

Full Length Research Paper

# Effects of genetically modified herbicide-tolerant (GMHT) rice on biodiversity of weed in paddy fields

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To detect potential changes in properties of weed communities in fields of GMHT rice Bar68-1, trials were carried out from 2007-2008 at Changsha, China with conventional *indica* rice D68 served as control. The average richness of weed community measured by species accumulation curve tended to be identical in the fields of Bar68-1 and D68 as the number of sampling points increased. There were no significant differences ( $p > 0.05$ ) in diversity indices which included numbers of species ( $S$ ), Shannon-Wiener ( $H'$ ), Pielou evenness ( $J'$ ), Simpson diversity ( $D$ ) and evenness ( $E$ ) indices. Species composition for these two weed communities was roughly comparable. The top four weed species, sorted by individual abundance, were *Monochoria vaginalis* (Burm. f.) Presl ex Kunth, *Lindernia procumbens* (Krock.) Philcox, *Cyperus difformis* L. and *Juncellus serotinus* (Rottb.) .B. Clarke in the fields of Bar68-1 and D68. ABC curves showed that the weed communities were "unpolluted". The data above confirm the hypothesis that the difference between the effect of GMHT rice Bar68-1 on biodiversity of weed in paddy fields and that of non-GM rice D68 was not significant.

**Key words:** Transgenic rice, community diversity, weeds species diversity, biosafety, environmental risk assessments, herbicide-resistant crops.

## INTRODUCTION

Herbicide-tolerance has been the predominant trait of genetically modified (GM) crops since their commercialization. In 2008, herbicide-tolerant soybean, maize, canola, cotton and alfalfa occupied 63% of the global area of GM crops (James, 2008; Marshall, 2009). The breeding of genetically modified herbicide-tolerance (GMHT) rice has been studied widely (Datta et al., 1992; Huang et al., 1998; Toldi et al., 2000; Cao et al., 2004; Endo et al., 2007; Xiao et al., 2007). On one hand, GMHT rice could be an effective means for weed control, especially for the management of rapid emergence of weedy rice. And GMHT rice could also be used in hybrid

seed production, to raise purity of parents of hybrid rice, to shorten the time for detection of hybrid rice seeds, to increase purity of hybrid rice, and to boost profits. On the other hand, many scientists and the public have concerns that GMHT rice would bring reduction of biodiversity, then affect the balance of agro-ecosystem; that exogenous gene of GMHT rice would escape to cultivated rice, weedy rice and its wild relatives through gene flow; and also that GMHT rice would become a weed or invasive natural habitats. However, few investigations of effects of GMHT rice on biodiversity have been conducted, most of the recent papers report on gene flow (Messeguer et al., 2001, 2004; Gealy et al., 2003; Chen et al., 2004; Lu and Snow, 2005; Jia et al., 2007; Shivrain et al., 2007; Espinoza-Esquivel and Arrieta-Espinoza, 2007). To meet people's demand of food and ensure its safety to people and environment, the research about effects of GMHT rice on biodiversity in paddy rice field becomes urgent and important.

Cerdeira and Duke (2006) pointed out that glyphosate-

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**Abbreviations:** GM, Genetically modified; GMHT, genetically modified herbicide-tolerance; SACs, species accumulation curves; FSEs, farm-scale evaluations.

**Table 1.** Arrangement of field experiments (2007-2008).

Year	Field	Sowing	Transplant	Harvest	Sample	Paddy field area (m <sup>2</sup> )	Plot number per field	Plot number per rice variety	Plot area (m <sup>2</sup> )	Hill space
2007	A	May 2	May 27	Aug 15	Aug 19, Sept 2.	850	6	3	120	20 × 20 cm
2007	B	May 18	Jun 5	Aug 29	Aug 5, Sept 4.	2145	12	6	120	20 × 20 cm
2008	A	May 2	Jun 2	Aug 12	Aug 22, Sept 1.	850	6	3	120	20 × 20 cm
2008	B	May 25	Jun 20	Sept 5	Aug 26, Sept 9.	2145	12	6	120	20 × 20 cm

