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RESEARCH ARTICLE

Baseline Sensitivity of *Scirpus juncoides* and *Monochoria vaginalis* Populations to HPPD Inhibitors in Korea

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Abstract

The study was conducted to evaluate the baseline sensitivity index (BSI) of *Scirpus juncoides* and *Monochoria vaginalis* populations to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, benzobicyclon, and mesotrione and to estimate the potential risk of the HPPD inhibitor resistance in paddy fields in Korea. The seeds of matured *S. juncoides* and *M. vaginalis* biotypes were collected from a total of 105 sites and a whole plant dose-response test was performed. Nonlinear fit curve (DoseResp) analysis revealed that GR_{50} values of *S. juncoides* biotypes ranged from 9.6 to 16.5 g a.i. ha^{-1} to mesotrione and 13.1 to 26.4 g a.i. ha^{-1} to benzobicyclon. BSI was 1.72 and 2.01 for mesotrione and benzobicyclon respectively. The GR_{50} values of *M. vaginalis* populations ranged from 10.9 to 16.3 g a.i. ha^{-1} to mesotrione and 11.7 to 21.1 g a.i. ha^{-1} to benzobicyclon. BSI was 1.48 for mesotrione and 1.78 for benzobicyclon. No shift in mesotrione and benzobicyclon sensitivity was observed suggesting that these HPPD herbicides can still be used very effectively to control *S. juncoides* and *M. vaginalis* populations in rice fields in South Korea. However, we strongly suggest that constant monitoring and baseline sensitivity studies need to be conducted continuously to detect the evolution of herbicide-resistant weed biotypes.

Keywords: Baseline sensitivity, GR_{50}, HPPD inhibitors, *Monochoria vaginalis*, *Scirpus juncoides*

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Introduction

The constant use of the same herbicide or herbicides that affect a single target area leads to a selection of herbicide-resistant weed populations. Resistance to acetolactate synthase (ALS) inhibitors was first recorded in 1998 in *Monochoria korsakowii* in Korea (Park et al., 1999). Since then, 15 weed species were confirmed resistant to different herbicides, which is nearly 20% of the total weed species occurring in rice fields (Bo et al., 2017; Bo et al., 2019; Lee et al., 2017b). A survey in 2011 showed that 72,736 ha of rice fields in Chungcheongnam-do, 35,194 ha in Jeollabuk-do, and 21,410 ha in Jeollanam-do were infested with herbicide-resistant weeds (Lee et al., 2012). Another survey in 2017 reported that the area infested with herbicide-resistant weeds in Chungcheongnam-do, Jeollabuk-do, and Jeollanam-do provinces increased up to 85,978, 81,494, and 91,543 ha respectively (Jeong et al., 2018; Won et al., 2018). The occurrence and distribution of herbicide-resistant weed species were investigated (Choi et al., 2018; Han et al., 2019; Jeong et al., 2018; Lee et al., 2017a; Lee et al., 2019a; Lee et al., 2019b) and reported that the incidence of herbicide-resistant weeds is significant in Korea, and if this continues, we expect more affected areas in the future.

Scirpus juncoides is the third most noxious weed after *Echinochloa spp* and *Monochoria vaginalis* in the paddy field of Korea (Lee et al., 2017b). A high density of herbicide-resistant *S. juncoides* resulted in up to 40% loss of rice yield (Kuk et al., 2002; Sada et al., 2013). The prevalence of herbicide-resistant S. *juncoides* in paddy fields has increased from 7,032 ha in 2012 to 14,963 ha in 2018 in Chungcheongnam-do province of Korea (Lee et al., 2012; Won et al., 2018).The excessive and unreasonable use of herbicides that inhibit acetolactate synthase (ALS) has led to the evolution of ALS inhibitor-resistant *S. juncoides*. In Korea, *S. juncoides* first evolved resistance to ALS inhibiting herbicides in 2001. Research has confirmed that Jeollabuk-do, Kimjea accession was resistant and cross-resistant to sulfonylurea (SU) herbicides (azimsulfuron, bensulfuron-methyl, and imazosulfuron) and imidazolinone herbicides (imazapyr and imazaquin) (Yong et al., 2002).

