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#### A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

# Baseline sensitivity of *Echinochloa crus-galli* collected in Korea

 $\mathbf{BY}$ 

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FEBRUARY, 2013

MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY

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THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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#### UNDER THE DIRECTION OF PROFESSOR DO SOON KIM SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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#### **ABSTRACT**

## Baseline sensitivity of *Echinochloa crus-galli* collected in Korea

Aceotolactate (ALS) and acetyl CoA carboxylase (ACCase) inhibitor resistant Echinochloa species now become problematic in Korean rice cultivation. Alternative herbicides with different modes of action can be used to control these herbicide resistant Echinochloa species. However, continuous uses of these alternative herbicides will eventually make the Echinochloa species become resistant to these herbicides as well. Therefore, this study was conducted to evaluate baseline sensitivity of Echinochloa crus-galli to the alternative herbicides selected for managing herbicide resistant Echinochloa species. 63 accessions of Echinochloa crus-galli collected across Korea were tested by whole plant assay. Four VLCFAs inhibitors, mefenacet, pretilachlor, fentrazamide, cafenstrole, one PPO inhibitor, oxadiargyl, and one herbicide with unknown mode of action, oxaziclomefone, were directly applied to the flooded paddy soil at a range of their doses when Echinochloa reached the 2 leaf stage. GR<sub>80</sub> values of mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone ranged 12.67 - 3544.84 g a.i. ha<sup>-1</sup>, 12.20 - 372.98 g a.i. ha<sup>-1</sup>, 2.68 - 58.16 g a.i. ha<sup>-1</sup>, 34.35 - 95.21 g a.i. ha<sup>-1</sup>, 4.68 - 461.50 g a.i. ha<sup>-1</sup>, and 0.34 - 25.81 g a.i. ha<sup>-1</sup>, respectively. Selectivity indices were 250.68, 30.57, 21.70, 2.77, 111.25, and 81.68 for mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone, respectively, suggesting that mefenacet has the greatest potential risk of resistance evolution, followed by oxadiargyl, while cafenstrole has the lowest risk of resistance evolution. However,

continuous use of these herbicides may eventually result in herbicide resistance to these herbicides.

**Keywords**: ALS inhibitor, ACCase inhibitor, baseline, *Echinochloa crus-galli*, herbicide resistance, resistance risk, VLFAs inhibitor

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#### 1. INTRODUCTION

Herbicide resistance has been a serious problem in the farming system around the world since 1950 (Holt and Lebaron 1990, Warwick 1991). For managing herbicide resistant weeds, alternative herbicides with different modes of action have been developed registered and used. However, extensive and continuous use of alternative herbicides resulted in another herbicide resistance to these herbicides. EPPO (European and Mediterranean Plant Protection Organization) suggested that baseline test should be include in weed management system for herbicide resistance management (EPPO 1999). When a new herbicide with a different mode of action is first introduce to the area where the herbicide has never been used before, baseline test gives basic information for population of a target weed in their herbicide sensitivity and its variation. If a target area already has resistance population, farmer can use other pesticide that has another mode of action (Patzoldt et al. 2002).

Baseline test can useful information of establish pesticide management systems (Olson et al. 2000, Hsiang et al. 1997, Jang et al. 2009). Baseline test is to investigate variation in the dose response of target pest population to certain pesticide and thus to estimate sensitivity variation. Researchers have tried to predict evolving resistance through monitoring dynamics of sensitivity and comparing sensitivities between regions (Schaub et al. 2002).

Since the first report of herbicide resistant weed in Korea, being resistant to

sulfonylurea (SU) herbicides, many SU resistant weeds were reported in paddy fields year by year; M. vaginalis in 1999 (Hwang et al. 2001), Cyperus difformis in 2003 (Kuk et al. 2004), Scirpus juncoides in 2001(Kuk et al. 2002). However, recent report of herbicide resistant Echinochloa spp. (Im et al. 2009) sparked general publics' and policy makers' interests in herbicide resistance in Korea as Echinochloa spp. is one of most frequently found weeds in paddy fields and causes serious rice yield due to its high competitiveness (Moon 2010, Moon et al. 2011). Researchers rapidly tested and recommended alternative herbicides with different modes of action for controlling herbicide resistant Echinochloa spp. (Bae et al. 2011). It is mefenacet, pretilachlor and fentrazamide (very long chain fatty acid synthase (VLCFAs) inhibitor), oxadiargyl (protoporphyrine IX oxidase (PPO) inhibitor), and oxaziclomefone (unknown mode of action) that controlled herbicide resistant Echinochloa spp. (Bae et al. 2011). However, as we have previously experienced, sole reliance, heavy and continuous use of these alternative herbicides with a single mode of action may result in another resistance in the existing resistant weed, i.e., multiple resistance. Even the above herbicides showing good activity against resistant Echinochloa spp. have relatively long history in Korean paddy fields. It is assumed that sensitivity of *Echinochloa* spp. may vary due to their natural variation, experience of herbicide exposure, herbicide mode of action, and so on. It is necessary to examine sensitivity of *Echinochloa* spp. to these herbicides early before these herbicides are widely used to manage herbicide resistant Echinochloa spp. However, no study has been conducted in this regard. Therefore,

baseline study of *E. crus-galli* accessions collected nationwide in Korea may give us better understanding of sensitivity variation and resistance risk in advance. This study may also provide a guideline of baseline study for paddy weeds in Korean paddy fields.

#### 2. LITERATURE REVIEW

#### 2.1. Herbicide resistance in *Echinochloa* spp.

Heavy reliance on same class herbicides and continuous use of same mode of action herbicides have led to evolve herbicide resistance in weed populations (Holt et al. 1993). Studies have confirmed resistant *Echinochloa* spp. to many herbicides with modes of action, ACCase inhibitor, ALS inhibitor, and PS II inhibitor, auxinic herbicide (Heap 1997). *Echinochloa* spp. has already reported to have multiple resistance to some herbicides. Propanil and quinclorac in *Echinochloa* spp. in America (Talbert and Burgos 2007), cyhalofop-butyl and penoxsulam resistant in South Korea (Im et al. 2009, Kang et al. 2010). Late watergrass (*E. phyllopogon*) has also confirmed to have multiple resistance to bispyribac-sodium, fenoxaprop-pethyl, molinate, penoxsulam and thiobencarb (Bakkali et al. 2007, Fischer et al. 2000).

## 2.2. Candidate herbicides for the management of herbicide resistant *Echinochloa* spp.

When a weed species is found to be resistant to one herbicide with a specific mode of action, it is common to use different herbicides with different modes of action. Developing a completely new herbicide to manage herbicide resistant weeds is difficult and impractical, weed scientists usually screen existing herbicides with different modes of action and recommend some of them showing good herbicidal

efficacy against herbicide resistant weed. *Echinochloa crus-galli* resistance to ALS and ACCase inhibitors led us to search other herbicides with different modes of action and commercially available. One of the most potential candidates is very long chain fatty acid synthesis inhibitor as it has long been used to control *Echinochloa* spp. in Korean paddy field with no report of herbicide resistance evolution.

Echinochloa spp. control in Korean paddy field can be divided into preemergence and post-emergence controls. For pre-emergence control, farmers usually apply herbicide at the same time as rotary tillage with water or transplanting rice. Herbicides belonging to pre-emergence control commonly include sulfonylurea herbicide. For post-emergence control, more diverse herbicides are available and mainly used in mixture with other herbicides with different modes of action and weed control spectrum. The mixture of two or three herbicides with different modes of action enables to control various weeds by a single application of pre-mix herbicide product, so we call this mixture as "one-shot herbicide", of which application timing can be divided into early post at 5~7 days after transplanting rice (DAT), early to mid-post at 10~12 DAT, mid post at 15 DAT, and late-post at around 20 DAT. The one-shot herbicide usually contains one sulfonylurea herbicide, a typical ALS inhibitor for perennial weed control, one grass killer such as mefenacet and fentrazamide mainly for early to mid-post control and pyriminobac-methyl, flucetosulfuron, penoxulam and metamifop for mid to late post control, and one

herbicide for sulfonylurea resistant weed control such as benzobicyclone, carfentrazone, tefuryltrione, bromobutide, etc.

Very long chain fatty acid synthesis (VLCFAs) inhibitor herbicides attack formation of very long chain fatty acid, and they inhibit growth of early stage plant, like seedling, but not seed (Böger et al. 2000). Resistance to VLCFAs inhibitor herbicides is extremely rare due to its broad spectrum of elongation fatty acids inhibition with different function (Trenkamp et al. 2004). In morphological, and anatomical investigation, fentrazamaide showed inhibition of the cell elongation and cell division of *Echinochloa* spp., and mefenacet also show similar phenomenon (Ito et al. 2008). Previous experiment was proven about reason of rare resistance evolution by broad spectrum of inhibition of VLCFAs formation (Trenkamp et al. 2004).