tolerant crops is highly unlikely to bring a risk to wild plant populations. Lu et al. (2008) stated that the existing GMHT (*EPSPS* and *Bar* gene) rice should not bring obvious adverse effect to the biodiversity of rice ecosystem. However, Yu et al. (2005) investigated transplanting rice fields under the conditions of no weeding and hand weeding in 2001, and found that the GMHT *japonica* rice "99-1" with *Bar* gene was more competitive to the weeds than conventional rice "Xiushui 11", which showed GMHT rice significantly inhibited the individual number of seedling and plant biomass of *Cyperus difformis* L., *Juncellus serotinus* (Rottb.) C.B. Clarke and that of total weeds (Yu et al., 2005). Is the impact of GMHT *indica* rice Bar68-1 on biodiversity of weed different from that of its conventional counterpart D68? A field experiment is needed to make it clear.

Weeds are parts of agro-biodiversity, they are indicators for biodiversity, and provide a large untapped reservoir of genetic diversity (Spahillari et al. 1999; Riches and Valverde, 2002), a habitat for some beneficial insects (Booth et al., 2003), and a major food resource for seed-eating birds (Watkinson et al., 2000). The purpose of this paper was to investigate the changes in the properties of weed communities and find out whether the effects of GMHT *indica* rice Bar 68-1 on biodiversity of weed communities in rice paddy fields are similar to that of its conventional counterpart D68. The data collected in these experiments provides useful information to assess the ecological risk of GMHT rice to related agro-ecosystem and biological diversity.

## MATERIALS AND METHODS

### Materials

The GMHT rice cultivar Bar68-1, which integrated the bar gene, was developed from conventional *indica* rice cultivar D68 (Xiao et al., 2007). D68 was served as control.

### Experimental design and crop management

The experiment was carried out at Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China (28.20N, 113.08E) in 2007 and 2008 with approval document No. 2006-060. A regular field management practice was applied as described earlier (Xiao et al., 2006). To compare the impact of GMHT and

non-GM rice on weed communities, neither herbicide was sprayed nor were other weed control methods such as hand weeding conducted. Field experimental arrangement was listed in Table 1. The field experiment was composed of two parts, one with GMHT, the other with conventional rice. The same rice varieties were planted at the same locations in the next year. Plots were randomized by using a random selection of numbers.

### Sampling

Weeds were sampled (Table 1) by the "Inverted W" (Thomas, 1985) method with nine squares each plot. All weeds rooted within the boundary of the squares (0.5 × 0.5 m) were cut at ground level, sorted into species, counted and weighed fresh plants individually.

### Statistical analysis

Species accumulation curves (SACs), diversity indices and abundance/biomass comparison curves (ABC curves) are used to compare community properties. The SACs (rarefaction curve) describes the rise in cumulative number of species with increase in the number of sample sites (plots) (Kindt and Coe, 2005). The calculation of diversity index is by the methods from Magurran (2004): Species richness (*S*) is the number of species recorded in the sample area. Species diversity was expressed by Shannon-Weaver diversity index (*H'*):

$$H' = -\sum_{i=1}^S p_i \ln(p_i)$$

*p<sub>i</sub>*: the relative abundance of each group of organisms. Community evenness calculates as Pielou evenness index (*J'*):

$$J' = \frac{H'}{\log 2S}$$

And calculate Simpson dominance index (*D*) and Simpson evenness index (*E*) as follows:

