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Control of Sulfonylurea Herbicide-Resistant *Lindernia dubia* in Korean Rice Culture

Yong In Kuk*†

*Biotechnology Research Institute, Chonnam National University, Gwangju 500-757, Korea

ABSTRACT ; A *Lindernia dubia* (L.) Pennell var. *dubia* accession from Jeonnam province, Korea was tested for resistance to sulfonylurea (SU) herbicides, imazosulfuron and pyrazosulfuron-ethyl in whole-plant response bioassay. The accession was confirmed resistant to both herbicides. The GR_{50} (herbicide concentration that reduced shoot dry weight by 50%) values of resistant accession were 264 and 19 times higher to imazosulfuron and pyrazosulfuron-ethyl, respectively, than that of the standard susceptible accession. The surviving resistant *L. dubia* after pyrazosulfuron-ethyl + molinate application can be controlled by sequential applications of soil-applied herbicides, butachlor, dithiopyr, pyrazolate, and thiobencarb and foliar herbicides, bentazon. Sulfonylurea-based mixtures such as mixtures of azimsulfuron + anilofos, bensulfuron-methyl + oxadiazon, pyrazosulfuron-ethyl + fentrazamide, and pyrazosulfuron-ethyl + anilofos + carfentrazon can also be used to control the surviving resistant *L. dubia*. However, use of these mixtures should be restricted to a special need basis. Thus, we suggest that sequential applications of non- SU-based mixtures such as butachlor + pyrazolate and MCPB + molinate + simetryne be used to control the surviving resistant *L. dubia* after SU herbicide applications. Rice yield was reduced 24% by resistant *L. dubia* that survived after the pyrazosulfuron-ethyl + molinate application compared with pyrazolate + butachlor in transplanted rice culture. *In vitro* ALS activity of the resistant biotype was 40 and 30 times more resistant to imazosulfuron and pyrazosulfuron-ethyl, respectively, than the susceptible biotype. Result of *in vitro* ALS assay that the resistance mechanism of *L. dubia* to SU herbicides may be due, in part, to an alteration in the target enzyme, ALS.

Keywords : Acetolactate synthase (ALS) inhibitor, *Lindernia dubia* (L.) Pennell var. *dubia*, sulfonylurea-resistant weed, rice.

Sulfonylurea (SU) herbicide-based mixtures have been widely used in the paddy fields of Korea and Japan to manage broadleaf and grass weeds. Repeated use of the same SU herbicide-based mixtures resulted in the development of resistance in several rice weed species. In 1987, the first case of resistance to SU-herbicide was reported in *Lactuca serriola* L. (prickly lettuce) in monoculture *Triticum aestivum* L. (wheat) fields treated with the same herbicides for five years (Mallory-Smith *et al.*, 1990). Incidence of resistance has increased due to repeated use of the same herbicides. Resistance to acetolactate synthase (ALS; EC 4.1.3.18) inhibitors, such as sulfonylurea, imidazolinone, triazolopyrimidine sulfonanilide, sulfamoylurea, and pyrimidinyl thiobenzoate has been reported in over 70 plants throughout the world (Heap, 2002). Most of the SU-resistant weeds were found in fields where dryland winter *T. aestivum* was grown and where chlorsulfuron or chlorsulfuron + metsulfuron-methyl had been applied for 3 to 5 years (Thill *et al.*, 1991). Since 1992, SU-resistant weeds (*Cyperus difformis* L. and *Sagittaria montevidensis* L. ssp. *calycina*) were also found in paddy fields in the U.S. (Pappas-Fader *et al.*, 1993) and Japan (*Monochoria korsakowii* Regel *et* Maack, *Limnophila sessiliflora* Blume, *Lindernia* spp, and *Scirpus juncoides* Roxb.) (Itoh *et al.*, 1997; Kohara *et al.*, 1998; Wang *et al.*, 1997; Wang *et al.*, 2000). In addition, SU-resistant biotypes of *M. korsakowii* (Park *et al.*, 1999), *M. vaginalis* (Kwon *et al.*, 2000), L. *dubia* (Park *et al.*, 2000), and *Rotala indica* (Willd.) Koehne (Kwon *et al.*, 2001) were observed recently in paddy fields in Korea. Among these resistant weeds, *L.* spp. including *L. procumbens, L. dubia, L. dubia* var. major, and *L. micrantha* were also confirmed to be resistant to sulfonylureas based on their whole-plant herbicide dose-response relationships (Itoh *et al.*, 1997; Itoh *et al.*, 1999; Uchino *et al.*, 1997) in Japan. However, date, only *L. dubia* Pennell var. dubia among *L.* spp. was confirmed to be resistant to sulfonylurea herbicides in Korea (Park *et al.*, 2000). *L. dubia* is distributed in paddies and swamps to warm-temperature regions of southern Korea (Park *et al.*, 2000). Rice paddy fields infested by the resistant biotype of the *L. dubia* have been found throughout the province of Jeonnam, Korea. These paddy fields were in monoculture rice production using SU herbicide-based mixture for eight consecutive years since 1990. Resistant *L. dubia* can grow as vigorously as the susceptible biotypes and, if left uncontrolled, may cause serious yield loss in direct-seeded or transplanted rice.

