r	S
	T5-20	r	S	r	S
Kimjae	K1-06	R	r	S	S
	K1-07	S	S	r	S
	K1A-01	S	S	R	r
	K1A-06	S	S	S	r
	K1-A08	r	S	S	S
	K1-A09	r	S	S	S
	K2-01	S	S	r	S
	K2-02	S	S	r	S
	K2-03	S	r	S	S
	K2-06	R	S	r	S
	K2-07	S	S	r	S
	K2-09	r	S	S	S
	K2-10	r	r	r	S
	K3-01	r	r	r	S
	K3-04	r	S	r	S
	K4-01	S	S	r	S
	K5-01	R	R	r	S
	K5-04	r	r	S	S
	K5-05	r	S	r	S
	K5A-05	S	S	S	r
	K5A-06	S	S	r	S
	K5A-07	r	S	S	S
Other area	C1-04	r	S	S	S
	Y1-03	r	S	S	S
	Y2-07	R	S	S	S

[†]SU: flazasulfuron; IMI: imazaquin; CHD: sethoxydim; AOPP: fluazifop-p-butyl; [††]R: highly resistant; r: moderately resistant; S: susceptible.

resistant to ALS inhibitors. However, 8.2% of the population from both sites was resistant to ALS and ACCase inhibitors.

ALS inhibitors (sulfonylurea and imidazolinone herbicides) and ACCase inhibitors (cyclohexanedione, aryloxyphenoxypropionate herbicides) were used to determine the cross- and multiple-resistance of barnyardgrass. Barnyardgrass highly resistant to sulfonylurea herbicide (flazasulfuron) was identified from two accessions (Accession no. T3-06 and T5-05) from Taeahn and three accessions (Accession no. K1-06, K2-06 and K5-01) from Kimjae (Table 2). One barnyardgrass accession (Accession no. T2-01) from Taeahn and one accession (Accession no. K1A-01) from Kimjae were highly resistant to ACCase herbicide (sethoxydim).

Cross-resistance is defined as a population that is resistant to two or more herbicides due to a single mechanism of resistance. Cross-resistance to ALS inhibiting herbicides was identified at one barnyardgrass accession (Accession no. T5-08) from Taeahn and five accessions (Accession no. K1-06, K2-10, K3-01, K5-01 and K5-04) from Kimjae, and cross-resistance to ACCase inhibiting herbicides was also identified at two barnyardgrass accessions (Accession no. T2-01 and T3-02) from Taeahn and one accession (Accession no. K1A-01) from Kimjae (Table 2).

The biotypes of *E. crus-galli* var. *crus-galli* demonstrated resistance to cyhalofop-butyl and were also confirmed to be cross-resistant to other ACCase inhibiting herbicides, fenoxaprop-p-ethyl and metamifop (Im et al., 2009). Kaloumenos et al (2013) reported that *Echinochloa oryzicola* accessions were found to be cross-resistant to penoxsulam, bispyribac-sodium, imazamox, foramusulfuron, nicisulfuron and rimsulfuron. *E. oryzicola* cross-resistance to ALS-herbicides was due to the result of Trp574Leu mutation. Barnyardgrass biotypes from Arkansas (AR1 and AR2) and Mississippi (MS1) have evolved cross-resistance to ALS-inhibiting herbicides, imazamox, imazethapyr, penoxsulam and bispyribac-sodium (Riar et al., 2013). The cross-resistance between two or more herbicides in an herbicide resistant weed biotype is conferred by a single mechanism. The target-site resistance is the major cause of herbicide resistance to ALS- and ACCase-inhibiting herbicides, and the expression levels of ALS and ACCase genes were higher in organs containing metabolically active meristems (Iwakami et al., 2012).

Multiple-resistance in an herbicide resistant biotype is defined by two or more mechanisms, and is usually the result of a sequential herbicide site of action selection or the accumulation of resistant alleles. Multiple-resistance to ALS inhibiting herbicide (flazasulfuron) and ACCase inhibiting herbicide (sethoxydim) were identified at four barnyardgrass accessions (Accession no. T3-06, T5-05, T5-15 and T5-20) from Taeahn and six accessions (Accession no. K2-06, K2-10, K3-01, K3-04, K5-01 and K5-05) from Kimjae (Table 2).

The continuous use of these herbicides in rice fields will increase the population of herbicide-resistant barnyardgrass. *E. oryzicola* accession from rice grown in California has developed multiple-resistance to the herbicides molinate, thiobencarb, clomazone, fenoxaprop-ethyl and bispyribac-sodium (Fischer et al., 2000).