$$D = 1 - \sum \left( \frac{n_i(n_i - 1)}{N(N - 1)} \right)$$

$$E = \frac{1}{S(1 - D)}$$

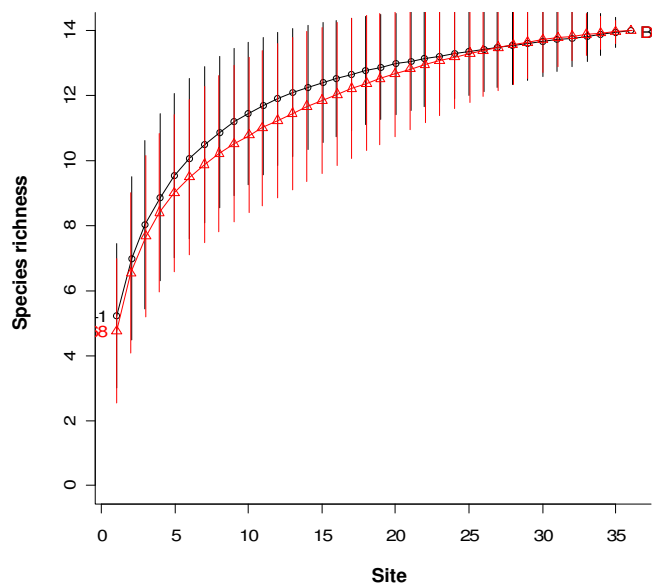


Figure 1. Sample-based species accumulation curve

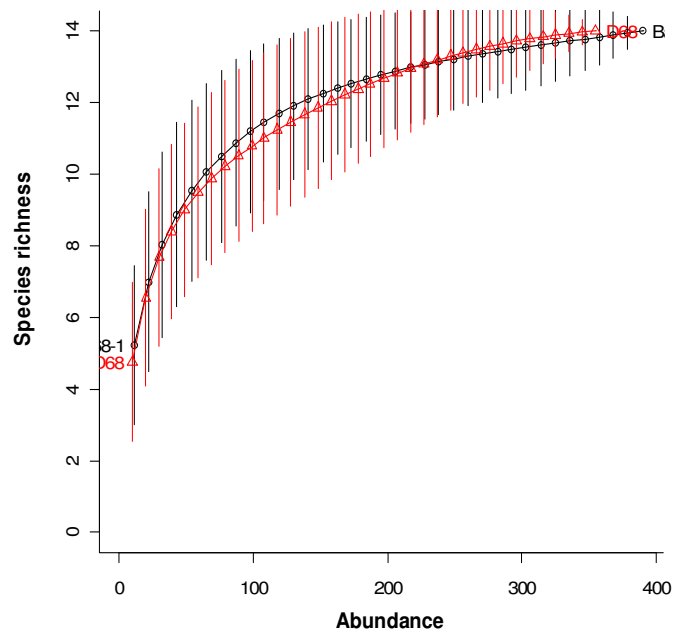


Figure 2. Individual-based species accumulation curve.

$n_i$ : number of individuals in the species;  $N$ : total number of individuals. The diversity indices were calculated by each plot separately total number of various subsets.

In the ABC curves, species are ranked from most to least important (either number of individuals or biomass) along the (logged) x-axis. The y-axis displays the cumulative abundance (as a percentage) of these species (Magurran 2004). ABC curve examine the entire species abundance distribution and a summary statistic -  $W$  can be calculated as follows:

$$W = \sum_{i=1}^S \frac{(B_i - A_i)}{50(S - 1)}$$

Where  $B_i$  = the biomass value of each species rank ( $i$ ) in the ABC curve;  $A_i$  = the abundance (individuals) value of each species rank ( $i$ ).  $A_i$  and  $B_i$  do not necessarily refer to the same species since species are ranked separately for each abundance measure.

SACs are drawn with R 2.8.1 (R Development Core Team 2008) and its package vegan (Oksanen et al. 2008) and BiodiversityR (Kindt and Coe, 2005). Origin 8.0 (OriginLab Corporation, Suite 303 Northampton, MA, USA) draws ABC curves. Means comparing are made by SPSS 15.0 (SPSS Inc., Chicago, IL, USA) using T-test. Variability around the mean are represented as  $\pm$  standard error of sample mean (SE).

## RESULTS AND ANALYSIS

### Species richness

SACs shows in Figures 1 and 2. Figure 1 displays the average richness of Bar68-1 and D68 computed by sample-based. For all possible combinations of 10 sites, their average richness was 11.45 and 10.79, whereas for all possible combinations of 36 sites, the average richness was 13.94 and 13.97. Figure 2 shows the results of SACs based on the number of plants surveyed. Figure 1 and 2

show similar patterns. If both curves of the individual-based and sample-based species accumulation have similar patterns, species are distributed at random over the sites (Kindt and Coe, 2005).

