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Identification of Sulfonylurea-Resistant Biotype of *Scirpus planiculmis* in Reclaimed Paddy Fields, Korea

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Abstract : A suspected biotype of *Scirpus planiculmis* to be resistant to sulfonylurea(SU) herbicides was identified in Seosan reclaimed paddy fields in Korea, in 2004. The fields have been cultivated for monocultural rice production with wet-direct seeding method and continuously treated with SU-based herbicide mixtures for thirteen years since 1990. In greenhouse studies, 6 different SU herbicides, such as azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron imazosulfuron and pyrazosulfuron-ethyl, completely controlled the Musan assession of *Scirpus planiculmis* at the recommended dose of each herbicide, however, the Seosan accession of *S. planiculmis* biotype was survived 20 to 45% even treated with 5 times higher dose of each recommended rate of all herbicides treated. The GR_{50} values of 6 SU herbicides for Seosan accession of *S. planiculmis* were 47 to 100 times higher than those for Musan accession of *S. planiculmis*. The I_{50} values pyrazosulfuron-ethyl to acetolactate synthase(ALS) extracted from Sesan and Muan accession of *S. planiculmis* were 409 nM and 0.8 nM, respectively. The I_{50} value of Seosan was 511 times higher than that of Muan accession. These results suggested that the Seosan accession of *S. planiculmis* have strong resistant characteristics to 6 SU herbicides, respectively, indicating that resistance might be due to the alteration in the target site of ALS. (Received 24, september 2004; accepted 20, december 2004)

Introduction

According to Dyer *et al.*, (1993) and Malik *et al.*, (1996), the development of herbicide-resistant weeds was first observed in 1960. Since the first report of this resistance, various herbicide-resistant weeds have been reported including resistance to herbicides with different modes of action(Carol, 1994 ; Cavan & Mosss, 1997 ; Park *et al.*, 1999 ; Wang *et al.*, 2002) In recent decades, the most significant development was the discovery of sulfonylurea(SU) herbicides, which requires very low-rate with extremely low mammalian toxicity and kills plants by inhibiting the enzyme acetolactate synthase(ALS) that catalyses the first step in branched amino acid biosynthesis(Donald, 1992 ; Gressel *et al.*, 1990 ; Hensley, 1997 ; Leckie *et al.*, 1993 ; Van *et al.*, 1992). Since the introduction of first SU herbicide in the early 1980s, chlorsulfuron, SU herbicides have been widely used in major cereal-growing areas to control or suppress broad leaf weeds and sedges. With the repeated use of the same SU-based herbicides, several weed species have developed resistance to SU herbicides(Heap, 1998 and 2003).

For weed management, mixtures containing bensulfuron-methyl(BSM) and pyrazosulfuron-ethyl(PSE) have been very extensively used for 15 years as one-shot-herbicides such as PSE/molinate, BSM/ mefenacet, BSM/esprocarb, and PSE/mefenacet mixtures in rice paddies of Korea. Like in Korea, SU-based herbicides have been extensively used in Japan for more than 15 years. As a results of repeated use of SU-based herbicides, SU-resistant biotypes of major rice paddy weeds have been confirmed such as *Monochoria korsakowii*, *Monochoria vaginalis*, *Lindernia dubia*, *Rotala indica*., *Scirpus*

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juncoides and *Cyperus difformis* L. (Itoh *et al.*, 1994 ; Itoh *et al.*, 1999 ; Kohara *et al.*, 1999 ; Koarai, 2000 ; Yoshida *et al.*, 1999). Among them, the most serious herbicide resistant weeds in rice field are *Monochoria vaginalis* and *Scirpus juncoides* in Korea and Japan considering their abundance and competitiveness over rice(Park, 1999 ; Itoh, 1999).

SU-based herbicides have been treated for about twenty years in the reclaimed paddy fields of about 10,000 ha in Seosan, the mid-western coastal area in Korea. The paddy field has mainly been practiced with the wet-direct seeding rice culture. Since 2002, it has been reported that *Scirpus planiculmis* in the fields was not controlled effectively with SU-based herbicides. Therefore, we collected corms of those *S. planiculmis* not effectively controlled and tested to confirm herbicide resistance and its mechanism.