Monochoria vaginalis is a monocot weed in the Pontederiaceae family. *M. vaginalis* is one of the most dominant paddy weeds globally and evolved resistance to ALS inhibitor bensulfuron-methyl in Japan, China, and Korea (Heap, 2022). Although its competitive effect on rice is not as high as *Echinochloa crus-galli* it is still a weed of equal importance to *E. crus-galli* (Won et al., 2014). Since the first report of herbicide-resistant *M. vaginalis* in Korea in 1999 (Kwon et al., 2000), all newly developed herbicides had to contain active ingredients capable of controlling herbicide-resistant *M. vaginalis.*

4-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors are a class of herbicides that inhibit plant growth by blocking 4-hydroxyphenylpyruvate dioxygenase, which is an enzyme in plants that breaks down the amino acid tyrosine into molecules. These groups of herbicides were primarily used in rice production in Japan. HPPD inhibitors have been used for corn, soybean, and cereal in Europe and North America since the late 1990s, and have become more important as weeds have shown resistance to glyphosate and other herbicides. However, constant use of the HPPD inhibiting herbicides leads to a selection of the HPPD inhibiting herbicide-resistant weed population. The first HPPD inhibitor resistance case have been detected in *Amaranthus tuberculatus* in a corn field in 2009 and 14 species of weeds have been reported to be resistant to HPPD inhibiting herbicides worldwide to date (Heap, 2022). HPPD inhibitors have been registered (KCPA, 2007) and used in paddy fields of Korea, but no weed species have been reported so far as resistant to these herbicides. Even so, early detection of resistance risk to this group (HPPD) of herbicides in paddy weed species (*S. juncoides* and *M. vaginalis*) is also important as part of herbicide resistance management.

Baseline sensitivity is the beginning point of the average sensitivity of the weed to the specific chemical compound and can ensure herbicide resistance criteria of any herbicide (Espeby et al., 2011). Espeby et al. (2011) and Kanetis et al. (2008) pointed out that the main purpose of the baseline sensitivity study is the determination of natural variation in herbicide or pesticide sensitivity of the population in the target area. Basic and important information such as application timing and/or recommended dose of newly registered herbicides (Paterson et al., 2002) or insecticides (Wise et al., 2008; Wong et al., 2010; Yuan et al., 2006) can be obtained by a baseline sensitivity study. In addition, the baseline sensitivity study can be put into practice to predict the risk of development of herbicide resistance even before new herbicides are registered and this can be one way to establish effective weed management strategies (Paterson et al., 2002; Vidotto et al., 2007). The response of each weed species to a particular herbicide varies between populations (Lim et al., 2021) and the possible herbicide resistance in the species is closely linked with baseline sensitivity and genetic diversity (Blows and Hoffmann, 2005; Moss, 2017). Thus, understanding the baseline sensitivity of certain weeds to specific herbicides is important and baseline sensitivity allows evaluation of the potential risk of developing herbicide resistance in the particular weed. Therefore, this study was conducted to evaluate the baseline sensitivity of *S. juncoides* and *M. vaginalis* to HPPD inhibitors, benzobicyclon, and mesotrione to estimate the potential risk of herbicide resistance in rice paddies of Korea.

Materials and Methods

Seed source

The seeds of *S. juncoides* and *M. vaginalis* at maturity were collected from paddy fields in Korea before rice harvesting in October 2020 (Fig. 1). Global positioning system (GPS) information and addresses of sampling sites were recorded using ICE CPS 100c (Supplemental data 1 and 2). To break dormancy, seeds were placed in a 50-mL tube (SPL Life Science Co., Ltd., Seoul, Korea) and kept dipping in the water for one month in the refrigerator at 4°C. The water in the tube was changed to distilled water once a week regularly.

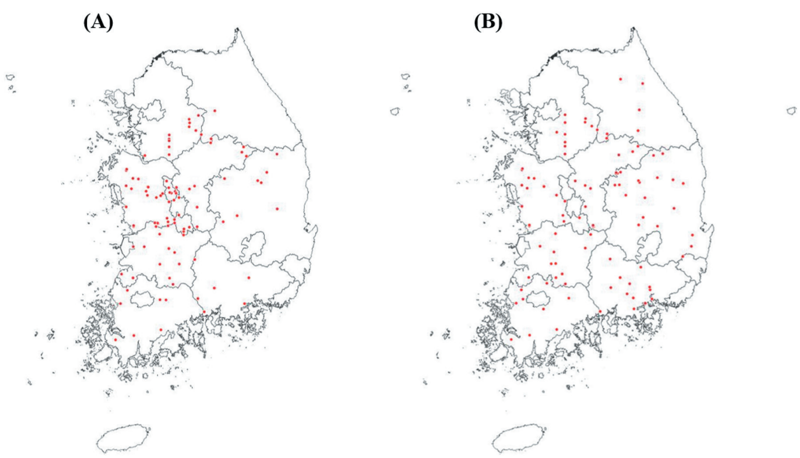

Fig. 1. Map of sampling sites from distributed *Scirpus juncoides* (A) and *Monochoria vaginalis* (B) in Korea.