### Diversity indices

Diversity indices of different subsets of weed communities were calculated by the total number of various subsets or compared the mean value of each plot (Table 2) and compared at different sample time (Figure 3). The results show there were no differences ( $p > 0.05$ ) in diversity indices between the subsets in the fields of Bar68-1 and D68. These indices included number of species ( $S$ ), Shannon-wiener index ( $H'$ ), and Pielou evenness index ( $J$ ), Simpson diversity index ( $D$ ) and Simpson evenness index ( $E$ ).

### Species composition and rank

The top four weed species sorted by individual abundance were *Monochoria vaginalis* (Burm. f.) Presl ex Kunth, *Lindernia procumbens* (Krock.) Philcox, *Cyperus difformis* L. and *Juncellus serotinus* (Rottb.) B. Clarke both in the fields of Bar68-1 and D68 (Table 3). The top four weed species sorted by relative fresh weight (%) in the fields of Bar68-1 were *Monochoria vaginalis* (Burm. f.) Presl ex Kunth, *Echinochloa phyllopogon* (Stapf) Koss., *Cyperus difformis* L., and *Leptochloa chinensis* (L.) Nees. Those in the fields of D68 were *Monochoria vaginalis* (Burm. f.) Presl ex Kunth, *Echinochloa phyllopogon* (Stapf) Koss.,

**Table 2.** Biodiversity indices of weed communities in GMHT and non-GMHT rice paddy fields.

Year	Field	Index	Calculated by total number of subsets		Compared the mean value of each plot			
			Bar68-1	D68	Df	Bar68-1	D68	p-value
<b>2007</b>	<b>A</b>				<b>10</b>			
		<i>S</i>	9.00	9.00		4.50 ± 0.72	4.00 ± 0.26	0.53
		<i>H'</i>	1.62	1.44		1.19 ± 0.14	1.07 ± 0.07	0.45
		<i>J'</i>	0.74	0.79		0.82 ± 0.04	0.78 ± 0.05	0.57
		<i>D</i>	0.76	0.78		0.66 ± 0.05	0.63 ± 0.05	0.60
		<i>E</i>	0.15	0.14		0.38 ± 0.06	0.43 ± 0.06	0.60
<b>2007</b>	<b>B</b>				<b>22</b>			
		<i>S</i>	10.00	10.00		3.08 ± 0.34	2.83 ± 0.24	0.55
		<i>H'</i>	1.73	1.55		0.86 ± 0.10	0.87 ± 0.08	0.96
		<i>J'</i>	0.63	0.67		0.81 ± 0.04	0.86 ± 0.02	0.29
		<i>D</i>	0.64	0.67		0.59 ± 0.05	0.64 ± 0.04	0.41
		<i>E</i>	0.16	0.15		0.70 ± 0.10	0.66 ± 0.10	0.77
<b>2008</b>	<b>A</b>				<b>10</b>			
		<i>S</i>	9.00	10.00		4.17 ± 0.54	3.83 ± 0.48	0.65
		<i>H'</i>	0.83	0.67		1.12 ± 0.16	1.15 ± 0.11	0.89
		<i>J'</i>	1.81	1.78		0.80 ± 0.07	0.90 ± 0.03	0.21
		<i>D</i>	0.81	0.77		0.65 ± 0.08	0.69 ± 0.03	0.60
		<i>E</i>	0.14	0.13		0.49 ± 0.15	0.43 ± 0.09	0.75
<b>2008</b>	<b>B</b>				<b>22</b>			
		<i>S</i>	8.00	8.00		2.58 ± 0.23	2.33 ± 0.19	0.41
		<i>H'</i>	1.40	1.43		0.81 ± 0.07	0.73 ± 0.09	0.53
		<i>J'</i>	0.77	0.69		0.89 ± 0.03	0.88 ± 0.04	0.80
		<i>D</i>	0.64	0.64		0.63 ± 0.03	0.62 ± 0.07	0.89
		<i>E</i>	0.20	0.19		0.69 ± 0.07	0.87 ± 0.13	0.24
<b>Total</b>					<b>70</b>			
		<i>S</i>	14.00	14.00		3.33± 0.23	3.03 ± 0.17	0.18
		<i>H'</i>	1.80	1.80		0.94± 0.06	0.90 ± 0.05	0.57
		<i>J'</i>	0.72	0.68		0.84± 0.02	0.86 ± 0.02	0.31
		<i>D</i>	0.80	0.76		0.62± 0.02	0.64 ± 0.03	0.77
		<i>E</i>	0.09	0.09		0.61± 0.05	0.65 ± 0.06	0.74

S, Number of species; *H'*, Shannon-Wiener index; *J'*, Pielou evenness index; *D*, Simpson diversity index; *E*, Simpson evenness index.