The SU herbicides inhibit ALS, the first enzyme that catalyzes the biosynthesis of branched-chain amino acids, valine, leucine, and isoleucine (Brown, 1990; Ray, 1984). In most

†Corresponding author: (Phone) +82-62-530-2052 (E-mail) yikuk@chonam.chonnam.ac.kr <Received August 16, 2002>

cases, resistance to ALS-inhibiting herbicides is due to an insensitive site of action caused by point mutation(s) in the ALS gene (Hwang *et al.*, 2001; Lovell *et al.*, 1996). On the other hand, a biotype of *Lolium rigidum* Gaud. (annual ryegrass) shows metabolism-based resistance to the SU herbicide, chlorsulfuron (Christopher *et al.*, 1991).

This research was conducted to (1) confirm resistance of *L. dubia* from Korea to imazosulfuron and pyrazosulfuron-ethyl; (2) determine the effect of resistant *L. dubia* on yield of transplanted rice; (3) identify alternative herbicides for the control of resistant *L. dubia*; and (4) determine whether ALS enzyme insensitivity is the mechanism of resistance.

MATERIALS AND METHODS

Plant materials

Seeds of suspected SU-resistant *L. dubia* were collected from locations throughout Jeonnam province, Korea in 2000. The fields where samples were collected had been treated with SU herbicide-based mixture, mainly pyrazosulfuron-ethyl + molinate, for eight consecutive years. *L. dubia* seeds were also collected from paddy fields untreated with herbicides in the city of Daejon, hereafter referred to as "susceptible biotype". To break dormancy, seeds were stored at 4°C for one month.

Response to SU herbicides

Seeds were sown in plastic pots (280 cm^2 surface area) filled with paddy soil (clay loam), placed in a greenhouse maintained at 30/25°C and a 14/10 h day/night period. Two-leaf seedlings, growing in 3-cm deep simulated flood, were treated with two SU herbicides, imazosulfuron (0.6, 1.2, 2.3, 4.7, 9.4, 18.8, 37.5, 75, 150, 300, 600, 1,200, and 4,800 g ai ha^{-1}) and pyrazosulfuron-ethyl (0.3, 0.7, 1.3, 2.6, 5.3, 10.5, 21, 42, 84, 168, and 336 g ai ha^{-1}). The recommended rates of imazosulfuron and pyrazosulfuron-ethyl are 75 and 21 g ai ha^{-1}, respectively. Thirty days after application (DAA), the plants were cut at the soil surface, oven-dried for 48 h at 60°C, and shoot dry weights were recorded. Experiments were repeated two or three times. Data were expressed as percentage of untreated control. Logistic equations:

Equation 1:

$$Y = \frac{a}{1 + (X/X_0)^b}$$

was used to describe the response of suspected SU-resistant *L. dubia* accession to SU herbicides using Statistical Analysis Systems (SAS, 2000) software. Fitted regression equations are shown in Fig. 1. GR_{50} values were calculated from the regression equations.