## 2.3. Baseline sensitivity and its implication in herbicide resistance management

Baseline test is basically investigating response of a species to some treatments, for example, zooplankton to effluent, pest to pesticide, and even human to drugs. It has been conducted to investigate sensitivity of treatments, for example, drugs for each disease (Lautt et al. 1998), pesticide for pest (Wise et al. 2008). In case of plant protection, baseline sensitivity test gives information about the level of resistance to

a particular plant protection product in the pest, weed, fungi or insect population and allows comparisons among different populations and between the same populations at different times, allowing the evaluation in sensitivity changes both between populations and along a period of time (McConnachie et al. 2011, Kendall et al. 1993).

A main objective of baseline sensitivity test is to investigate natural variation of sensitivity of a pest species to pesticide in a target area (Espeby et al. 2011, Han et al. 2008, Tang et al. 2011). Robertson et al. (1995) defined the variation between samples of the same population as 'natural variation'. The population means assortment of same biotype samples in same collection area. When a new pesticide is introduced, researchers conduct baseline test (Tang et al. 2011, Kanetis et al. 2008, Olaya and Köller 1999) to investigate of efficacy for target pest in specific area. The area is often including region of main farmland of plant, for example, the north central region of Spain for *Bromus diandrus* and *Lolium rigidum* by dalapon (Barroso et al. 2010), including whole nation, Israel for Emmer wheat (Snape et al. 1991), and even including whole Europe for *Papaver rhoeas* populations by florasulam (Tang et al. 2011).

Baseline test can be used to check the level or potential risk of resistance, find suitable pesticide, and establish pest management strategy. Baseline test gives basic information of population of target pest, and target area, and can establish resistance population (Cahill et al. 1996). If target area has resistance population, farmer can

use pesticide with different mode of action (Patzoldt et al. 2002) or establish other strategy (Patzoldt et al. 2002). In case of no resistance in population, baseline test is also helpful to establish pest management strategies (Vidotto et al. 2007, Patzoldt et al. 2002). Also, when farmer want to use a new herbicide, baseline test can establish baseline of proper dose recommendation (Tang et al. 2011).

Regular baseline test in the same area may help more accurate pest management strategies based on population dynamics in temporal sensitivity change. Baseline test can give information of population dynamics (Vidotto et al. 2007). Baseline test allow to compare between the same populations at different times and monitor pesticide resistance evolution (Schaub et al. 2002, Kendall et al. 1993). Diagnosing herbicide-resistant weeds as a first step in resistance management and monitoring their nature, distribution, and abundance demands efficient and effective screening tests (Beckie et al. 2000). Screening test is a part of baseline test, but baseline test gives more information of population of weed for resistance management. Therefore, baseline test is very helpful for herbicide resistance management.

#### 3. METHODS AND MATERIALS

#### 3.1. Collection of Echinochloa crus-galli

Collecting population from various locations is one of the most important processes for baseline sensitivity study. Across Korea in autumn between 2009 and 2011, we collected more than two hundred accessions of *Echinochloa crus-galli*, of which 61 accessions were chosen for this study by considering their representation and regional distribution in Korea paddy fields (Figure 1 and Table A1). One ACCase resistant accession collected in Seosan (Im et al. 2009), and one collected in Japan in 2010 were also include as a reference.



**Figure 1.** Collection sites of *E. crus-galli* accessions. 62 accessions were collected in Korea and one accession in Japan. Further information including name of collection site, latitude and longitude are summarized in Figure A1 in Appendices

#### 3.2. Preparation of plant materials

Seeds of 63 *E. crus-galli* accessions were soaked in tap water in a multi-well acryl box designed for germination and placed in the incubation room maintained at 30/20°C for 72 hours before germination started. Pre-germinated seeds were then transplanted at a density of 3 plants per well to a paddy soil fertilized with urea N fertilizer at 43 kg N ha<sup>-1</sup>. Each accession was kept isolated from the other accessions by diving them using a transparent acryl multi-well box placed on the paddy soil contained a plastic tray (48 cm x 38.5 cm x 18 cm). Each well was 8.41 cm<sup>2</sup> rectangular. Plants were then placed in the glasshouse maintained at 30/20°C (day/night). All experiments were consisted with three replications of a completely randomized block design.

#### 3.3. Herbicide treatment

Four VLCFAs inhibitors, carfenstrole, fentrazamide, mefenacet, and pretilachlor, one PPO inhibitor, oxadiargyl, and one herbicide with unknown mode of action, oxaziclomefone, were selected as they showed good efficacy against herbicide resistant *Echinochloa* spp. (Bae et al. 2011). Application dose rates were 30 – 240 g a.i. ha<sup>-1</sup>, 11.9 – 95.0 g a.i. ha<sup>-1</sup>, 65.6 - 525.0 g a.i. ha<sup>-1</sup>, 69.4 – 555 g a.i. ha<sup>-1</sup>, 8.5 – 68.0 g a.i. ha<sup>-1</sup>, and 7.5 – 60.0 a.i. ha<sup>-1</sup> for cafenstrole (1 % SC, Bayer CropScience), fentrazamide (1.9 % EW, Bayer CropScience), mefenacet (3.5 % EW, Bayer

CropScience), pretilachlor (14 % EC, Syngenta), oxadiargyl (1.7 % EC, Bayer CropScience), and oxaziclomefone (17 % SC, Bayer CropScience), respectively. Application was directly made to the flooded soil maintained at 4 to 5 water depth at 7 days after transplanting pre-germinated seeds. Water depth was maintained to the end of the each experiment by regular irrigation.

#### 3.4. Assessment

Visual efficacy was recorded at 10 days, and 20 days after treatment (DAT). Visual efficacy was scored base on visual symptom and mortality ranging from 0 (dead) to 10 (unaffected). Fresh weight was measured at 20 DAT.

#### 3.5. Statistical analyses

All the data were subjected to analysis of variance (ANOVA) and fitted to the standard dose-response model (Streibig, 1980) to estimate  $GR_{50}$  and  $GR_{80}$  values as follows,

$$y = \frac{C}{1 + \left[\frac{x}{GR_{50}}\right]^B} \tag{1}$$

where, y and x denote plant growth and the herbicide dose, respectively, C and B denote the maximum plant growth at no herbicide treatment and the slope of the curve, respectively, and  $GR_{50}$  value is the herbicide dose that reduces plant growth by 50%.  $GR_{80}$  value was calculated by estimated standard dose-response model. The sensitivity index (SI) value was calculated by dividing the greatest  $GR_{80}$  values  $(GR_{80 \text{ max}})$  by the lowest  $GR_{80}$  value  $(GR_{80 \text{ min}})$  of each herbicide using the following equation (eqn. 2).

$$SI = \frac{GR_{80} max.}{GR_{80} min.} \tag{2}$$

The greatest  $GR_{80}$  value ( $GR_{80}$  max) was also divided by the standard recommended dose (S) of each herbicide to examine how much the greatest  $GR_{80}$  value exceeds the standard recommended dose as follows,

$$y = \frac{GR_{80}max.}{S} \tag{3}$$

All the statistical analyses were conducted by using Genstat 5 (Genstat Committee 1997).

#### 4. REEULTS AND DISSCUSION

#### 4.1. Baseline sensitivity of *E. crus-galli* to each herbicide

A total of the 63 accessions of *E. crus-galli* were assessed for their sensitivity to mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone. GR<sub>80</sub> values of mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone ranged 12.67 - 3544.84 g a.i. ha<sup>-1</sup>, 12.20 - 372.98 g a.i. ha<sup>-1</sup>, 2.68 - 58.16 g a.i. ha<sup>-1</sup>, 34.35 - 95.21 g a.i. ha<sup>-1</sup>, 4.68 - 461.50 g a.i. ha<sup>-1</sup>, and 0.34 - 25.81 g a.i. ha<sup>-1</sup>, with mean values of 525.06, 122.36, 24.86, 53.31, 90.66, and 7.25 g a.i. ha<sup>-1</sup>, respectively (Figure 2).

Most of GR<sub>80</sub> values for mefenacet were lower than its standard dose 1050 g a.i. ha<sup>-1</sup> in Korea with two accessions from Gimje (accession code: 06.034) and Iksan (08.048) of Jeonbuk province showing greater GR<sub>80</sub> values with 3176.4 and 1107.3 g a.i. ha<sup>-1</sup>, respectively (Figure 2A and Table A2). Accessions from Andong (08.133) of Kyeongbuk province, Sunchang (08.237) of Jeonbuk province and Nampyung (06.057) of Jeonnam province also showed high GR<sub>80</sub> values. Contrastingly, some accessions mainly from Jeonnam province and Chungbuk province showed very low GR<sub>80</sub> values, lower than 200 g a.i. ha<sup>-1</sup>, 5 times lower than the standard dose of mefenacet. The accession from Boseong of Jeonnam province (08.245) showed extremely sensitive to mefenacet with the GR<sub>80</sub> value of 12.7 g a.i. ha<sup>-1</sup>, about 250 times difference from the value of the greatest value of Gimje accession (06.034).