The dose-response assay

The whole plant dose-response experiment was conducted at the experimental glasshouse (E127°35'4.0" longitude and N36° 36'99.5" latitude) of Chungnam National University located in Daejeon, South Korea in 2021. Three to five seeds of each biotype were sown into a perforated tray (equipped the 105 holes) filled with paddy soil (Seoul Bio Co., Ltd., Seoul, Korea). These trays were placed in a rectangular polystyrene pot (L: 56 cm, W: 35 cm, H: 15 cm) under flooded conditions (3 cm water depth) and incubated with metal halide light to provide a 12-h photoperiod from 6 a.m. to 6 p.m. at 25/30°C light/dark in the greenhouse. Two HPPD inhibitors including mesotrione and benzobicyclon were applied as water surface application when the weeds were at the 2-3 leaf stage. Mesotrione (40% EC; Tenor city^{TM}, Syngenta Co., Ltd., Seoul, Korea) was applied at 5, 10, 20, 40, 80, 160 g a.i. ha^{-1} and benzobicyclon (3.5% EC; Najima^{TM}, KyungNong Co., Ltd., Seoul, Korea) was applied at 4.4, 8.8, 17.5, 35, 70, 140 g a.i. ha^{-1} to both *S. juncoides and M. vaginalis* biotypes. Without herbicide was used as untreated control. The aboveground fresh weight of the treated weeds was measured 14 days after treatment (DAT). The experiment was a Completely Randomized Block design with three replications.

Statistical analysis

Non-linear regression analysis was performed by fitting the fresh weight of samples measured 14 DAT. The log-logistic model estimated the GR_{50} (the dose requiring 50% fresh weight reduction) values of *S. juncoides and M. vaginalis*.

The data were expressed as the percentages of untreated control estimates. A non-linear regression dose-response equation (1) (Streibig, 1980) was used in OriginPro 8.1 (OriginLab, 2021) program.

$$y = d + \frac{a-d}{\left[1+\left(\frac{x}{c}\right)^{b}\right]} \quad (1)$$

where y is fresh weight; x is the dose rates of herbicides; a is the minimum value; d is the maximum value; c is GR_{50} (the herbicide dose required for 50% biomass reduction compared with the untreated); b is proportional to the slopes around the dose of GR_{50}.

The baseline sensitivity index (BSI) was calculated by dividing the greatest GR_{50} value (GR_{50max}) by the smallest GR_{50} value (GR_{50min}) as follows,

$$BSI = GR_{50max}/GR_{50min} \quad (2)$$

One-way ANOVA statistical analysis was performed using OriginPro 8.1 (OriginLab, 2021).

Results and discussion

Whole-plant assays and nonlinear regression analysis revealed the dose responses and BSI of *S. juncoides* and *M. vaginalis* to benzobicyclon and mesotrione. In *S. juncoides*, over 90% growth reduction was observed in 105 tested biotypes at the recommended dose of mesotrione (160 g a.i. ha^{-1}) and 104 out of 105 biotypes at the recommended dose of benzobicyclon (140 g a.i. ha^{-1}) (Fig. 2). The GR_{50} values of *S. juncoides* ranged from 9.6 to 16.5 g a.i. ha^{-1} to mesotrione and 13.1 to 26.4 g a.i. ha^{-1} to benzobicyclon, resulting in the BSI of 1.72 and 2.01 (Table 1).