Rice yield as affected by SU-resistant *L. dubia*

A field experiment was conducted in a paddy field infested with herbicide-resistant *L. dubia* at Jeonnam Agricultural Research and Extension Service, Korea. The field had been treated with SU-herbicide based mixtures of pyrazosulfuron-ethyl + molinate for eight consecutive years. Rice "Ilmibyeo", 10-day-old seedlings were transplanted on May, 2001 by hand at a distance of 15 cm between plants and 30 cm between rows. Plot size was 4 m by 5 m. The experimental design was a randomized complete block with three replications. Urea fertilizer was used at 110 kg N ha^{-1}. Fifty percent of N was applied pre-plant incorporated (PPI), 30% at 5-leaf stage, and 20% at panicle formation stage. Phosphorus and potassium were also applied PPI at 70 and 80 kg ha^{-1}, respectively. Other cultural management practices were carried out in accordance with the standard rice cultivation method of the Rural Development Administration (RDA, 1998). The herbicides tested (Tables 1 and 2) were applied at recommended rates at 10 days after transplanting (2-leaf *L. dubia*). The number of survivors and shoot dry weight m^{-2} were recorded 50 DAA. Rice yield samples were harvested from three, 4.2-m^2 quadrants per plot. Yield parameters number of panicles, spikelets per panicle, ripened grain (%), and 1,000 grain weight were determined from these samples. Rough rice yield was calculated based on grain weight adjusted to 15% moisture. Data was analyzed using analysis of variance (ANOVA) procedure in SAS (2000) program. Treatment means were separated using Fishers protected LSD (P=0.05).

Control of resistant *L. dubia* by non-ALS inhibitor herbicides

A field experiment was conducted in a paddy field infested with herbicide-resistant *L. dubia* at Jeonnam Agricultural Research and Extension Service, Korea. Eight herbicides with different modes of action were tested for their efficacy on SU-resistant *L. dubia*. The herbicides used are shown in Table 3. Pyrazosulfuron-ethyl + molinate was applied 10 days after transplanting followed by butachlor, dimepiperate, dithiopyr, esprocarb, molinate, pretilachlor, pyrazolate, and thiobencarb granule were applied 20 or 30 days after transplanting (DAT) at respective 1.5 and 4.5 leaf-stage of *L. dubia*. However, bentazon soluble concentrate was applied 40 DAT, at 5.5 to 6-leaf stage of *L.dubia*. Plot size was 1 m by 1.2 m. The experimental design was a randomized complete block with three replications. The number of survivors, shoot dry weight, and rice injury were recorded 40 DAA. Visual ratings for rice injury were recorded using a scale of 0 to 9, where 0 equals no injury and 9 equals complete kill. Other procedures were the same as those described in above section, rice yield as affected by SU-resistant *L. dubia.*

Control of resistant *L. dubia* by SU herbicide-based mixtures

Seven SU herbicide-based mixtures and two non-SU herbicide-based mixtures were tested for efficacy on SU-resistant *L. dubia.* The herbicides used are shown in Table 4. Other procedures were the same as those described in above section, rice yield as affected by SU-resistant *L. dubia.*