The GR<sub>80</sub> values of accessions from Yeonggwang (08.262), Gurye (08.241) and Yeongam (08.256) of Jeonnam province were close to 120 g a.i. ha<sup>-1</sup>, about 26 times difference from the value of Gimje accession. The GR<sub>80</sub> values of accessions from Yangpyeong (08.315) of Gyeonggi province, and Goesan (08.213) and Boeun (08.219) of Chungbuk province were also approximately 170 g a.i. ha<sup>-1</sup>, about 19 times difference in comparison with the greatest GR<sub>80</sub> value of the above Gimje accession. This high variation in GR<sub>80</sub> values thus results in high sensitivity index of 250. As the GR<sub>80</sub> value of Gimje accession was extremely low, it needs to be reevaluated. However, even excluding this accession, sensitivity index calculating by comparing the greatest GR<sub>80</sub> and the second lowest GR<sub>80</sub> of Yeonggwang (08.262) still gives high sensitivity index of 26, suggesting potential risk of resistance to mefenacet particularly when mefenacet is continuously and widely being used.

In case of pretilachlor,  $GR_{80}$  values were lower than its standard dose 560 g a.i. ha<sup>-1</sup> (Figure 2B and Table A3). Accessions from Yeongam (08.256) of Jeonnam province, Okcheon (08.221) of Chungbuk province, and Pocheon (08.083) of Gyeonggi province showed high  $GR_{80}$  values with 373.0, 320.6, and 272.5 g a.i. ha<sup>-1</sup>, respectively. After the greatest group of  $GR_{80}$  values, next  $GR_{80}$  values were composed a series to  $GR_{80}$  value from Geoje (08.168) of Gyeongnam province with 64.40 5 g a.i. ha<sup>-1</sup>. Accession from Ulsan (08.155) of Gyeongbuk province, Yanggu

of Gangwon province (08.185), and Boseong of Jeonnam province (08.245) showed the lowest GR<sub>80</sub> values with 12.2, 14.5, and 15.5 g a.i. ha<sup>-1</sup>, respectively.

All of GR<sub>80</sub> values of fentrazamide were also lower than its standard dose 95 g a.i. ha<sup>-1</sup> (Figure 2C and Table A4). Accessions from Dangjin (08.297) of Chungnam province showed GR<sub>80</sub> values with 58.2 g a.i. ha<sup>-1</sup> showed extremely greater than other accessions, and accession from Suwon (01.015) of Gyeonggi province and Seosan (05.005) of Chungnam province showed high GR<sub>80</sub> values with 42.1, and 40.1 g a.i. ha<sup>-1</sup>, respectively. Accession of Seosan was confirmed resistance to ACC inhibitor, it could be herbicide resistance by metabolic enhanced, but it needs to be reevaluated. Accessions from Boseong (08.245), Jangseong (08.266), and Gurye (08.241) of Jeonnam province showed the lowest group of GR<sub>80</sub> values with 2.68, 9.62, and 11.70 g a.i. ha<sup>-1</sup>, respectively.

All of GR<sub>80</sub> values of cafenstrole were lower than its standard dose 240 g a.i. ha<sup>-1</sup> (Figure 2D and Table A5). Accessions from Sokcho (08.111) of Gangwon province, Gimje (06.034) of Jeonbuk, Japan (07.016), Andong (08.133) of Cyeongbuk province showed GR<sub>80</sub> values with 95.2, 81.1, 84.7, and 80.6 g a.i. ha<sup>-1</sup>, respectively. Accessions that had great GR<sub>80</sub> values were not focused any province. And accessions showed low GR80 values from Cheongwon (08.217) of Chungbuk province, Cheonyang (08.306) of Chungnam province, Iksan (08.048) of Jeonbuk province, Namwon (08.234) of Jeonbuk province with 34.4, 37.5, 37.9, and 39.7 g a.i. ha<sup>-1</sup>, respectively.

Unlike other herbicide's GR<sub>80</sub> values and standard dose, many accession's GR<sub>80</sub> values of oxadiargyl were greater than its standard dose 68 g a.i. ha-1 due to late treatment timing. (Figure 2E and Table A6). Accessions from Gimcheon (08.180) of Gyeonbuk province, Andong (08.133) of Gyeongbuk province, Nampyeong (06.057) of Jeonnam province Gimje (06.034) of Jeonbuk province showed GR<sub>80</sub> values with 520.6, 380.8, 349.6, and 340.5 g a.i. ha<sup>-1</sup>, respectively. Accessions from Seosan (05.003) of Chungbuk province, Uiseong (08.136) of Cyeongbuk province Chungju (08.209) of Chungbuk province showed low GR<sub>80</sub> values with 4.7, 4.7, and 13.2 g a.i. ha<sup>-1</sup>, respectively.

All of  $GR_{80}$  values of oxaziclomefone were lower than its standard dose 60 g a.i.  $ha^{-1}$  (Figure 2F and Table A7). Accessions from Iksan (08.048) of Jeonbuk province, Busan (08.159), Pohang (08.140), and Yeongam (08.256) of Jeonnam province showed  $GR_{80}$  values with 25.81, 21.65, 20.58, and 18.83 g a.i.  $ha^{-1}$ , respectively. Accessions from Icheon (08.194) of Gyeonggi province, Japan (07.016), Yeonggwang (08.262) of Jeonnam province showed low  $GR_{80}$  values with 0.32, 0.45, and 0.54 g a.i.  $ha^{-1}$ , respectively.

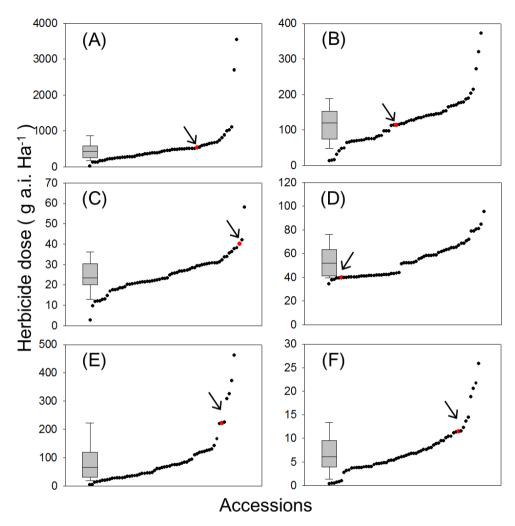


Figure 1. Distribution of  $GR_{80}$  values of the 63 accessions of *E. cruss-galli* collected in Korea for mefenacet (A), pretilachlor (B), fentrazamide (C), cafenstrole (D), oxadiargyl (E), oxaziclomefone (F). Bar graph shows range of  $GR_{80}$  values by box plot. Scatter graph shows  $GR_{80}$  values, herbicide dose required for 80% growth reduction. Each spot represents the  $GR_{80}$  value of each accession. The arrow shows  $GR_{80}$  value of ALS inhibitor herbicide resistant accession.

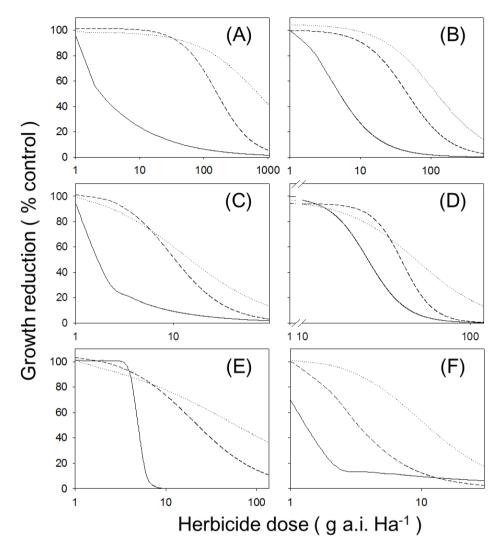


Figure 3. Dose response curves of mefenacet (A), pretilachlor (B), fentrazamide (C), cafenstrole (D), oxadiargyl (E), and oxaziclomefone (F). dotted, dashed, and solid lines represent dose response curves of accessions with the greatest  $GR_{80}$ , median  $GR_{80}$ , and the lowest  $GR_{80}$ , respectively.