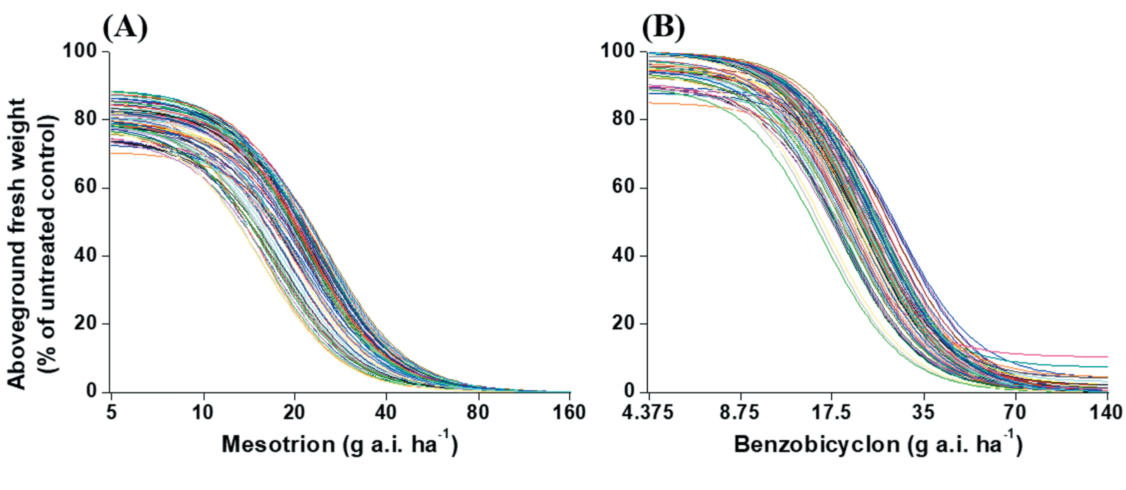

Fig. 2. Dose-response for the fresh weight (% of untreated control) of the *Scirpus juncoides* with a range dose of mesotrione (A) and benzobicyclon (B).

Table 1. Baseline sensitivity indices of *Scirpus juncoides* populations to mesotrione and benzobicyclon in Korea.

Herbicide	*GR_{50} (g a.i. ha^{-1})		Sensitivity index
	Minimum	Maximum	
Mesotrione	9.6	16.5	1.72
Benzobicyclon	13.1	26.4	2.01

*GR_{50}: The rate causing a 50% growth reduction.

The experimental data showed that all of the 105 (100%) tested *M. vaginalis* biotypes were significantly controlled with over 90% growth reduction in fresh weight compared to untreated control at the 1x recommended dose of both herbicides (Fig. 3). The value of GR_{50} in *M. vaginalis* ranged from 10.9 to 16.3 g a.i. ha^{-1} to mesotrione and 11.7 to 21.1 g a.i. ha^{-1} to benzobicyclon. The BSI was 1.48 and 1.78 for mesotrione and benzobicyclon respectively (Table 2).

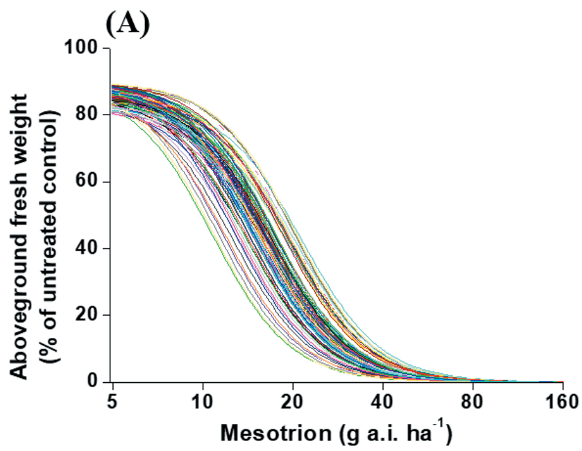

Fig. 3. Dose-response for the fresh weight (% of untreated control) of the Monochoria vaginalis with a range concentration of mesotrione (A) and benzobicyclon (B).

Table 2. Baseline sensitivity indices of *Monochoria vaginalis* populations to mesotrione and benzobicyclon in Korea.

Herbicide	*GR_{50} (g a.i. ha^{-1})		Sensitivity index
	Minimum	Maximum	
Mesotrione	10.9	16.3	1.48
Benzobicyclon	11.7	21.1	1.78

*GR_{50}: The rate causing a 50% growth reduction.

To compare variations in the sensitivity of weed biotypes to mesotrione and benzobicyclon, the GR_{50} values of *S. juncoides* (Fig. 4) and *M. vaginalis* (Fig. 5) were arranged from the lowest to highest. GR_{50} values among biotypes of both species to mesotrione and benzobicyclon were not statistically differed in one-way ANOVA Scheffe's test.