In vitro ALS assay

Enzyme extraction and assay were done based on the method of Ray (1984), with modifications. Four grams of leaf tissue harvested from young seedling were frozen in liquid nitrogen and ground in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate (TPP), 10 μM flavin adenine dinucleotide, 0.5% polyvinylpyrrolidone, and 10% (v/v) glycerol. The homogenate was filtered through four layers of Miracloth, then centrifuged at 20,000 g for 20 min at 4°C. ALS in the supernatant was precipitated by ammonium sulfate 45%. The crude extract was placed on ice for 30 min and centrifuged at 20,000 g for 20 min at 4°C. The pellet was suspended in 1 ml elution buffer. The extract was desalted on Sephadex G-25 column (PD-10) equilibrated with elution buffer [70 mM potassium phosphate buffer (pH 7.5) containing 80 mM sodium pyruvate, 0.5 mM $MgCl_2$, and 0.5 mM TPP], and the crude enzyme preparation was collected in 2.5 ml tube. Two hundred μl of enzyme extract and 10 μl of herbicide solution were added to 790 μl of assay buffer [70 mM potassium phosphate buffer (pH 7.5) containing 80 mM sodium pyruvate, 0.5 mM $MgCl_2$, and 0.5 mM TPP], and the solution was incubated at 37°C for 1 h. The final concentration of herbicides in the assay solutions were 0, 0.001, 0.01, 0.1, 1, 10, and 100 μM (imazosulfuron and pyrazosulfuron-ethyl). At the end of reaction time, 50 μl of 6 N H_2SO_4 was added to 500 μl of each solution and the solutions were incubated at 60°C for 30 min to convert acetolactate to acetoin (pink color). To evaluate the color change due to ALS activity, 50 μl of 2 N NaOH was added to 500 μl of the remaining supernatant instead of H_2SO_4, and a reference absorbance was measured according to the following procedure. Acetoin was quantified by a modified colorimetric assay (Westerfeld, 1945). The color was developed by adding 0.5 ml of 0.5% (w/v) creatine and 0.5 ml of 5% (w/v) α-naphthol prepared in 2.5 N NaOH, then heated at 60°C for 15 min. After cooling, absorbance was measured by spectrophotometer at 530 nm. Total protein content in crude ALS extracts was determined by the method of Bradford (1976).

Data was analyzed by using nonlinear regressions, Logistic equation 1, using the SAS (2000) program. I_{50} values were calculated from the regression equations.

RESULTS AND DISCUSSION

Confirmation of resistance

Accession originated from paddy fields used for monoculture rice production, which were routinely treated with SU