Table 1. Summary of baseline sensitivity data and distribution analysis

Herbicide –	Variation			Distribution <sup>b</sup>				
	Range	Mean	Median	$SD^a$	Mean	SD	Skewness <sup>c</sup>	Kurtosis <sup>d</sup>
Mefenacet	12.67 – 3176.42	484.53	411.08	421.97	484.54	421.97	4.34	24.44
Pretilachlor	12.20 – 372.98	122.36	119.42	66.18	120.41	67.41	1.11	2.50
Fentrazamide	2.68 - 58.16	24.86	23.36	8.85	24.86	8.85	0.63	1.93
Cafenstrole	34.35 – 95.21	53.31	52.05	14.13	53.31	14.13	0.82	-0.12
Oxadiargyl	4.68 – 520.55	110.90	68.93	115.48	110.90	115.48	1.72	2.32
Oxaziclomefone	0.31 - 25.81	7.14	5.82	5.10	6.91	5.17	1.46	2.54

a: SD means standard deviation

b: Distribution was estimated by using the software Genstat 5 (Genstat Committee 1997).

c : Skewness presented bias of data. Positive number of skewness meant data had long right tail.

d: Kuritosis presented concentration of data around its mean.

#### 4.2. Distribution of $GR_{80}$ values of E. crus-galli to each herbicide

To analyze distribution of sensitivity, GR<sub>80</sub> values were fitted to the cumulative distribution function. All distributions of sensitivity of each herbicide were confirmed to be normal distribution (Table 1). Skewness on sensitivity to mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 4.34, 1.11, 0.63, 0.82, 1.72, and 1.46, with kurtosis of 24.44, 2.50, 1.93, -0.12, 2.32, and 2.54, respectively. All distributions of sensitivity were right-skewed and sensitivity of mefenacet and oxadiargyl were more skewed than the others. According to previous study (Espeby et al. 2011), skewness could be a clue of creeping resistance. Therefore, skewed distributions of mefenacet and oxadiargyl suggest that evolution of resistance to these two herbicides is now in progress. Contrarily, distributions of cafenstrole and fentrazamide suggest low risk of resistance evolution to these herbicides.

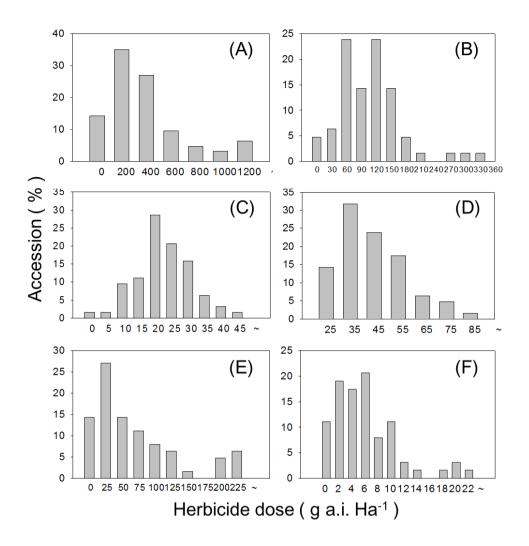


Figure 4. Frequency distribution of E. cruss-galli accessions  $GR_{80}$  values of mefenacet (A), pretilachlor (B), fentrazamide (C), cafenstrole (D), oxadiargyl (E), and oxaziclomefone (F).

#### 4.3. Implication of sensitivity index

Sensitivity index value by herbicide was very important factor in the measuring resistance risk (Paterson et al. 2002, Zelaya and Owen 2005), and other pesticides, fungicide and insecticide, also used similar factor, named resistance factor. Sensitivity index values by mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 279.75, 30.57, 21.70, 2.77, 98.63, and 75.33, respectively. Sensitivity index value was greatest in mefenacet, followed by oxadiargyl. Therefore, resistance risk of mefenact and oxadiargyl was greater than other herbicides. On the other hand, Sensitivity index values of cafenstrole and fentrazamide were relatively lower than other herbicides. So, it could suggest lower resistance risk.

To study about relation between standard dose and baseline sensitivity data, greatest  $GR_{80}$  was compared by standard dose. This value could implicate risk of resistance in standard dose environment. Some accessions that had higher  $GR_{80}$  than standard dose could alive after herbicide treatment, and those could make their strong offspring in standard dose treatment condition. In this study, values that were dividing greatest  $GR_{80}$  by standard dose by mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 3.03, 0.67, 0.61, 0.40, 7.66, and 0.43, respectively. Values of  $GR_{80}$  by pretilachlor, fentrazamide, cafenstrole, and oxadiargyl were lower than standard dose, but values of  $GR_{80}$  by mefenacet and oxadiargyl were higher than standard dose of each herbicide. In case of oxadiargyl, herbicide treatment was conducted at the 2 leaf stage, but standard dose was legislated for preemergence treatment. So,  $GR_{80}$  could be larger than standard dose. In case of mefenacet,

greatest  $GR_{80}$  was almost 3 times higher than standard dose. So, resistance risk of *Echinochloa* species of mefenacet reached danger level in Korea, moreover, it could be declared resistance could exist already.

In many cases, particularly in the case of cross-resistance, sensitivity to the pesticides in the same class showed high correlation in their activity to the same accession (Jang et al. 2009, Hsiang et al. 1997, Olson et al. 2000)). However, the sensitivity of 63 accessions showed low correlation among each other even between the herbicides in the same class, VLFAs. Low correlation among sensitivities of accessions to the herbicides tested may be due to their different ecological and physiological nature in responding to the herbicides tested. Nonetheless, low correlation may indicate genetic diversity of *E crus-galli* in responding to herbicides even belonging to the same mode of action.

To investigate relationship between herbicide use and potential resistance risk based on sensitivity index, herbicide application areas of these 6 herbicides were estimated from 1985 to 2011 (Figure A1 in Appendix). Mefenacet and pretilachlor have long history of use in Korean paddy field since 1985 and 1990, respectively, but show different resistance risk; a bit higher resistance risk in mefenacet than pretilachlor considering their sensitivity indices and distribution (Table 2 and Figure 4, respectively). We suppose that the reason of this difference may be related with their length of use history and something unknown mechanism which we have not found yet. Further study may be required to investigate the reason of this difference more in detail.

Table 2. Sensitivity index and relation between GR<sub>80</sub> and standard dose

Herbicide	Sensitivity index <sup>a</sup>	Standard dose b (g a.i. ha <sup>-1</sup> )	GR <sub>80 max</sub> /S <sup>c</sup>	
Mefenacet	250.0	1050	3.03	
Pretilachlor	30.6	560	0.67	
Fentrazamide	21.7-	95	0.61	
Cafenstrole	2.8	240	0.40	
Oxadiargyl	111.3	68	7.66	
Oxaziclomefone	81.7	60	0.43	

a: Sensitivity index – greatest  $GR_{80}$ /lowest  $GR_{80}$ 

Table 3. Correlation among GR<sub>80</sub> values of all E. curs-galli accessions for each herbicide

Herbicide	Mefena.	Pretil.	Fentra.	Cafens.	Oxadia.
Pretilachlor	-0.026	-	-	-	-
Fentrazamide	0.260	0.170	-	-	-
Cafenstrole	0.174	0.015	-0.050	-	-
Oxadiargyl	0.332	0.126	0.079	0.198	-
Oxaziclomefone	0.069	0.140	-0.032	-0.166	0.098

b: Registered in Korea

c: Greatest GR<sub>80</sub> was divided by the standard dose

#### 5. CONCLUSION

This study was conducted to evaluate baseline sensitivity of Echinochloa crus-galli to alternative herbicides selected for managing herbicide resistant Echinochloa species. GR<sub>80</sub> values of mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone ranged 12.67 - 3544.84 g a.i. ha<sup>-1</sup>, 12.20 - 372.98 g a.i. ha<sup>-1</sup>, 2.68 - 58.16 g a.i. ha<sup>-1</sup>, 34.35 -95.21 g a.i. ha<sup>-1</sup>, 4.68 - 461.50 g a.i. ha<sup>-1</sup>, and 0.34 - 25.81 g a.i. ha<sup>-1</sup>, respectively. Skewness on sensitivity to mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 4.34, 1.11, 0.63, 0.82, 1.72, and 1.46, respectively. Skewness could be clue of creeping resistance. Therefore, skewed distributions of mefenacet and Oxadiargyl suggest that evoluation of resistance to these two herbicides are now in progress. SI values of mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 279.75, 30.57, 21.70, 2.77, 98.63, and 75.33, respectively. SI value was greatest in mefenacet, followed by oxadiargyl. Therefore, resistance risk of mefenacet and oxadiargyl was greater than other herbicides. Low correlation among herbicides could be caused by concentration of sensitivities in low level, and no clue of resistance accessions. Values that were dividing greatest GR<sub>80</sub> by standard dose by mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, and oxaziclomefone were 3.03, 0.67, 0.61, 0.40, 7.66, and 0.43, respectively. The greatest GR<sub>80</sub> values of pretilachlor, fentrazamide, cafenstrole, and oxadiargyl were lower than their recommended standard dose, but those of mefenacet and oxadiargyl were higher than their standard dose, suggesting that mefenacet and oxadiargyl have high potential risk of herbicide resistance. The high GR<sub>80</sub> value of oxadiargyl might be related with late application

of this herbicide as it is mainly used for pre-emergence use. However, the value of mefenacet, almost 3 times greater than the standard dose and its high sensitivity index suggest high resistance risk of *Echinochloa* species to mefenacet in Korea. It is too early to say but to imply that mefenacet resistant Echinochloa may have already developed in Korean paddy fields oxadiargylExcept mefenacet and oxadiargyl, the other alternative herbicides can be incorporated in to program to manage ALS inhibiter and ACC inhibitor herbicide resistance *Echinochloa crus-galli* oxadiargyl. This study is the first approach to investigate baseline sensitivity of *E. crus-galli* in Korea and should be applied to other weeds and herbicides. Further studies may be required not only for regular monitoring of herbicide resistance in a specific weed species but also for understanding herbicide resistance evolution and geographical distribution.