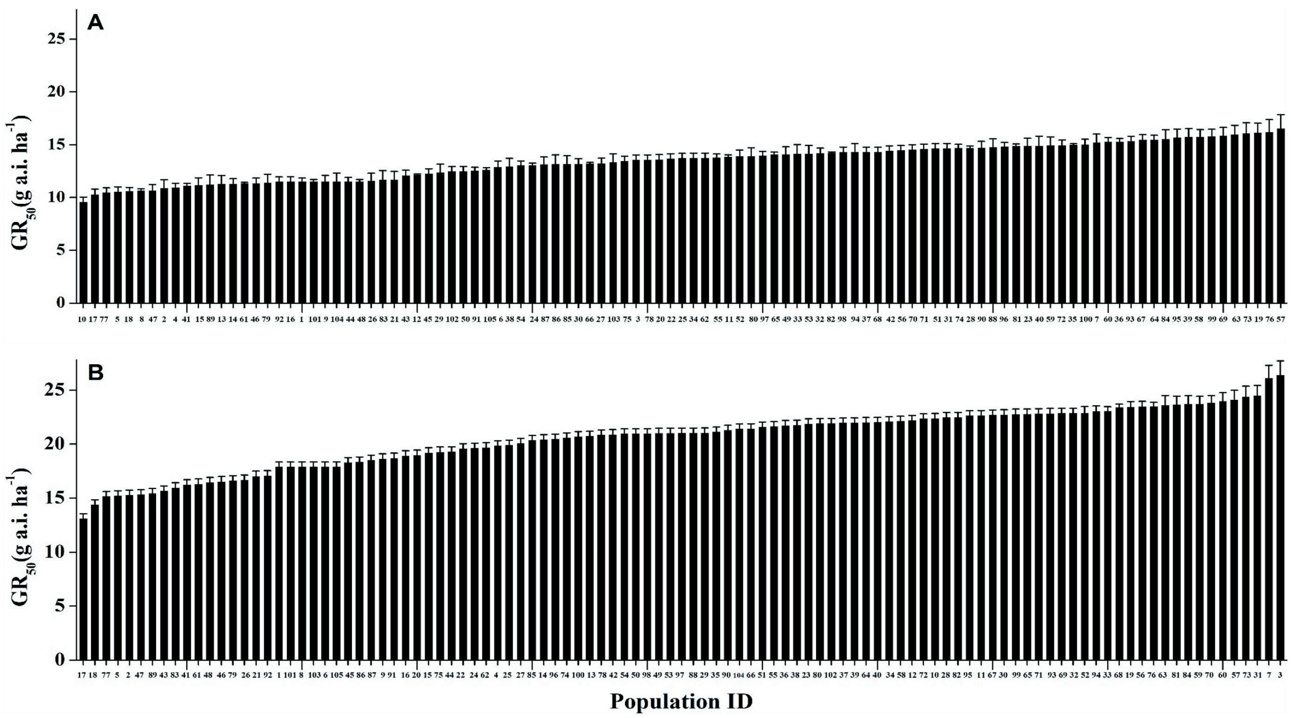

Fig. 4. GR_{50} values of *Scirpus juncoides* populations to mesotrione (A) and benzobicyclon (B) in Korea. Vertical bars represent the means of three replications (n=3). *P*-values (>0.05) were not statistically significant in the one-way ANOVA Scheffe's test. GR_{50}: The rate causing a 50% growth reduction.