Material and methods

Plant materials

Two accessions of *S. planiculmis* corms were collected from Seosan reclaimed paddy fields in Chungnam province where SU-based herbicides had been consecutively treated for 13 years, and from the herbicide-untreated area in Muan, Chonnam province in March 2003. The corms of 2 accessions were germinated in a growth chamber(E-15, Conviron's, Canada) maintained at 30±2℃ and 75% relative humidity, and 12 hrs photoperiod (dark/light). Twenty-five germinated corms of each accession having plumule of 2 or 3 cm in length were transplanted to a plastic pot(45 cm×35 cm×25 cm) containing clay loam paddy soil and maintained the water level to 5 cm depth. The herbicides were treated at 10 days after transplantation.

Herbicide treatment

Six different SU herbicides, azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron, imazosulfuron, and pyrazosulfuron-ethyl formulated granular were separately treated to each accession of *S. planiculmis* at 10 days after transplantation. GR_{50} values were calculated from exhibiting 50% reduction of fresh weight against *S. planiculmis* treated with 1/20, 1/10, 1/5, 1, 2, 5 and 10 times of recommended rate of respective herbicide. At 20 days after herbicide treatment, survival rates of the treated plants were determined. All treatments for each measurement were triplicated.

ALS Extraction and Assay

ALS was extracted from the shoots of 10-day-old seedlings which were grown under the growth chamber (E-15, Conviron's, Canada) maintained at 25℃. The shoot were homogenized in 3 volumes of buffer containing 0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine-pyrophosphate, 10 μM FAD and 10% v/v glycerol. The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 27,000 xg for 20 min. ALS was precipitated from the supernatant fluid with $(NH4)_2SO_4$. The enzyme was collected at 25 to 50% saturation by centrifugation and the pellet dissolved in buffer containing 0.1 M K_2HPO_4, pH 7.5, 20 mM pyruvate, and 0.5 mM $MgCl_2$ and desalted on small column of Sephadex G-25 (Pharmacia PD-10) equilibrated with the same buffer. The desalted enzyme was used immediately for assays.

ALS assays were carried out in a final volume of 0.5 ml at 30℃. The final reaction mixture contained 20 mM K_2HPO_4, 20 mM sodium pyruvate, 0.5 mM thiamine-pyrophosphate, 0.5 mM $MgCl_2$, and 10 μM FAD and, its pH was adjusted to be 7.0. Then, by dissolving a range of amounts of pyrazosulfuron-ethyl in the mixtures, 0, 0.0001, 0.001, 0.01, 0.1, 1, 10 μM concentrations were prepared. Assays were prepared as described above to avoid the introduction of organic solvents into the reaction mix. Assays were initiated by adding enzyme (100 μl) and terminated by adding 50 μl of 6 N H_2OS_4. ALS was determined as described by Westerfield(1945) with the follow-ing modifications. The acidified reaction mixtures were heated for 15 min at 6 0℃ after which 0.5 ml of 0.5℃ w/v creatine was added. Next, 0.5 ml of 5% w/v α-naphthol, freshly prepared in 2.5 N NaOH, was added and the solutions were heated for an additional 15 min at 60℃. The absorbances of the solutions were then determined at 525 nm by using a spectrophotometer(UV4, Unicam, USA). Protein was determined by the method of Lowry *et al.* (1951). The I_{50} value for inhibition is defined as the concentration of herbicides which inhibits ALS activity by 50%.

Result and discussion

History of herbicide use in control efficacy

Table 1 shows the history of herbicides used and efficacy on *S. planiculmis* in reclaimed paddy fields. *S. planiculmis* growing in this area has been effectively controlled by SU-based mixtures for about 13 years since the initial use of the herbicides in 1990. However, the efficacy for control of *S. planiculmis* in the fields has been greatly declined since 2000 at which was the 21^{th} year of the consecutive use of the SU-based herbicide mixtures. This observation indicates that SU-resistant accession of *S. planiculmis* has been occurred in Seosan reclaimed paddy field.

Dose-response to SU herbicide

In order to confirm if the suspected biotype of *S. planiculmis* is truly resistant to SU herbicides, the emerged seedlings of the biotype at 10 days after planting were treated with 6 different SU herbicides, azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron imazosulfuron and pyrazosulfuron-ethyl formulated as granule at a range of application dose rate, 1/20, 1/10, 1/5, 1, 2, 5 and 10 times rate of their recommended doses. The response of the putative resistant biotype was then compared with that of a standard susceptible biotypes. The putative resistant biotype of *S. planiculmis* was not controlled even with 5 times higher dosage of the dosage of respective recommended rate of all herbicides treated, but the susceptible biotype was controlled very effectively at the recommended dose of the respective herbicide(Fig. 1). The putative resistant biotype of *S. planiculmis* was found to be resistant to all the SU herbicides tested, suggesting that the biotype has cross resistance to SU herbicides.