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## **APPENDICES**

Table A1. Echinochloa crus-galli accessions used in this baseline sensitivity study

(	Collected area		Latitude	Longitude	Resistance	Year of	
Province	City or county	code <sup>a</sup>	Lantago	2011911446	110010101100	collection	
	Suwon-si	SNU-E-01.015	37°16'8.40"N	126°59'24.19"E	S	2009	
	Paju-si Gyoha-eup	SNU-E-08.070	37°45'15.49"N	126°45'32.90"E	U	2011	
	Pochun-gun Youngbuk-myeon	SNU-E-08.083	38° 6'30.59"N	127°17'11.20"E	U	2011	
Gyeonggi-do	Icheon-si Sindun-myeon	SNU-E-08.194	37°18'19.83"N	127°24'44.56"E	U	2011	
	Yeoju-si Neunseo-myeon	SNU-E-08.199	37°17'51.41"N	127°33'59.66"E	U	2011	
	Yangpyeong-gun yangseo-myeon	SNU-E-08.315	37°30'42.58"N	127°22'51.38"E	U	2011	
	Gimpo-si Pungmu-dong	SNU-E-08.324	37°36'26.93"N	126°44'8.83"E	U	2011	
	Hoengseong-gun Anheung-myeon	SNU-E-08.105	37°25'11.81"N	128°10'21.28"E	U	2011	
	Injae-gun Buk-myeon	SNU-E-08.107	38° 7'12.48"N	128°12'14.11"E	U	2011	
	Goseong-gun Ganseong-eup	SNU-E-08.109	38°20'7.54"N	128°22'40.68"E	U	2011	
Congruen de	Sokcho-si Nohak-dong	SNU-E-08.111	38°11'17.01"N	128°33'40.58"E	U	2011	
Gangwon-do	Yangyang-gun Yangyang-eup	SNU-E-08.114	38° 5'42.82"N	128°37'32.43"E	U	2011	
	Samcheok-si Geundeok-myeon	SNU-E-08.120	37°23'20.20"N	129°13'14.10"E	U	2011	
	Yanggu-gun	SNU-E-08.185	38° 5'37.81"N	127°59'15.64"E	U	2011	
	ChunCheon-si sin-dong	SNU-E-08.319	37°56'21.22"N	127°43'8.45"E	U	2011	
	Eumseong-gun Gamgok-myeon	SNU-E-08.205	37° 6'10.56"N	127°38'18.65"E	U	2011	
	Chungju-si Sinni-myeon	SNU-E-08.209	37° 0'1.93"N	127°43'13.13"E	U	2011	
Chungcheongbuk-do	Goesan-gun Gammul-myeon	SNU-E-08.213	36°50'13.15"N	127°52'10.21"E	U	2011	
Chungcheongbuk-do	Chungwon-gun Miwon-myeon	SNU-E-08.217	36°38'54.38"N	127°40'26.78"E	U	2011	
	Boeun-gun Boeun-eup	SNU-E-08.219	36°30'51.00"N	127°43'31.66"E	U	2011	
	Okcheon-gun Cheongsan-myeon	SNU-E-08.221	36°20'14.07"N	127°48'1.07"E	U	2011	
	Seosan-si	SNU-E-05.003	36°35'48.00"N	126°28'27.04"E	S	2009	
	Seosan-si	SNU-E-05.005	36°39'45.46"N	126°20'4.79"E	R	2009	
	Nonsan-si Seong-dong	SNU-E-06.003	36°14'26.47"N	127° 2'46.94"E	S	2009	
Chungahaananan	Gongju-si Teabong-eup	SNU-E-08.292	36°24'49.35"N	127° 5'37.16"E	U	2011	
Chungcheongnam-	Dangjin-gun Dangjin-eup	SNU-E-08.297	36°53'16.57"N	126°36'4.29"E	U	2011	
do	Seosan-si Jangheung-dong	SNU-E-08.298	36°47'21.66"N	126°28'51.55"E	U	2011	
	Yesan-gun Yesan-eup	SNU-E-08.304	36°40'8.68"N	126°52'44.79"E	U	2011	
	Cheongyang-gun Ungok-myeon	SNU-E-08.306	36°32'5.55"N	126°50'53.70"E	U	2011	
	Seocheon-gun Seocheon-eup	SNU-E-08.308	36° 5'23.56"N	126°40'59.04"E	U	2011	

	Sacheon-si Seopo-dong	SNU-E-08.277	35° 2'15.89"N	127°58'6.72"E	U	2011
	Hamyang-gun Hamyang-eup	SNU-E-08.290	35°31'6.04"N	127°43'53.89"E	Ŭ	2011
	Youngyang-gun Youngyang-eup	SNU-E-08.126	36°39'28.49"N	129° 8'36.51"E	Ū	2011
	Bonghwa-gun Bonghwa-eup	SNU-E-08.127	36°52'7.96"N	128°44'22.80"E	Ū	2011
0	Andong-si Pungsan-eup	SNU-E-08.133	36°35'5.36"N	128°33'31.52"E	U	2011
Gyeongsangbuk-do	Uiseong-gun Uiseong-eup	SNU-E-08.136	36°21'0.09"N	128°40'28.08"E	U	2011
	Pohang-si Buk-gu	SNU-E-08.140	36° 6'25.91"N	129° 8'2.08"E	U	2011
	Gyeonju-si Yul-dong	SNU-E-08.154	35°48'31.27"N	129°11'32.69"E	U	2011
	Gimcheon-si Nongso-myeon	SNU-E-08.180	36° 6'17.70"N	128°11'1.53"E	U	2011
	Mungyeong-si jeomchon-dong	SNU-E-08.188	36°34'43.56"N	128°12'39.11"E	U	2011
	Geoje-si Dundeok-myeon	SNU-E-08.168	34°50'13.11"N	128°30'32.98"E	U	2011
Cucanacananam da	Uiryeong-gun Uiryeong-eup	SNU-E-08.170	35°19'19.86"N	128°16'38.74"E	U	2011
Gyeongsangnam-do	Ulsan Ulju-gun	SNU-E-08.155	35°33'49.62"N	129° 8'0.18"E	U	2011
	Busan Gijang-gun	SNU-E-08.159	35°14'32.85"N	129°13'42.57"E	U	2011
	Jangeup-si Bujeon-dong	SNU-E-08.272	35°31'26.37"N	126°53'50.16"E	U	2011
	Gimje-si Buryang-myeon	SNU-E-06.034	35°43'59.62"N	126°50'18.84"E	S	2009
	Gimje-si Buryang-myeon	SNU-E-06.035	35°44'19.53"N	126°49'28.27"E	S	2009
	Buan-gun Heangan-myeon	SNU-E-06.039	35°44'59.51"N	126°43'33.67"E	S	2009
Jeollabuk-do	Iksan-si Sinheung-dong	SNU-E-08.048	35°56'16.04"N	126°59'20.69"E	U	2011
	Jinan-gun Jinan-eup	SNU-E-08.229	35°46'45.58"N	127°29'8.44"E	U	2011
	Imsil-gun Goanchon-myeon	SNU-E-08.231	35°39'27.34"N	127°17'2.83"E	U	2011
	Namwon-si Sangok-dong	SNU-E-08.234	35°25'28.43"N	127°22'12.12"E	U	2011
	Sunchang-gun Jeokseong-myeon	SNU-E-08.237	35°24'56.02"N	127°14'55.83"E	U	2011
	Nampyeong-gun	SNU-E-06.057	35° 1'41.70"N	126°51'44.32"E	S	2009
	Gokseong-gun Ogok-myeon	SNU-E-08.238	35°14'3.84"N	127°21'59.73"E	U	2011
	Gurye-gun Gurye-eup	SNU-E-08.241	35°11'21.42"N	127°27'49.36"E	U	2011
Jeollanam-do	Boseong-gun Joseong-myeon	SNU-E-08.245	34°48'48.40"N	127°15'15.64"E	U	2011
	Haenam-gun Okcheon-myeon	SNU-E-08.251	34°34'1.73"N	126°39'7.57"E	U	2011
	Mokpo-si Deayang-dong	SNU-E-08.254	34°50'16.59"N	126°25'42.64"E	U	2011
	Yeongam-gun Sampo-eup	SNU-E-08.256	34°44'3.18"N	126°30'34.14"E	U	2011
	Yeonggwang-gun Bulgap-eup	SNU-E-08.262	35°12'36.27"N	126°30'23.64"E	U	2011
	Jangseong-gun Hwangyong-eup	SNU-E-08.266	35°18'2.36"N	126°45'50.13"E	U	2011
Japan	Usa-si	SNU-E-07.016	33°31'55.06"N	131°23'26.58"E	U	2010