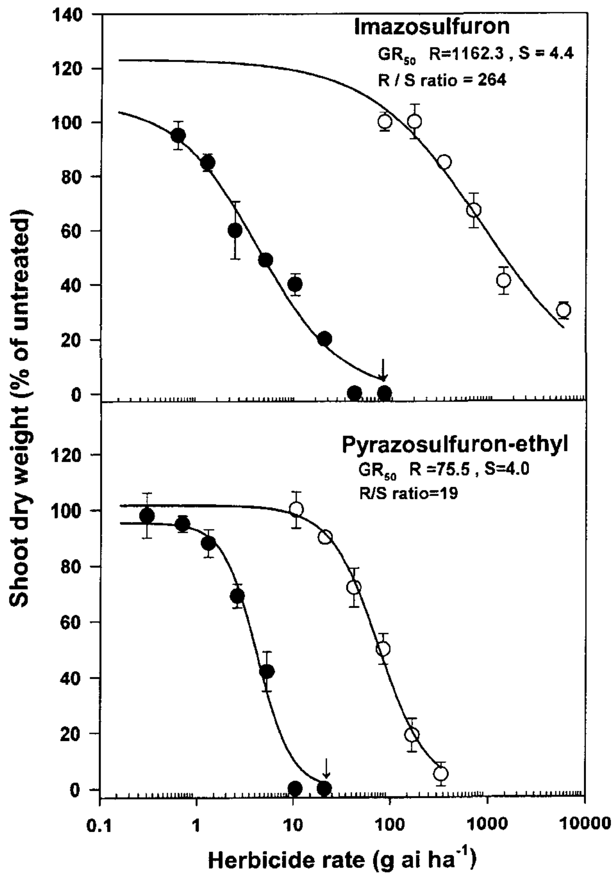

Fig. 1. Shoot dry weight of susceptible (●) and resistant (○) biotypes of herbicides in whole-plant response assay. The herbicides were applied to 2-leaf *Lindernia dubia* (L.) and shoot dry weight was determined 30 days after application. Vertical bars represent standard errors of the mean. Arrow (↓) indicates the recommended rate of imazosulfuron (75 g ai ha^{-1}) and pyrazosulfuron-ethyl (21 g ai ha^{-1}) for rice paddy fields in Korea. Regression equations for imazosulfuron were $Y=107.2/[1+(X/3.9)^{1.03}$, $R^2=0.97$ for susceptible and $Y=123.2/[1+(X/732.2)^{0.8}$, $R^2=0.96$ for resistant biotype. Regression equations for pyrazosulfuron-ethyl were $Y=95.5/[1+(X/4.2)^{2.4}$, $R^2=0.98$ for susceptible and $Y=101.4/[1+(X/75.7)^{1.7}$, $R^2=0.99$ for resistant biotype.

herbicide-based mixtures for 8 consecutive years since 1990. *L. dubia* in the fields has been controlled by SU herbicides for seven years. However, efficacy of the herbicides on *L. dubia* had declined greatly since 1997 and *L. dubia* has become the dominant weed in these fields. This observation indicates that resistant biotype of *L. dubia* has evolved in these fields.

The accession exhibited higher levels of resistance to imazosulfuron and pyrazosulfuron-ethyl in whole-plant response assay (Fig. 1). The susceptible biotype of *L. dubia* was completely controlled with 1/2 of the respective recommended rates of the aforementioned herbicides, but the resistant biotype was not completely controlled by 16 to 32 times the recommended rates. The resistant biotype based on GR_{50} was 265 and 19 times more resistant to imazosulfuron and pyrazosulfuron-ethyl, respectively, than the susceptible biotype. Similar to our result, *L. dubia* was reported resistant to SU- herbicides in Japan (Uchino *et al.*, 2000).

Rice yield as affected by SU-resistant *L. dubia*

Results of field experiments showed that SU-resistant *L. dubia* in transplanted fields was only controlled 16% by pyrazosulfuron-ethyl + molinate, but was completely inhibited by the recommended rates of pyrazolate + butachlor in transplanted rice culture (Table 1). The densities (plants m^{-2}) of *L. dubia* in transplanted rice paddies were 1,683 in untreated plots 50 DAT. SU-resistant *L. dubia* was the main weed species in these fields. Rice yield in plots treated with pyrazosulfuron-ethyl + molinate was similar to yield from untreated plots in transplanted fields (Table 2). Yield reduction caused by escaped *L. dubia*, compared to pyrazolate + butachlor, about 24% in transplanted culture.

The number of panicles m^{-2} and spikelets per panicle in plots treated with pyrazosulfuron-ethyl + molinate were 19 and 17% respectively less than plots treated with pyrazolate + butachlor in transplanted culture. However, other yield components such as ripened grain and 1,000 grain weight were the same in all treatments. Therefore, it was concluded that rice yield loss was directly due to reduction of the number of rice panicles and spikelets per panicle produced per plant. High density of escaped *L. dubia* severely limited the tillering capacity of rice plants, thereby reducing rice yield. Although we do not study yield loss caused by escaped *L. dubia* in directed culture, the yield loss in directed culture may be more serious than in transplanted culture. For example, rice yield was reduced 70% by resistant *M. vaginalis* that escaped the pyrazosulfuron-ethyl + molinate compared with hand weeding in direct-seeded rice culture (Kuk *et al.*, 2002). On the other hand, rice yield was reduced 44% by resistant *M. vaginalis* that survived the pyrazosulfuron-ethyl + molinate treatment compared with pyrazolate + butachlor in transplanted rice culture.

Control of resistant *L. dubia* in field experiments

A paddy field was infested with the herbicide-resistant *L.dubia* used for this study. The surviving resistant *L. dubia* after pyrazosulfuron-ethyl + molinate application was controlled completely by sequential applications of respective recommended rates of soil-applied herbicides, butachlor, dithiopyr, pyrazolate, and thiobencarb when applied 20 DAT (Table 3). The surviving resistant *L. dubia* was also 82, 72 and 94% controlled by sequential applications of dimepiper-

Table 1. Effects of soil-applied herbicides on resistant biotype of *Lindernia dubia* in transplanted rice culture. The herbicides were applied 10 days after rice transplanting. Parameters were recorded at 50 days after transplanting.