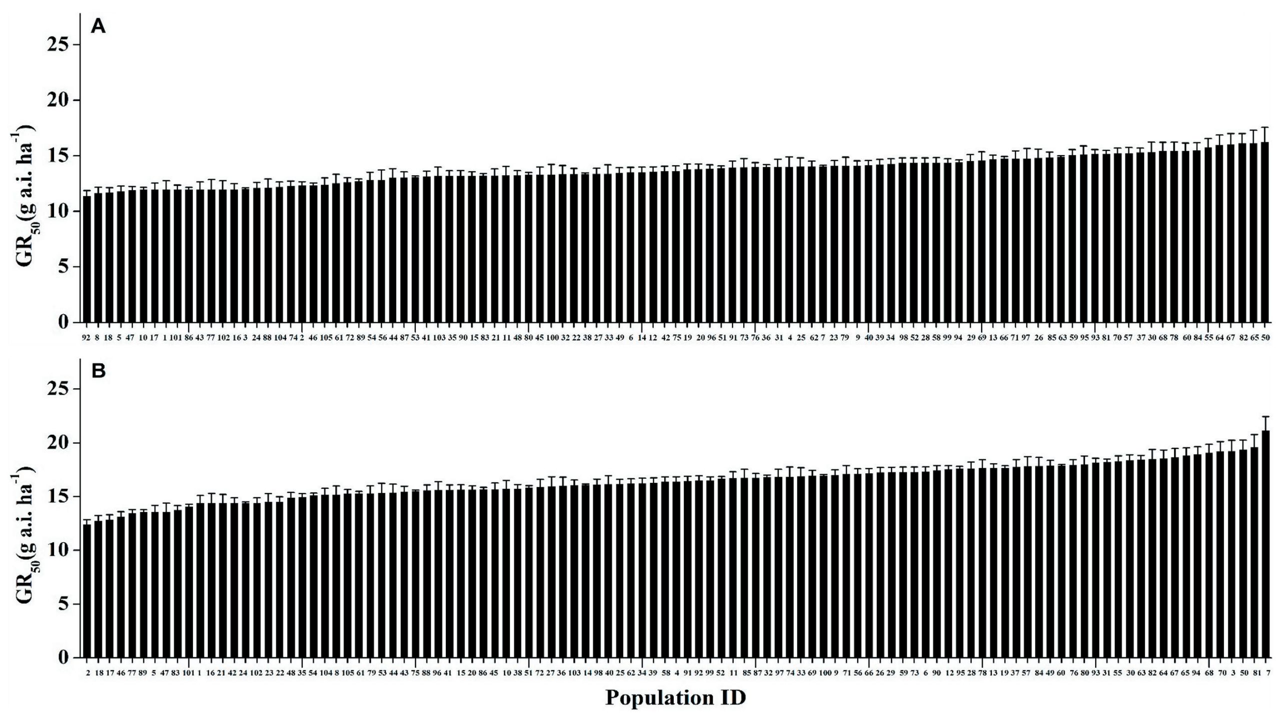

Fig. 5. GR_{50} values of *Monochoria vaginalis* populations to mesotrione (A) and benzobicyclon (B) in Korea. Vertical bars represent the means of three replications (n=3). *P*-values (>0.05) were not statistically significant in the one-way ANOVA Scheffe's test. GR_{50}: The rate causing a 50% growth reduction.

Our study found a less-potential occurrence of the mesotrione and benzobicyclon resistance cases in both weed species. In the case of *S. juncoides*, minimum and maximum GR_{50} values were not highly different and BSI for mesotrione and benzobicyclon was 1.72 and 2.01, respectively (Fig. 4). For comparison, the first SU-resistant biotypes of *S. juncoides* were found in rice fields located in Hokkaido Prefecture, Japan and the lethal dose of the resistant biotype was 40-140 fold for bensulfuron-methyl, 41-79 fold for pyrazosulfuron ethyl, and 75-93 fold higher for imazosulfurone than that of the susceptible biotype (Kohara et al., 1999). Tanaka (2003) reported that imazosulfuron controlled the sensitive *S. juncoides* biotype above 80% at the dosage of more than 10 g a.i. ha^{-1} but could not control the resistant biotype at 1,000 g a.i. ha^{-1}. The rates required to inhibit 50% growth of resistant biotype were 271 fold higher than that of susceptible one.

In our study, no existing resistance cases to mesotrione and benzobicyclon were observed in *M. vaginalis* biotypes in Korea. BSI was also relatively low, having 1.48 for mesotrione and 1.78 for benzobicyclon (Fig. 5). According to Kurniadie et al. (2021), the percentage of weed growth reduction varies from place to place and is influenced by several factors such as land-use history, farmers' herbicide application practices, frequency of herbicide application, and crop planting patterns. However, if one of the biotypes is not resistant to a particular herbicide, the difference between GR_{50max} and GR_{50min} values will not be significant. Kurniadie et al. (2021) performed a whole plant resistance test on *M. vaginalis* to ALS inhibitor, bensulfuron-methyl, and found resistant biotypes with resistance ratios ranging from 12.6 to 48.76. Kuk et al. (2002) reported that GR_{50} values of resistant *M. vaginalis* biotype to imazosulfuron were 1,112-3,172 fold higher compared with susceptible biotype in the whole-plant response bioassay. Several researchers have been conducted baseline sensitivity studies on different species to different herbicides. For instance, *Papaver rhoeas* to florasulam (Paterson et al., 2002), *Echinochloa crus-gall* to azimsulfuron, bensulfuron-methyl, cyhalofop-butyl, molinate, propa,nil and quinclorac (Vidotto et al., 2007), *Alisma plantago-aquatica*, *Cyperus difformis*, and *Schoenoplectus mucronatus* to penoxsulam (Loddo et al., 2018), *Lolium rigidum* and *Bromus diandrus* to glyphosate (Barroso et al., 2010) was conducted globally. In Korea, Lim et al. (2021) reported the baseline sensitivity index of *Echinochloa crus-gall* and *E. oryzicola* to florpyrauxifen-benzyl, a new synthetic auxin herbicide. The baseline sensitivities of *Echinochloa crus-galli* to the very-long-chain fatty acid synthase (VLCFAs) inhibitors (mefenacet, pretilachlor, fentrazamide, and cafenstrole), PPO inhibitor (oxadiargyl), and herbicide with unknown mode of action (oxaziclomefone) were also conducted (Lim, 2013).