a: R and S mean resistant and susceptible to ACCase inhibitor predetermined by Im et al. (2009) and U means unknown whether they are resistant or susceptible to ACCase inhibitor

Table A2. Summary of non-linear regression analysis to fit dose-responses of mefenacet to the standard dose-response model

Accession No.	B value <sup>a</sup>	r²	GR <sub>50</sub> <sup>b</sup>	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	1.42	0.90	416.55	1107.26	08.234	1.07	0.62	178.75	650.66
08.070	2.18	0.86	187.35	353.86	08.237	0.85	0.72	185.86	955.00
08.083	1.68	0.91	154.32	352.71	08.238	NA	NA	NA	NA
08.105	1.42	0.63	239.61	635.17	08.241	1.57	0.74	53.30	129.26
08.107	0.52	0.86	19.30	280.41	08.245	0.64	0.98	1.46	12.67
08.109	3.92	0.68	377.66	537.89	08.251	2.24	0.92	110.39	204.75
08.111	2.28	0.96	180.93	332.15	08.254	2.69	0.97	189.05	316.51
08.114	5.10	0.82	396.63	520.52	08.256	3.92	0.87	90.92	129.50
08.120	1.24	0.90	124.96	380.84	08.262	3.34	0.86	79.68	120.67
08.126	1.62	0.94	163.37	385.44	08.266	1.74	0.86	122.49	271.46
08.127	2.52	0.95	140.95	244.22	08.272	NA	NA	NA	NA
08.133	1.25	0.70	336.97	1019.70	08.277	2.26	0.92	260.34	480.39
08.136	4.04	0.92	190.09	267.91	08.290	2.38	0.87	413.56	740.28
08.140	0.37	0.68	11.02	486.72	08.292	2.70	0.86	131.11	219.08
08.154	4.84	0.98	343.06	456.94	08.297	3.80	0.94	348.56	502.01
08.155	4.19	0.72	185.12	257.82	08.298	1.36	0.70	292.66	811.66
08.159	1.91	0.95	137.28	283.78	08.304	5.61	0.82	311.06	398.26
08.168	2.97	0.68	173.82	277.08	08.306	5.26	0.92	376.61	490.17
08.170	1.55	0.95	157.91	387.11	08.308	2.76	0.94	194.05	320.95
08.180	1.65	0.81	259.04	600.15	08.315	2.81	0.93	104.17	170.54
08.185	7.62	0.81	176.97	212.28	08.319	1.17	0.60	149.75	489.74
08.188	3.00	0.94	266.83	423.89	08.324	6.54	0.74	388.39	480.09
08.194	0.73	0.45	71.66	478.81	06.003	1.03	0.21	229.75	880.34
08.199	3.83	0.86	317.67	456.21	06.057	1.50	0.78	395.84	996.22
08.205	0.99	0.45	167.84	685.68	06.039	5.48	0.93	182.45	234.97
08.209	2.10	0.71	131.50	254.46	06.035	3.55	0.74	401.82	593.78
08.213	3.22	0.98	115.93	178.29	01.015	4.22	0.54	481.06	668.15
08.217	$NA^c$	NA	NA	NA	06.034	0.94	0.68	721.26	3176.42
08.219	3.20	0.95	108.76	167.75	05.003	4.06	0.94	383.06	538.83
08.221	5.70	0.97	193.12	246.29	05.005	5.72	0.93	344.50	438.98
08.229	3.11	0.93	325.84	508.92	07.016	2.33	0.95	203.10	368.03
08.231	2.12	0.92	261.13	502.47					

a: B value means the slope of the standard dose-response curve.

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of *E. crus-galli*, respectively.

c: NA means not available due to failure of raising seedlings for herbicide dose-response study

Table A3. Summary of non-linear regression analysis to fit dose-responses of pretilachlor to the standard dose-response model

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Accession No.	B value <sup>a</sup>	r <sup>2</sup>	GR₅₀⁵	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	5.44	0.90	75.41	97.30	08.234	1.20	0.82	23.55	74.61
08.070	1.29	0.77	59.92	175.21	08.237	1.25	0.78	43.47	131.53
08.083	2.10	0.89	140.61	272.52	08.238	1.20	0.86	22.22	70.68
08.105	12.50	0.90	62.68	70.03	08.241	1.15	0.83	29.17	97.16
08.107	1.09	0.64	35.52	126.70	08.245	1.03	0.99	4.05	15.48
08.109	14.40	0.96	61.50	67.71	08.251	1.14	0.83	49.25	166.53
08.111	1.20	0.76	44.08	140.21	08.254	1.62	0.88	30.36	71.43
08.114	1.96	0.73	82.93	168.22	08.256	1.15	0.79	111.61	372.98
08.120	1.61	0.74	47.51	112.40	08.262	2.71	0.71	86.83	144.83
08.126	2.96	0.87	111.50	178.10	08.266	NA	NA	NA	NA
08.127	1.26	0.80	44.57	134.51	08.272	5.44	0.87	75.41	97.30
08.133	1.26	0.81	47.56	143.04	08.277	3.08	0.84	108.09	169.62
08.136	1.86	0.71	72.46	152.68	08.290	1.01	0.66	38.86	153.95
08.140	1.26	0.88	44.57	134.51	08.292	0.37	0.75	1.11	47.83
08.154	3.18	0.87	48.18	74.51	08.297	3.88	0.92	114.66	163.91
08.155	1.26	1.00	4.07	12.20	08.298	0.49	0.82	2.94	48.73
08.159	3.87	0.82	58.56	83.78	08.304	1.05	0.92	10.72	40.09
08.168	2.62	0.99	37.94	64.40	08.306	1.28	0.88	27.14	80.03
08.170	1.21	0.83	26.47	83.32	08.308	2.64	0.87	66.75	112.86
08.180	1.09	0.72	57.11	202.79	08.315	1.24	0.91	38.44	117.67
08.185	0.58	0.83	1.31	14.54	08.319	28.29	0.96	66.15	69.48
08.188	2.71	0.74	86.83	144.83	08.324	2.20	0.75	75.72	142.19
08.194	4.10	0.93	104.27	146.22	06.003	4.98	0.96	96.51	127.48
08.199	2.49	0.76	71.74	125.18	06.057	3.11	0.96	88.00	137.46
08.205	1.09	0.90	18.36	65.26	06.039	1.42	0.71	45.65	121.18
08.209	1.20	0.82	23.55	74.61	06.035	0.41	0.78	1.02	30.65
08.213	3.18	0.96	48.18	74.51	01.015	1.22	0.73	59.92	187.01
08.217	14.40	0.96	61.50	67.71	06.034	3.18	0.81	74.07	114.48
08.219	3.08	0.81	112.84	176.99	05.003	1.24	0.88	38.44	117.67
08.221	1.24	0.84	105.00	320.60	05.005	0.45	0.74	5.32	113.13
08.229	3.64	0.97	96.50	141.17	07.016	1.10	0.79	60.34	213.52
08.231	1.22	0.70	60.58	188.90					

a: B value means the slope of non-linear regression curve

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of E. crus-galli, respectively

c: NA means not available due to failure of raising seedlings for herbicide dose-response study.