Weed control treatment	Rate (g ai ha^{-1})	No. of individual (m^{-2})	Shoot dry weight (g m^{-2})	Herbicide efficacy (%)
Pyrazosulfuron-ethyl + molinate	21 + 1500	1,722	81	16
Pyrazolate + butachlor	1,800 + 1,050	0	0	100
Untreated control	-	1,924	96	0

Herbicide efficacy was based on reduction of shoot dry weight

Table 2. Yield reduction of rice as affected by competition with surviving resistant biotype of *Lindernia dubia* in transplanted fields. Herbicides were applied 10 days after transplanting.

Herbicide	Rate (g ai ha^{-1})	Panicles (No. m^{-2})	Spikelets per panicle	Ripened grain (%)	1,000 grain (g)	Yield (kg $10a^{-1}$)	Yield index
Pyrazosulfuron-ethyl + molinate	21 + 1,500	278 ^{b}	84^{b}	90.0^{a}	23.0^{a}	428^{b}	76.3
Pyrazolate + butachlor	1,800 + 1,050	343 ^{a}	101^{a}	93.5^{a}	22.4^{a}	561^{a}	100
Untreated control	-	270 ^{b}	85^{b}	91.0^{a}	22.5^{a}	419^{b}	74.6

Means within a column followed by the same letter are not significantly different at the 5% level according to the LSD test.

Table 3. Effects of sequential application at 20 days after transplanting (DAT) or 30 DAT of herbicides with other modes of action on surviving resistant *Lindernia dubia* biotype after pyrazosulfuron-ethyl + molinate application at 10 days after transplanting. Parameters were recorded 40 days after application.

Herbicide	Rate	No. of survivors[a]		Shoot dry weight[b] (g/m^2)		Rice injury[c] (visual rate, 0~9)	
		20 DAT[d]	30 DAT	20 DAT	30 DAT	20 DAT	30 DAT
		% reduction based on untreated check					
Bentazon[e]	1,600	-	100	-	100	0	0
Butachlor	1,500	100	69	100	86	1	0
Dimepiperate	2,100	71	65	82	73	0	0
Dithiopyr	90	100	87	100	95	0	0
Esprocarb	1,500	87	90	72	60	1	0
Molinate	1,500	30	33	27	15	0	0
Pertilachlor	600	77	59	94	79	1	0
Pyrazolate	1,800	100	94	100	91	0	0
Thiobencarb	2,100	100	56	100	85	0	0

[a]Number of individual in the untreated control averaged 1,683 per m^2.
[b]Shoot dry weight in the untreated control averaged 86g per m^2.
[c]A rating of represents 0 no rice injury and 9 indicates complete kill.
[d]Herbicide application at 20 days after transplanting (DAT) or 30 DAT.
[e]Bentazon was applied 40 DAT, at 5.5 to 6-leaf stage of *L. dubia.*

ate, esprocarb, and pretilachlor when applied 20, but molinate showed very poor control of *L. dubia.* On the other hand, efficacy of these herbicides was less when applied 30 DAT than when applied 20 DAT. Foliar-applied herbicide, sequential application of bentazon, also had excellent control of the surviving resistant *L. dubia* when applied 40 DAT. Related research showed that SU-resistant *L. dubia* also controlled by pretilachlor, carfenstrole, and bifenox (Uchino *et al.*, 2000). These results show that control of SU-resistant *L. dubia* is possible by rotating modes of action. In addition, rice injury by herbicides tested was not observed 40 DAA.

Even though butachlor, dithiopyr, pyrazolate, and tioben-

Table 4. Effect of sequential application at 20 days after transplanting (DAT) or 30 DAT of sulfonylurea-based mixtures on surviving resistant *Lindernia dubia* biotype after pyrazosulfuron-ethyl + molinate application at 10 days after rice transplanting. Parameters were recorded 40 days after application.