Based on the results obtained from our experiment, we predicted that there is a relatively low risk of the development of mesotrione and benzobicyclon resistant *S. juncoides* and *M. vaginalis* biotypes in Korea. The reason why resistance to the HPPD inhibiting herbicides has not yet evolved despite their quite long-term use in Korea might be the use of herbicides with a different mode of action in rice fields. However, we emphasize that farmers do not use sublethal doses or continuous use of mesotrione and benzobicyclon in the rice fields since repeated usage or sublethal doses of certain herbicide applications may lead to herbicide resistance (Ashworth et al., 2016). As an example, *Palmer amaranth* evolved resistance to dicamba as a result of lower dose application (Crespo et al., 2016) and the continuous use of 2,4-D for more than 12 years, resulting in a 2,4-D resistant *Amaranthus tuberculatus* population (Bernards et al., 2012). Interestingly, in the USA, HPPD inhibitor-resistant *Palmer amaranth* populations were observed in a cornfield, which had a history of continuous application and even with no previous history of use of HPPD herbicides (Sandell et al., 2012). This means, the population was not evolved resistance to HPPD inhibiting herbicides, but earlier used herbicides with different modes of action and exhibited cross-resistance to HPPD inhibiting herbicides. Lu et al. (2020) reported the cross-resistant wild radish population to the HPPD inhibiting herbicides mesotrione, tembotrione, and isoxaflutole which had shown 4 to 6.5-fold resistance compared to the susceptible population in the dose-response experiments.

Conclusion

In conclusion, the baseline sensitivity index for *S. juncoides* and *M. vaginalis* was relatively low (1.48-2.01) to HPPD inhibiting herbicide, mesotrione, and banzobicyclon. No shift in mesotrione and benzobicyclon sensitivity was observed suggesting that these HPPD inhibitors can still be used very effectively to control *S. juncoides* and *M. vaginalis* populations in rice fields in South Korea. However, HPPD inhibitor-resistant paddy weeds may occur considering that HPPD inhibiting herbicides have been used in cropping systems for more than 15 years (KCPA, 2007) in Korea. Therefore, constant monitoring is necessary to recognize the evolution of herbicide-resistant weed biotypes.

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Supplemental data 1. Sites of seed collection for *Scirpus juncoides* and their global positioning system (GPS) coordinators.

ID	GPS		ID	GPS		ID	GPS	
	N	E		N	E		N	E
1	35.15111	127.2018	36	126.5714	36.81276	71	127.3102	35.35012
2	36.12371	127.0733	37	126.5644	36.60197	72	127.6987	35.16933
3	36.13021	127.0654	38	126.6488	36.56456	73	127.5657	36.07823
4	36.13231	127.0233	39	126.5687	36.33317	74	127.2444	35.80604
5	36.08540	127.0412	40	126.6885	36.08365	75	127.245	37.00204
6	36.02340	127.4759	41	126.6885	36.09576	76	127.2443	37.10068
7	36.05530	127.4810	42	127.2308	36.14573	77	127.2446	37.00204
8	36.13230	127.3339	43	127.0993	35.98905	78	127.2440	37.20112
9	36.17501	127.3307	44	126.8583	35.82778	79	127.7128	37.50118
10	36.23231	127.4007	45	126.6861	35.42964	80	127.2442	37.17886
11	36.45510	127.4019	46	126.4946	35.10087	81	127.2453	37.25291
12	36.53451	126.8819	47	126.4236	34.63857	82	127.9076	37.15263
13	36.45490	127.0444	48	126.7063	34.69550	83	127.9146	37.20223
14	36.42280	127.3234	49	127.1247	34.76992	84	127.7578	37.25745
15	36.51261	127.2817	50	127.7964	34.99991	85	127.6711	37.30982
16	36.52010	127.2552	51	127.9624	35.30239	86	127.5644	37.35693
17	36.58050	127.2405	52	127.2444	36.40204	87	127.5714	37.40684
18	36.50560	127.1419	53	127.2450	36.40068	88	127.5644	37.45276
19	36.48251	126.9026	54	127.9537	35.30239	89	127.6488	36.60197
20	36.53350	127.3405	55	127.6508	35.66589	90	127.5687	36.56456
21	36.51161	127.3556	56	127.4750	35.97880	91	127.6885	36.33317
22	36.58520	127.3627	57	127.2146	36.19203	92	127.6885	36.08365
23	36.40204	127.2444	58	128.3195	36.22165	93	127.2308	36.09576
24	36.40068	127.2450	59	128.4037	37.03021	94	128.0993	36.14573
25	36.40204	127.2443	60	128.4314	37.09699	95	126.8583	36.98905
26	36.40112	127.2446	61	127.9761	37.55987	96	126.6861	35.82778
27	36.40118	127.2440	62	127.3345	35.75581	97	128.4946	35.42964
28	36.47886	127.1128	63	127.1044	35.60591	98	128.4236	35.10087
29	36.40291	127.2442	64	127.4074	35.60907	99	128.7063	36.63857
30	36.40263	127.2453	65	127.2054	36.66556	100	128.1247	36.69550
31	36.59223	126.9076	66	126.5062	35.47674	101	128.7964	36.76992
32	36.58745	126.9146	67	126.5723	35.44194	102	128.9624	36.99991
33	36.68982	126.7578	68	126.5994	35.27088	103	128.9537	36.30239
34	36.69693	126.6711	69	127.1083	35.15346	104	128.6508	36.66589
35	36.79684	126.5644	70	127.2559	35.42928	105	128.4750	36.97880