Table A4. Summary of non-linear regression analysis to fit dose-responses of fentrazamide to the standard dose-response model

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Accession No.	B value <sup>a</sup>	r <sup>2</sup>	GR₅₀⁵	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	5.16	0.90	25.64	33.54	08.234	1.34	0.75	10.44	29.41
08.070	3.34	0.88	13.42	20.33	08.237	1.38	0.77	8.00	21.81
08.083	24.70	0.94	12.29	13.00	08.238	3.17	0.96	17.26	26.72
08.105	1.24	0.86	3.96	12.12	08.241	1.50	0.96	4.65	11.70
08.107	1.62	0.88	9.87	23.19	08.245	0.75	0.96	0.42	2.68
08.109	23.46	0.93	12.09	12.82	08.251	3.28	0.89	14.64	22.35
08.111	3.47	0.88	20.91	31.17	08.254	3.32	0.83	18.69	28.38
08.114	3.39	0.87	14.61	22.00	08.256	3.32	-3.32	18.69	28.38
08.120	5.65	0.88	13.08	16.72	08.262	1.26	0.85	10.22	30.78
08.126	2.38	0.83	19.89	35.58	08.266	2.30	0.95	5.26	9.62
08.127	1.50	0.79	9.25	23.36	08.272	1.89	0.79	17.48	36.37
08.133	3.27	0.90	12.35	18.88	08.277	3.95	0.87	14.73	20.93
08.136	1.56	0.85	12.69	30.81	08.290	1.23	0.76	6.52	20.15
08.140	5.03	0.98	16.36	21.55	08.292	4.37	0.96	15.57	21.39
08.154	7.60	0.99	16.80	20.16	08.297	0.97	0.66	13.93	58.16
08.155	1.30	0.75	8.00	23.19	08.298	5.33	0.94	19.63	25.46
08.159	1.32	0.86	9.03	25.84	08.304	3.53	0.79	13.89	20.57
08.168	2.40	0.83	17.31	30.86	08.306	2.46	0.69	15.75	27.68
08.170	2.97	0.89	20.17	32.16	08.308	6.86	0.95	14.54	17.80
08.180	4.28	0.81	15.47	21.40	08.315	3.89	0.90	12.28	17.54
08.185	3.84	0.89	20.41	29.28	08.319	1.45	0.97	6.71	17.45
08.188	2.39	0.76	14.45	25.82	08.324	1.80	0.75	14.03	30.29
08.194	1.53	0.83	10.97	27.14	06.003	1.97	0.74	14.88	30.08
08.199	24.45	0.95	11.31	11.97	06.057	2.01	0.78	15.24	30.40
08.205	1.33	0.76	9.38	26.67	06.039	3.97	0.94	15.96	22.64
08.209	1.46	0.86	7.18	18.56	06.035	2.70	0.86	17.80	29.74
08.213	1.45	0.91	13.00	33.91	01.015	1.25	0.82	13.93	42.08
08.217	1.25	0.89	4.86	14.68	06.034	2.06	0.74	19.24	37.72
08.219	3.09	0.76	13.46	21.09	05.003	3.57	0.94	16.83	24.81
08.221	4.98	0.94	16.37	21.63	05.005	1.71	0.83	17.83	40.11
08.229	3.31	0.93	17.76	26.99	07.016	1.46	0.88	14.76	38.10
08.231	1.46	0.77	7.13	18.45					

a: B value means the slope of the standard dose-response curve.

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of *E. crus-galli*, respectively.

Table A5. Summary of non-linear regression analysis to fit dose-responses of cafenstrole to the standard dose-response model

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Accession No.	B value <sup>a</sup>	r <sup>2</sup>	GR₅₀⁵	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	14.60	1.00	34.43	37.86	08.234	4.49	0.93	29.14	39.68
08.070	5.39	0.89	42.95	55.55	08.237	6.92	0.97	33.32	40.72
08.083	6.84	0.81	57.57	70.51	08.238	4.81	0.97	30.16	40.24
08.105	7.58	0.98	34.37	41.27	08.241	2.33	0.80	30.81	55.92
08.107	3.47	0.95	27.16	40.51	08.245	2.75	0.84	37.56	62.18
08.109	2.12	0.76	35.69	68.75	08.251	3.24	0.86	42.27	64.84
08.111	2.01	0.74	47.75	95.21	08.254	1.32	0.86	27.49	78.65
08.114	3.27	0.87	41.51	63.43	08.256	5.16	0.96	32.39	42.37
08.120	6.98	0.95	32.13	39.19	08.262	3.41	0.95	29.19	43.83
08.126	2.22	0.78	34.95	65.19	08.266	1.46	0.86	22.20	57.52
08.127	1.28	0.91	19.83	58.81	08.272	4.63	0.90	43.21	58.29
08.133	1.32	0.87	28.30	80.64	08.277	5.02	0.97	39.43	51.97
08.136	1.97	0.84	39.10	78.99	08.290	3.70	0.73	49.50	72.01
08.140	3.99	0.98	28.83	40.81	08.292	2.50	0.88	33.48	58.26
08.154	5.97	0.96	33.13	41.78	08.297	6.49	0.97	32.48	40.22
08.155	1.26	0.80	17.78	53.28	08.298	1.43	0.72	24.02	63.21
08.159	8.60	0.95	34.99	41.11	08.304	2.92	0.96	26.92	43.26
08.168	3.97	0.96	36.78	52.17	08.306	6.90	0.91	30.70	37.53
08.170	6.77	0.97	33.65	41.30	08.308	2.12	0.88	35.69	68.75
08.180	5.97	0.98	33.13	41.78	08.315	3.19	0.89	39.77	61.39
08.185	4.81	0.94	31.22	41.65	08.319	1.29	0.88	22.90	66.90
08.188	10.40	0.98	34.40	39.30	08.324	7.90	0.99	33.40	39.80
08.194	6.49	0.95	32.48	40.22	06.003	4.71	0.96	38.78	52.05
08.199	5.10	0.96	39.90	52.36	06.057	6.70	0.96	34.27	42.15
08.205	4.38	0.97	31.25	42.88	06.039	2.14	0.83	22.13	42.29
08.209	1.44	0.88	19.53	51.25	06.035	3.05	0.84	38.55	60.78
08.213	3.58	0.93	35.35	52.07	01.015	3.46	0.90	39.41	58.83
08.217	3.71	0.96	23.64	34.35	06.034	3.46	0.93	54.30	81.09
08.219	4.38	0.88	31.25	42.88	05.003	2.40	0.82	36.53	65.02
08.221	9.40	0.99	34.43	39.90	05.005	8.67	0.98	33.75	39.60
08.229	4.20	0.92	41.89	58.27	07.016	2.44	0.85	47.99	84.70
08.231	7.58	0.97	34.36	41.26					

a: B value means the slope of the standard dose-response curve.

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of *E. crus-galli*, respectively.

Table A6. Summary of non-linear regression analysis to fit dose-responses of oxadiagyl to the standard dose-response model

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Accession No.	B value <sup>a</sup>	r <sup>2</sup>	GR₅₀ <sup>b</sup>	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	1.75	0.59	40.94	90.60	08.234	1.34	0.66	24.53	69.30
08.070	1.56	0.62	32.72	79.75	08.237	6.83	0.76	23.50	28.79
08.083	1.02	0.47	73.04	285.47	08.238	1.25	0.76	46.85	142.16
08.105	0.64	0.90	35.41	307.88	08.241	2.13	0.79	8.64	16.58
08.107	0.82	0.24	14.20	77.26	08.245	0.42	0.39	1.65	44.04
08.109	2.02	0.73	12.11	24.05	08.251	1.36	0.72	16.69	46.16
08.111	0.66	0.37	55.59	461.50	08.254	1.13	0.38	20.15	68.93
08.114	1.08	0.51	23.15	83.76	08.256	1.04	0.34	14.37	54.56
08.120	0.99	0.50	32.10	130.12	08.262	1.56	0.50	11.59	28.25
08.126	1.17	0.50	33.55	109.49	08.266	1.86	0.78	13.18	27.74
08.127	0.56	0.33	18.92	225.87	08.272	1.22	0.44	19.55	61.13
08.133	0.81	0.57	69.20	380.76	08.277	0.82	0.47	40.41	219.57
08.136	0.57	0.81	0.41	4.73	08.290	1.17	0.44	13.63	44.38
08.140	1.45	0.75	13.37	34.80	08.292	1.05	0.46	20.19	75.97
08.154	1.70	0.67	27.63	62.52	08.297	1.57	0.53	8.26	20.03
08.155	0.50	0.42	7.61	121.83	08.298	2.03	0.73	10.43	20.64
08.159	1.16	0.59	36.05	118.75	08.304	1.17	0.31	10.51	34.53
08.168	0.74	0.39	37.83	248.78	08.306	3.06	0.72	9.53	14.98
08.170	0.87	0.77	60.52	300.01	08.308	1.48	0.58	29.20	74.35
08.180	0.61	0.39	53.84	520.55	08.315	0.91	0.50	32.98	151.32
08.185	0.66	0.66	6.67	54.63	08.319	2.04	0.64	13.63	26.87
08.188	9.79	0.98	24.22	27.90	08.324	1.14	0.71	27.66	93.72
08.194	2.63	0.71	18.01	30.53	06.003	0.99	0.45	31.25	127.30
08.199	0.84	0.42	23.15	119.64	06.057	0.98	0.50	84.35	349.62
08.205	1.80	0.61	20.09	43.39	06.039	1.05	0.53	17.65	66.19
08.209	1.36	0.71	4.78	13.21	06.035	1.77	0.42	38.55	84.37
08.213	0.92	0.51	16.48	74.60	01.015	0.94	0.73	35.34	153.31
08.217	1.34	0.58	16.84	47.25	06.034	0.78	0.29	57.97	340.53
08.219	4.87	0.28	27.66	36.77	05.003	8.25	0.41	3.96	4.68
08.221	1.93	0.64	16.76	34.34	05.005	0.68	0.21	28.62	222.46
08.229	27.84	0.48	34.64	36.41	07.016	1.29	0.66	22.09	64.96
08.231	1.05	0.62	5.96	22.34					

a: B value means the slope of the standard dose-response curve.