*Alternanthera philoxeroides* (Mart.) Griseb and *Echinochloa crusgalli* (L.) Beauv (Table 4).

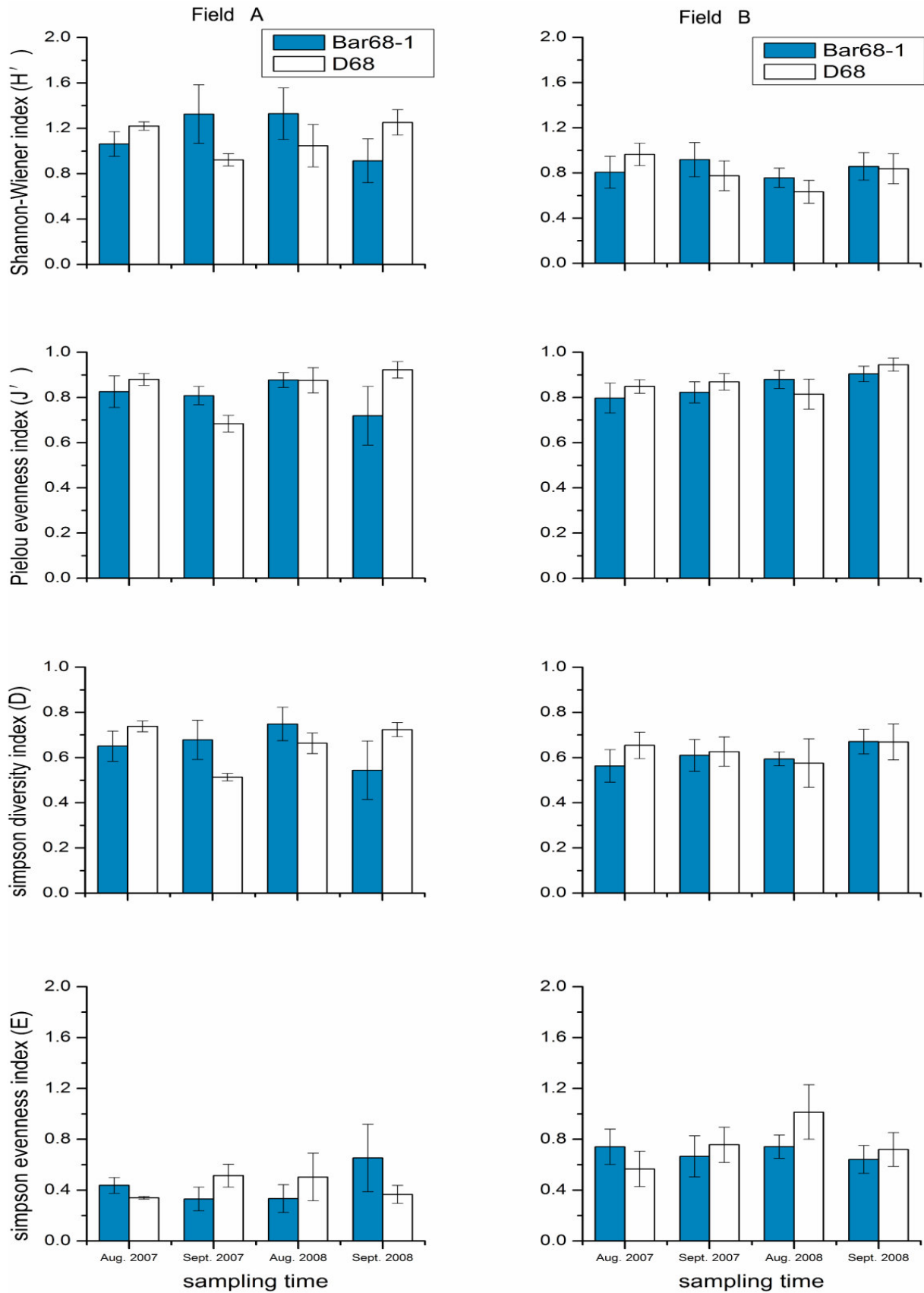
### Disturbance degree

Figures 4 and 5 show ABC curves of the weed communities in the rice paddy fields of Bar68-1 and D68 respectively. ABC curves in both Figure 4 and 5 shows "unpolluted" conditions. The W values related to each curve were positive. These results give support to the interpreting of graphing.

### DISCUSSION

On the basis of experimental data collected in fields of GMHT rice Bar68-1 and its unmodified counterpart D68,

SACs show the average richness of weed species increased and tended to be identical as sample sites increased; differences of diversity indices were not significant in various subsets of Bar68-1 and D68; species composition for these two weed communities was very similar; ABC curves show "unpolluted" conditions for the level of disturbance affecting the assemblage (The placement of curves based on species abundance and based on biomass in ABC curves is used to make inferences about the levels of disturbance, pollution-induced or otherwise, affecting the assemblage, named by "unpolluted," "moderately polluted," and "grossly polluted" conditions (Magurran, 2004). In undisturbed assemblages, it will be characterized by species that have large body size and long life spans and these species are unlikely to be numerically dominant but are expected to be dominant in terms of biomass (Magurran, 2004). The