Herbicide	Rate	No. of survivors[a]		Shoot dry weight[b]		Rice injury[c] (visual rate, 0~9)	
		20 DAT[d]	30 DAT	20 DAT	30 DAT	20 DAT	30 DAT
		% reduction based on untreated check					
SU-based mixture							
Azimsulfuron + anilofos	15 + 450	96	78	98	90	0	0
Bensulfuron-methyl + molinate	51 + 1,500	57	38	49	25	0	0
Bensulfuron-methyl + oxadiazon	39 + 240	79	59	94	96	0	0
Imazosulfuron + molinate	75 + 1,500	40	37	27	20	0	0
Pyrazosulfuron-ethyl + fentrazamide	21 + 300	100	69	100	88	0	0
Ethoxysulfuron + benfurasate + molinate	21 + 360 +1,500	0	30	45	40	1	0
Pyrazosulfuron-ethyl + anilofos + carfentrazon	15 + 450 + 30	100	46	100	87	1	0
Non-SU-based mixture							
Butachlor + pyrazolate	1,050 + 1,800	100	59	100	89	0	0
MCPB + molinate + simetryne	160 +1,600 + 300	47	80	97	89	1	0

[a]Number of individual in the untreated control averaged 1,683 per m^2.
[b]Shoot dry weight in the untreated control averaged 86g per m^2.
[c]A rating of represents 0 no rice injury and 9 indicates complete kill.
[d]Herbicide application at 20 days after transplanting (DAT) or 30 DAT.

carb could control the SU-resistant biotype of *L. dubia* when applied 20 DAT, these herbicides do not control perennial weeds. Paddy fields infested with the resistant biotype of *L. dubia* may also be infested with perennial weeds such as *Scirpus juncoides* Roxb., *Eleocharis kuroguwai* Ohwi, and *Sagittaria trifolia* L. To achieve control of annual and perennial species in rice paddies, soil-applied herbicides such as molinate and pyrazolate for control of annual weeds are routinely used with SU herbicides. Thus, various SU herbicide-based mixtures were tested for control of the surviving resistant *L. dubia* after pyrazosulfuron-ethyl + molinate application (Table 4). Some SU herbicide-based mixtures did not control resistant *L. dubia* when applied at recommended rates of bensulfuron-methyl + molinate, imazosulfuron + molinate, and ethoxysulfuron-ethyl + benfurasate + molinate irrespective of application time. However, mixtures of SU herbicides, azimsulfuron + anilofos, bensulfuron-methyl + oxadiazon, pyrazosulfuron-ethyl + fentrazamide, and pyrazosulfuron-ethyl + anilofos + carfentrazon showed fair control (87 to 100% biomass reduction) of the resistant *L. dubia.* On the other hand, herbicides with different modes of action, butachlor + pyrazolate and MCPB + molinate + simetryne controlled the resistant *L. dubia* 89 to 100%. In addition, rice injury by herbicides tested was not observed 40 DAA. Although application of some SU herbicide-based mixture improved the control of the surviving resistant *L. dubia* after pyrazosulfuron-ethyl + molinate application, use of herbicides with different modes of action, such as MCPB + molinate + simetryne and butachlor + pyrazolate, is necessary to manage SU-resistant *L. dubia.*