Out of 155 barnyardgrass accessions, 42 accessions were resistant to at least one of ALS and ACCase inhibitors. Six and three accessions were cross-resistant to ALS and ACCase inhibitors, respectively. In case of cross-resistance, herbicide mixtures and sequences within a growing season, or the herbicide rotation with different modes of actions across growing seasons, are recommended to manage the herbicide-resistant barnyardgrass populations. However, 10 accessions of barnyardgrass showed multiple-resistance to both ALS and ACCase inhibitors indicating reduced herbicide options to rotate. To better understand and manage these barnyardgrass populations, dose response and resistance mechanism of each accession should be conducted in future.

Acknowledgement

This study was financially supported by research fund of Chungnam National University in 2012.

References

Fischer, A.J., Ateh, C.M., Bayer, D.E. and Hill, J.E. 2000. Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. Weed Sci. 48(2):225-230.

Heap, I. 2012. International survey of herbicide resistant weeds. http://www.weedscience.com (Accessed on Sep. 10. 2012).

Im, I.B. 2009. Control and emergence of herbicides resistant *Echinochloa oryzicola* in paddy field of Korea. Korean J. Weed Sci. 29(2):103-104. (In Korean)

Im, S.H., Park, M.W., Yook, M.J. and Kim, D.S. 2009. Resistance to ACCase inhibitor cyhalofop-butyl in *Echinochloa crus-galli* var. *crus-galli* collected in Seosan, Korea. Korean J. Weed Sci. 29(1):178-184. (In Korean)

Iwakami, S., Uchino, A., Watanabe, H.W. and Yamasue, Y. 2012. Isolation and expression of genes for acetolactate synthase and acetyl-CoA carboxylase in *Echinochloa phyllopogon*, a polyploidy weed species. Pest Manag. Sci. 68(7):1098-1106.

Kaloumenos, N.S., Chatzilazaridou, S.L., Mylona, P.V., Polidoros, A.N. and Eleftherohorinos, L.G. 2013. Target-site mutation associated with cross-resistance to ALS-inhibiting herbicides in late watergrass (*Echinochloa oryzicola* Vasing.). Pest Manag. Sci. 69(7):865-873.

Maxwell, B.D. and Mortimer, A.M. 1994. Selection for herbicide resistance. In: Powles, S. B. and J. A. M. Holtum (eds): Herbicide resistance in plants; Biology and biochemistry. Lewis Publishers, Florida, USA. pp. 1-26.

Osuna, M.D., Vidotto, F., Fischer, A.J., Bayer, D.E. and Ferrero, A. 2002. Cross-resistance to bispyribac-sodium, and bensulfuron-methyl in *Echinochloa phyllopogon* and *Cyperus difformis*. Pestic. Biochem. Physiol. 73(1)9-17.

Park, T.S., Kim, C.S., Park, J. E., Oh, Y.K. and Kim, K.U. 1999. Sulfonylurea-resistant biotype of *Monochoria korsakowii* in reclaimed paddy fields in Seosan, Korea. Korean J. Weed Sci. 19(4):340-344.

Park, T.S., Ku, B.I., Kang, S.K., Choi, M.K., Park, H.K., Lee, K.B. and Ko, J.K. 2010. Response of the resistant biotype of *Echinochloa oryzoides* to ACCase and ALS inhibitors and effect of alternative herbicides. Korean J. Weed Sci. 30(3):291-299. (In Korean)

Riar, D.S., Norsworthy, J.K., Srivastava, V.S., Nandula, V.N., Bond J.A. and Scott R.C. 2013. Physiological and molecular basis of acetolactate synthase-inhibiting herbicide resistance in barnyardgrass (*Echinochloa crusgalli*). J. Agric. Food Chem. 61(2):278-289.

Ruiz-Santaella, J.P., Prado, R.D., Wagner, J., Fischer, A.J. and Gerhards, R. 2006. Resistance mechanism to cyhalofop-butyl in a biotype of *Echinochloa phyllopogon* from California. J. Plant Dis. Protec. 20: 95-100.

Yasuor, H., Osuna, M.D., Ortiz, A., Saldain, N.E., Eckert, J.W. and Fischer, A.J. 2009. Mechanism of resistance to penoxsulam in late watergrass (*Echinochloa phyllopogon*). J. Agric. Food Chem. 57(9):3653-3660.