**Figure 3.** Diversity indices of weed communities at different sampling time in GMHT and non-GMHT rice paddy fields.

**Table 3.** Abundance percentage of weed communities in GMHT and non-GMHT rice paddy fields.

Weed species	Bar68-1			D68		
	Rank	Abundance	Proportion (%)	Rank	Abundance	Proportion (%)
<i>Monochoria vaginalis</i> (Burm. f.) Presl ex Kunth	1	135	34.62	1	143	40.28
<i>Lindernia procumbens</i> (Krock.) Philcox	2	82	21.03	2	85	23.94
<i>Cyperus diformis</i> L.	3	64	16.41	3	36	10.14
<i>Juncellus serotinus</i> (Rottb.) C.B. Clarke	4	37	9.49	4	23	6.48
<i>Echinochloa phyllopogon</i> (Stapf) Koss.	5	17	4.36	6	15	4.23
<i>Alternanthera philoxeroides</i> (Mart.) Griseb	6	15	3.85	5	15	4.23
<i>Cyperus iria</i> L.	7	13	3.33	7	13	3.66
<i>Jussiaea linifolia</i> Vahl	8	8	2.05	9	3	0.85
<i>Fimbristylis dichotoma</i> (L.) Vahl	9	5	1.28	15	0	0.00
<i>Leptochloa chinensis</i> (L.) Nees	10	4	1.03	10	2	0.56
<i>Echinochloa crusgalli</i> (L.) Beauv.	11	4	1.03	8	13	3.66
<i>Marsilea quadrifolia</i> L.	12	4	1.03	16	0	0.00
<i>Lindernia crustacea</i> (L.) F. Muell	13	1	0.26	17	0	0.00
<i>Ammannia baccifera</i> L.	14	1	0.26	12	2	0.56
<i>Scirpus juncooides</i> Roxb.	15	0	0.00	11	2	0.56
<i>Leersia hexandra</i> Swartz	16	0	0.00	13	2	0.56
<i>Jussiaea reppens</i> L.	17	0	0.00	14	1	0.28