The GR_{50} values of 6 SU herbicides tested required to reduce fresh weight accumulation by 50% were significantly higher for each of the resistant biotypes relative to the susceptible ones. Although GR_{50} values of the resistant and susceptible biotypes varied among herbicides treated, the largest extent of difference in GR_{50} values between resistant and susceptible biotypes was observed in the resistant biotype was 165-fold greater than that for the susceptible biotype. However, the GR_{50} values of bensulfuron-methyl and imazosulfuron for the resistant biotype were 47- and 49-fold greater than those for the susceptible biotype, respectively(Table. 3).

Inhibition ALS activity

An *in vitro* assay was conducted to compare dose-response of ALS enzyme extracted from the resistant and The inhibition acrivity of pyrazosulfuron-ethyl(PSE) to ALS extracted from Muan and Seosan accession of *S. planiculmis* was evaluated. PSE inhibited the ALS activity extracted from both accession, however, ALS activity was more sharply declined at Muan accession

Table 1. History of herbicide use and reportedl efficacy against *Scirpus planiculmis* in the Seosan reclaimed rice field where the resistant biotype was observed.

Year	Herbicide use	Efficacy
1986 ~ 1989	Nonsulfonylurea herbicides	Effective(not emerged)
1990	Bensulfuron+quinclorac	Effective(not emerged)
1991 ~ 1999	Pyrazosulfuron+molinate fb[a)] bentazon and fenoxaprop-P-ethyl	Effective(not emerged)
2000 ~ 2003	Imazosulfuron+thiobencarb fb[a)] bentazon and fenoxaprop-P-ethyl	No effect(emerged)

[a)]fb : follow by.

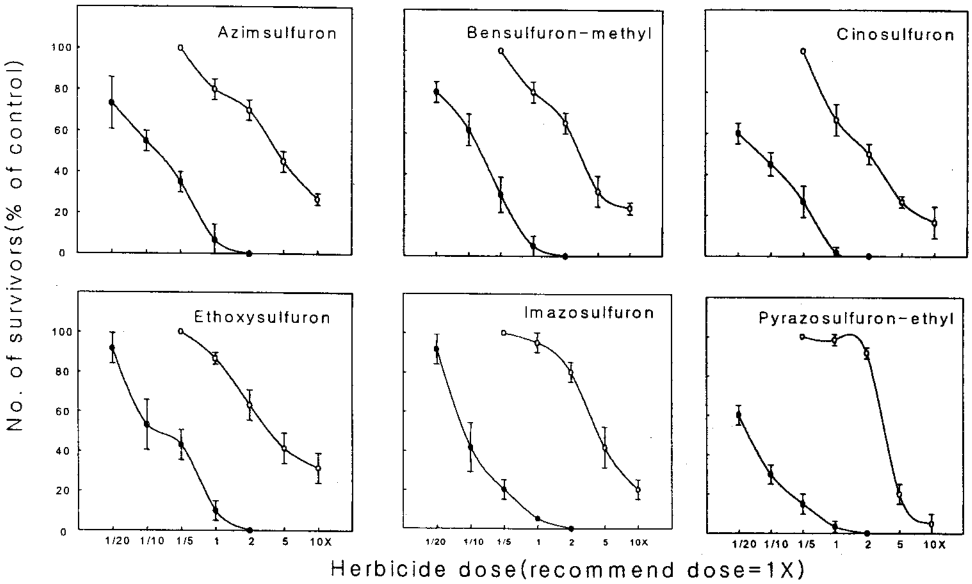

Fig. 1. Number of survivors of Muan accession(●) and Seosan accession(○) of *Scirpus planiculmis* as affected by different dose of sulfonylurea herbicides at 20 days after herbicide treatment. Herbicides were applied at 10 days after transplantation. Vertical bars represent standard errors of the mean.