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of *E. crus-galli*, respectively.

Table A7. Summary of non-linear regression analysis to fit dose-responses of oxaziclomefone to the standard dose-response model

Accession No.	B value <sup>a</sup>	r <sup>2</sup>	GR₅o <sup>b</sup>	GR <sub>80</sub>	Accession No.	B value	r <sup>2</sup>	GR <sub>50</sub>	GR <sub>80</sub>
08.048	1.42	0.59	9.72	25.81	08.234	NA	NA	NA	NA
08.070	2.03	0.72	4.80	9.51	08.237	3.72	0.75	3.11	4.52
08.083	0.42	0.67	0.42	11.55	08.238	0.57	0.73	0.28	3.20
08.105	1.82	0.69	5.28	11.31	08.241	1.38	0.63	4.50	12.30
08.107	NA	NA	NA	NA	08.245	2.16	0.87	2.94	5.58
08.109	1.74	0.72	3.55	7.87	08.251	0.60	0.89	0.06	0.64
08.111	2.89	0.59	3.29	5.32	08.254	1.98	0.81	2.27	4.57
08.114	4.44	0.86	5.84	7.97	08.256	1.59	0.66	7.87	18.83
08.120	29.84	0.64	3.80	3.98	08.262	0.59	0.81	0.05	0.54
08.126	0.76	0.86	0.63	3.94	08.266	0.80	0.90	0.13	0.76
08.127	4.91	0.97	4.78	6.35	08.272	1.54	0.90	1.79	4.39
08.133	1.38	0.93	1.44	3.93	08.277	0.53	0.73	0.37	5.18
08.136	2.00	0.77	3.55	7.10	08.290	2.28	0.71	5.48	10.06
08.140	1.37	0.58	7.46	20.58	08.292	4.39	0.81	4.11	5.64
08.154	0.43	0.69	0.19	4.59	08.297	0.52	0.67	0.40	5.82
08.155	1.51	0.90	4.15	10.38	08.298	2.18	0.77	5.51	10.41
08.159	1.16	0.62	6.55	21.65	08.304	2.68	0.97	1.63	2.74
08.168	18.51	0.99	3.25	3.50	08.306	2.15	0.91	4.68	8.92
08.170	2.32	0.85	3.17	5.76	08.308	1.38	0.94	1.44	3.93
08.180	2.07	0.78	7.40	14.45	08.315	1.10	0.59	3.87	13.63
08.185	22.18	0.93	3.49	3.71	08.319	2.97	0.63	2.98	4.75
08.188	0.53	0.91	0.05	0.74	08.324	1.07	0.71	3.05	11.17
08.194	0.38	0.91	0.01	0.32	06.003	1.69	0.79	2.11	4.79
08.199	0.87	0.78	0.76	3.72	06.057	23.05	0.96	3.56	3.78
08.205	1.43	0.83	2.28	6.01	06.039	1.47	0.87	2.94	7.56
08.209	1.64	0.70	3.79	8.84	06.035	4.65	0.95	5.04	6.79
08.213	0.46	0.74	0.18	3.77	01.015	1.88	0.81	3.24	6.78
08.217	1.70	0.96	1.65	3.74	06.034	1.24	0.77	2.22	6.80
08.219	0.68	0.71	0.98	7.62	05.003	1.62	0.74	3.12	7.34
08.221	21.46	0.79	3.61	3.85	05.005	1.74	0.79	5.14	11.42
08.229	3.64	0.94	4.53	6.63	07.016	1.04	0.98	0.12	0.45
08.231	2.71	0.79	4.65	7.76		-		-	

a: B value means the slope of the standard dose-response curve.

b: GR<sub>50</sub> and GR<sub>80</sub> represent herbicide doses required for 50% and 80% growth reduction of *E. crus-galli*, respectively.

c: NA means not available due to failure of raising seedlings for herbicide dose-response study.

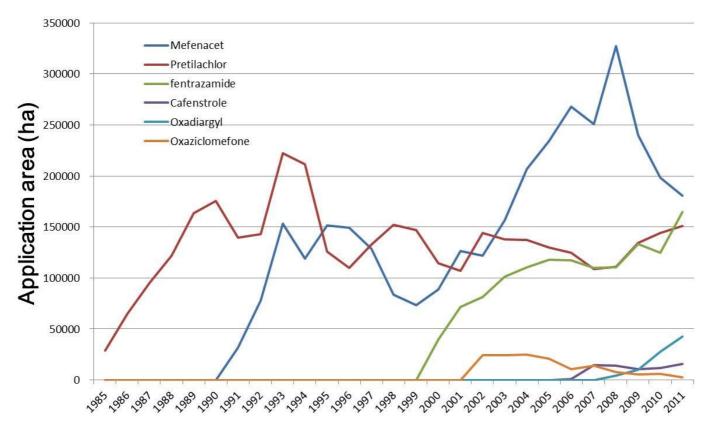


Figure A1. Application area of herbicides tested in this study from 1985 to 2011.

## 국문 요지

## 한국에서 수집한 물피의 제초제 Baseline sensitivity 연구

Aceotolactate (ALS)와 acetyl CoA carboxylase (ACCase)를 저해하는 제초 제에 대한 피의 저항성 문제는 한국의 벼 생산 체계에서 매우 문제시 되고 있다. 이에 따라 저항성 피를 관리하기 위하여 다른 작용기작을 가지고 있는 대체 제초 제들을 선발하여 사용하도록 추천하고 있다. 하지만 이들 대체 제초제들 또한 그 사용이 반복적으로 계속 된다면 피에 대한 저항성 문제가 역시 대두될 것으로 보 인다. 그러므로 본 연구는 한국 물피에 대한 이들 대체 제초제들의 baseline sensitivity를 평가하고 이를 바탕으로 저항성 위험도를 알아보기 위하여 수행되 었다. 전국에서 수집된 63개의 물피 수집종들을 담수조건에서 2엽기까지 키운 후 여섯 개의 초기처리 제초제인 mefenacet, pretilachlor, fentrazamide, cafenstrole, oxadiargyl, oxaziclomefone를 다양한 약량으로 처리하여 약량반응 을 평가하였으며, 비선형회귀분석을 통해 GR<sub>80</sub>값을 계산하여 이 값의 분포와 변 이폭을 비교하고 baseline sensitivity를 평가하였다. GR<sub>80</sub> 값은 mefenacet이 12.67 - 3544.84 g a.i.  $ha^{-1}$ , pretilachlor? 12.20 - 372.98 g a.i.  $ha^{-1}$ , fentrazamide7 2.68 - 58.16 g a.i. ha<sup>-1</sup>, cafenstrole 34.35 - 95.21 g a.i. ha<sup>-1</sup>, oxadiargyl이 4.68 - 461.50 g a.i. ha<sup>-1</sup>, 그리고 oxaziclomefone이 0.34 -25.81 g a.i. ha-1의 범위를 나타내었다. 피의 제초제에 대한 감수성의 분포 및 변 이폭을 나타내는 지수인 sensitivity index (SI)는 각각 279.75, 30.57, 21.70, 2.77, 98.63, 75.33으로서 mefenacet의 값이 가장 컸고, 그 다음으로 oxadiargyl이 높았다. 이들 두 제초제의 최대 GR<sub>80</sub>값도 추천약량보다도 높았기에 이들 제초제의 지속 사용시 저항성 위험이 큰 것으로 판단되었다. 반면에 다른 제

초제들은 최대  $GR_{80}$ 값이 이들 제초제의 추천약량보다도 낮아 저항성 위험은 높지 않은 것으로 판단되었다.