**Table 4.** Biomass percentage of weed communities in GMHT and non-GMHT rice paddy fields.

Weed species	Bar68-1			D68		
	Fresh weight per plant (g)	Sum of fresh weight (g)	Relative fresh weight (%)	Fresh weight per plant (g)	Sum of fresh weight (g)	Relative fresh weight (%)
<i>Monochoria vaginalis</i> (Burm. f.) Presl ex Kunth	33.1 ± 5.24	4468.03	55.45	36.57 ± 5.00	5229.53	60.76
<i>Lindernia procumbens</i> (Krock.) Philcox	1.24 ± 0.12	102.04	1.27	1.28 ± 0.12	108.53	1.26
<i>Cyperus diformis</i> L.	8.13 ± 1.52	520.63	6.46	9.85 ± 2.12	354.71	4.12
<i>Juncellus serotinus</i> (Rottb.) C.B. Clarke	7.17 ± 0.72	265.14	3.29	7.34 ± 0.68	168.92	1.96
<i>Echinochloa phyllopogon</i> (Stapf) Koss.	90.46 ± 22.35	1537.75	19.08	88.65 ± 16.76	1329.72	15.45
<i>Alternanthera philoxeroides</i> (Mart.) Griseb	22.01 ± 5.14	330.08	4.10	40.43 ± 8.13	606.39	7.05
<i>Cyperus iria</i> L.	12.52 ± 4.04	162.78	2.02	3.08 ± 1.22	40.10	0.47
<i>Jussiaea linifolia</i> Vahl	16.02 ± 12.14	128.17	1.59	54.75 ± 53.16	164.25	1.91
<i>Fimbristylis dichotoma</i> (L.) Vahl	10.08 ± 1.85	50.42	0.63	0.00	0.00	0.00
<i>Leptochloa chinensis</i> (L.) Nees	101.15 ± 29.72	404.61	5.02	72.44 ± 1.90	144.87	1.68
<i>Echinochloa crusgalli</i> (L.) Beauv.	12.29 ± 5.94	49.18	0.61	34.19 ± 8.45	444.51	5.16

Table 4. Cont.

<i>Marsilea quadrifolia</i> L.	0.01 ± 0.00	0.04	0.00	0.00	0.00	0.00
<i>Lindernia crustacea</i> (L.) F. Muell	0.00	0.00	0.00	0.33	0.33	0.00
<i>Ammannia baccifera</i> L.	0.36 ± 0.15	1.90	0.02	1.90	0.72	0.01
<i>Scirpus juncooides</i> Roxb.	0.00	0.00	0.00	6.38 ± 0.27	0.00	0.00
<i>Leersia hexandra</i> Swartz	37.01	37.01	0.46	11.21	11.21	0.13
<i>Jussiaea reprens</i> L.	0.00	0.00	0.00	3.16	3.16	0.04

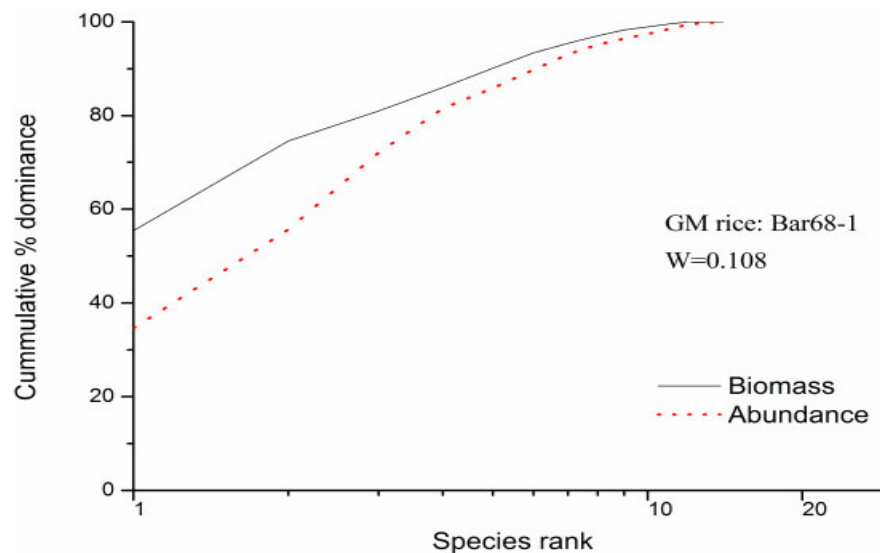


Figure 4. ABC curve of weed communities in GMHT rice paddy fields.