than Seosan accession of *S. planiculmis* with the increasing concentration of PSE. The I_{50} values of ALS inhibition by PSE to Musan and Seosan accessions were 0.8 nM and 409 nM, respectively. The resistance index of I_{50} values with ALS inhibition *in vitro* was 511 times between Muan and Seosan accessions of *S. planiculmis*. This differential inhibition to ALS activity is thought to be attribute to an alteration in the target site in SU-resistant Seosan accessions. and may be directly related to inhibition of ALS enzyme activity by SU herbicides. These results are consistent with the report of ALS inhibitor-resistant weeds including *Lindernia micratha*(Itoh, 1999), *Monochoria vaginalis* (Koarai, 2000), and *Monochoria korsakowii*(Park *et al*, 1999 ; Wang, 1999). Therefore, it can be concluded that the resistance was apparently due to the alteration in the target site from sensitive to insensitive to SU herbicides.

Table 2. Dose of respective herbicides exhibiting growth reduction of 50%(GR_{50}) by fresh weight against Muan (S) and Seosan(R) biotypes of *Scirpus planiculmis*

Herbicide	GR_{50}(g a. i./ha)		
	R	S	R/S
Azimsulfuron	156.8	1.4	62
Bensulfuron-methyl	149.6	3.2	47
Cinosulfuron	160.2	2.1	76
Ethoxysulfuron	149.3	1.5	100
Imazosulfuron	312.7	6.4	49
Pyrazosulfuron-ethyl	198.4	1.2	165

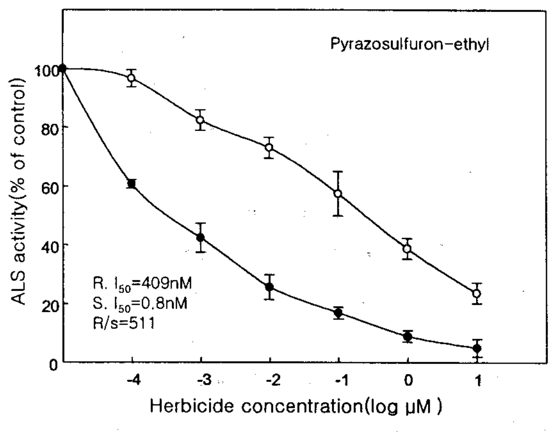

Fig. 2. Inhibition of ALS enzyme extracted from Muan (●) and Seosan(○) accession of *Scirpus planiculmis.* by pyrazosulfuron-ethyl at a range from 0.0001 to 10 μM. The vertical bars represent standard errors of the mean. The I_{50} values were the pyrazosulfuron-ethyl concentrations that reduced ALS activity by 50%.

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한국 서산 간척지 논에서 Sulfonylurea계 제초제에 대한 저항성 새섬매자기 발생

박태선(농업과학기술원 농업생물부 잡초관리과)

요약 : Sulfonylurea계 제초제에 대한 저항성 새섬매자기가 한국의 서산 간척지 논에서 발생한 것이 확인되었다. 제초제 저항성 새섬매자기가 발생한 논은 담수직파 양식으로 벼를 재배하여 왔으며, 1990년부터 13년동안 연속적으로 sulfonylurea계 혼합 제초제를 사용하고 있다. 온실 조건에서 서산과 무안에서 채취한 새섬매자기 구경을 이식 후 10일에 azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron, imazosulfuron 그리고 pyrazosulfuron-ethyl을 처리하였을 때 무안의 새섬매자기는 각 제초제 추천량에서 방제가 되었으나 서산의 새섬매자기는 추천량의 5배량에서도 20-40% 생존되었다. 생체중 50%를 억제하는 각 제초제의 농도(GR_{50})는 두 지역에서 채취한 새섬매자기 간에 현저한 차이가 있었으며, 서산의 매자기에 대한 6 종류의 GR_{50}은 무안의 새섬매자기 대한 GR_{50} 값 보다 47~100배 높게 나타났다. 또한 무안과 서산의 새섬매자기에서 추출한 acetolactate synthase(ALS)에 대한 pyrazosulfuron-ethyl의 50% 억제 농도(I_{50})각각 0.8 nM과 409 nM로 나타나, 서산의 새섬매자기가 무안의 새섬매자기 보다 511배 높은 저항성을 가지고 있음을 확인하였다.

Key words : Resistance, *Scirpus planiculmis*, sulfonylurea herbicide.

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