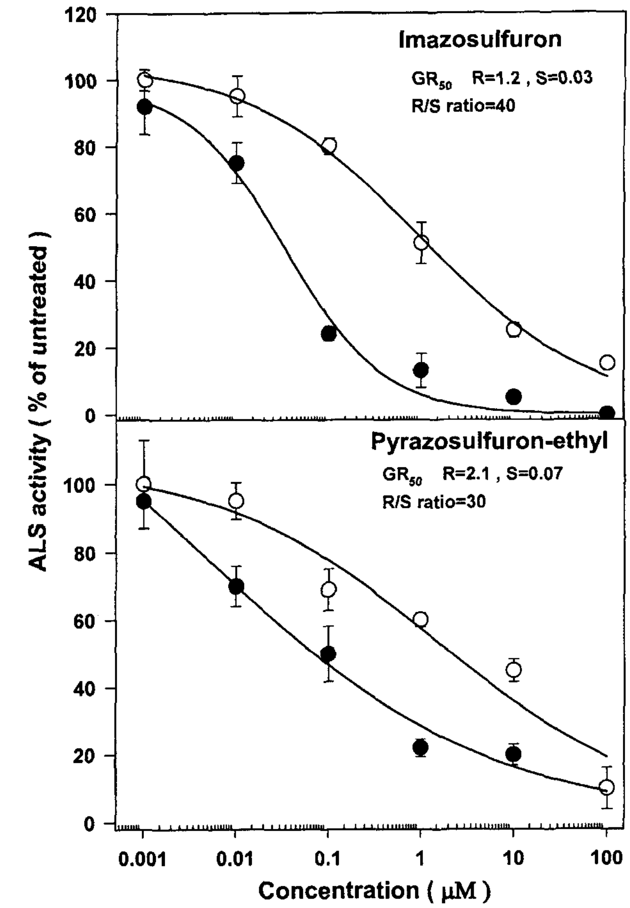

Fig. 2. Effects of imazosulfuron and pyrazosulfuron-ethyl on *in vitro* ALS activity of partially purified enzyme from susceptible (●) and resistant (○) biotypes of *L. dubia.* Each data point is the mean S.E of three replication. Regression equations for imazosulfuron were $Y=98.8/[1+(X/0.03)^{0.8}$, $R^2=0.99$ for susceptible and $Y=105.6/[1+(X/0.98)^{0.46}$, $R^2=0.99$ for resistant biotype. Regression equations for pyrazosulfuron-ethyl were $Y=157.0/[1+(X/0.0047)^{0.3}$, $R^2=0.99$ for susceptible and $Y=106.2/[1+(X/1.59)^{0.4}$, $R^2=0.95$ for resistant biotype.

ALS inhibition

The specific activity *in vitro* of ALS extracted from shoot tissue of resistant and susceptible *L. dubia* was similar, with 543 ± 75 for susceptible and 538 ± 62 for resistant biotype in terms of acetoin production (nmol hr^{-1}) mg^{-1} protein. This data indicates that resistance was not caused by overexpression of target site (ALS) in the resistant biotype. *In vitro* ALS activity from the resistant biotype was 40 and 30 times more resistant to imazosulfuron, and pyrazosulfuron-ethyl, respectively, than the susceptible biotype (Fig. 2). These data indicate that the mechanism of SU resistance for this biotype is an altered target enzyme. The basis of SU resistance in *L. dubia* appears to be similar to that of *Scirpus juncoides* Roxb., *M. vaginalis,* and *Lindernia* spp. wherein resistance to ALS inhibitor herbicides has been linked to a reduced sensitivity of the target enzyme (Kuk *et al.,* 2002; Shibuya et al., 1999; Uchino & and Watanabe, 1999).

We concluded that accession of *L. dubia* collected from Jeonnam province was resistant to imazosulfuron and pyrazosulfiron-ethyl. The surviving resistant *L. dubia* after pyrazosulfuron + molinate application can be controlled by sequential application of soil-applied herbicides, butachlor, dithiopyr, pyrazolate, and thiobencarb and foliar herbicides, bentazon. If, in some cases, a SU herbicide is badly needed to control other weed species in the paddy, mixtures of azimsulfuron + anilofos, bensulfuron-methyl + oxadiazon, pyrazosulfuron-ethyl + fentrazamide, and pyrazosulfuron-ethyl + anilofos + carfentrazon can be used to control the resistant *L. dubia.* Use of these mixtures should be restricted to a special need basis. ALS resistance can be managed by using an integrated system, which utilizes other herbicides as well as mechanical, cultural, and biological weed control methods. The occurrence of resistant *L. dubia* in southern

Korea can cause up to 24% rice yield loss; thus, continuous monitoring of SU-resistant *L. dubia*, including its distribution and spread, is imperative for resistance management.

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