individuals (or abundance) curve will be expected to lie below the biomass curve (Magurran, 2004). Our results indicate that GMHT *indica* rice Bar 68-1 didn't change weed biodiversity in the rice paddy fields. In recent years, more and more people concerned about the potential impact of GMHT crops on biodiversity (Dale et al., 2002; Butler et al., 2007). There are some reports related to the issue of GMHT crops on weed

biodiversity. Watkinson et al. (2000) simulated the effects of GMHT crops on weed populations, and predicted weed populations might be reduced to low-level under the management of GMHT crops. Field experimental results from farm-scale evaluations (FSEs) of GMHT crops in the UK indicated that weed diversity was little affected by GMHT crops with weeds controlled by a broad-spectrum herbicide, except for transient effects immediately

following herbicide application in the fields of GMHT sugar beet, maize and spring oilseed rape (Heard et al., 2003). A study conducted in Alberta, Canada found weed densities increased in conventional varieties, compared to GMHT canola sprayed with broader-spectrum herbicides (Cathcart et al., 2006). An experiment carried out in Argentina showed that weed richness in field of GMHT soybean decreased or remained stable in

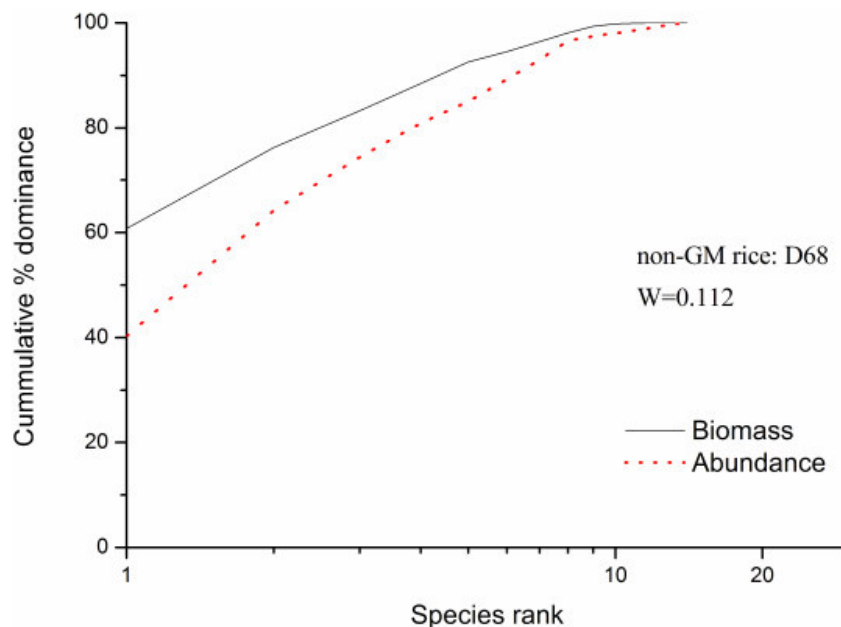


Figure 5. ABC curve of weed communities in non-GMHT rice paddy fields.

stable in the early planting season before glyphosate application but increased at harvest (Vitta et al., 2004). Weed diversity in GMHT soybean fields by limited use of glyphosate (single application  $\text{yr}^{-1}$ ) was equivalent to or even higher than that in non-GM systems (Scursoni et al., 2006).

There is an imperfection in the experiments showed above, in which the impacts of GMHT crops and that of herbicides are confounded, just as Andow (2003) pointed out: “the results do not demonstrate the ‘safety’ or ‘risk’ of the transgenes themselves, as their effect is not isolated from others”, while it refers to the experimental design of the FSEs. As herbicides were used, the results of these studies above would be the combined effects of GMHT crops and herbicides. In our experiments, no herbicide was used, so that the result was the impact of GMHT rice itself, and can be used to test whether the effect of GMHT rice Bar68-1 on biodiversity of weed in paddy fields is identical or similar to that of non-GM rice D68.

According to the results from species accumulation curves, diversity indices and ABC curves, we concluded that the effect of GMHT rice Bar 68-1 on biodiversity of weed communities has no significant difference from that of its unmodified counterpart, D68.

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