Supplemental data 2. Sites of seed collection for *Monochoria vaginalis* and their global positioning system (GPS) coordinators.

ID	GPS		ID	GPS		ID	GPS	
	N	E		N	E		N	E
1	36.40204	127.2444	36	37.55987	128.4314	71	35.82778	126.8583
2	36.40068	127.2450	37	35.75581	127.0761	72	35.42964	126.6861
3	36.40204	127.2443	38	35.60591	127.0345	73	35.10087	128.4946
4	36.40112	127.2446	39	35.60907	127.1044	74	36.63857	128.4236
5	36.40118	127.2440	40	36.66000	127.4074	75	36.69550	128.7063
6	36.47886	127.1128	41	35.47674	127.2054	76	36.76992	128.1247
7	36.40291	127.2442	42	35441941	126.5062	77	36.99991	128.7964
8	36.40263	127.2453	43	35.27088	126.5723	78	36.30239	128.9624
9	36.59223	126.9076	44	35.15346	126.5994	79	36.66589	128.9537
10	36.58745	126.9146	45	35.42928	127.1083	80	36.97880	128.6508
11	36.68982	126.7578	46	35.35012	127.2559	81	36.19203	128.4750
12	36.69693	126.6711	47	35.16933	127.3102	82	35.22165	128.2146
13	36.79684	126.5644	48	36.07823	127.6987	83	35.03021	128.3195
14	36.81276	126.5714	49	35.80604	127.5657	84	36.09699	128.4037
15	36.60197	126.5644	50	37.40204	127.2444	85	35.55987	128.4314
16	36.56456	126.6488	51	37.10068	127.2450	86	36.75581	128.0761
17	36.33317	126.5687	52	37.00204	127.2443	87	36.60591	128.0345
18	36.08365	126.6885	53	37.10112	127.2446	88	36.60907	128.1044
19	36.09576	126.6885	54	37.50118	127.244	89	36.66000	128.4074
20	36.14573	127.2308	55	37.27886	127.1128	90	36.47674	128.2054
21	35.98905	127.0993	56	37.25291	127.2442	91	36.44194	128.5062
22	35.82778	126.8583	57	37.15263	127.2453	92	35.27088	128.5723
23	35.42964	126.6861	58	37.20223	127.9076	93	35.15346	128.5994
24	35.10087	126.4946	59	37.25745	127.9146	94	35.42928	128.1083
25	34.63857	126.4236	60	37.30982	127.7578	95	35.35012	128.2559
26	34.6955	126.7063	61	37.35693	127.6711	96	35.16933	128.3102
27	34.76992	127.1247	62	37.40684	127.5644	97	36.07823	128.6987
28	34.99991	127.7964	63	37.45276	127.5714	98	35.30604	128.5657
29	35.30239	127.9624	64	36.60197	127.5644	99	37.89449	128.4893
30	35.66589	127.9537	65	36.56456	127.6488	100	37.95216	128.1355
31	35.97880	127.6508	66	36.33317	127.5687	101	36.76386	128.0557
32	36.19203	127.4750	67	36.08365	127.6885	102	36.61719	129.1140
33	36.22165	127.2146	68	36.09576	127.6885	103	37.29579	128.4085
34	37.03021	128.3195	69	36.14573	127.2308	104	35.95900	129.2447
35	37.09699	128.4037	70	36.98905	128.0993	105	35.81611	129.2251