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[Short Report]

# Tolerance of Rice (*Oryza sativa* L.) and *Echinochloa* Weeds to Growth Suppression by Rice Straw Added to Paddy Soil in Relation to Iron Toxicity

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**Abstract:** The effects of addition of rice straw to submerged soil on the emergence and growth of rice (*Oryza sativa* L.) and two paddy weeds (*Echinochloa oryzicola* Vasing. and *Echinochloa crus-galli* (L.) Beauv. var. *crus-galli*) were investigated. Rice straw suppressed both the emergence and growth of transplanted plants depending on the amount of rice straw added (0%, 0.3%, 0.6% and 0.9% (w/w)) in the order of *E. crus-galli* > *E. oryzicola* > rice. The severe suppression of emergence and growth of *E. crus-galli* in the presence of 0.9% rice straw in hydroponic culture was thought to be due to high Fe content of the shoots. Since the difference in tolerance for the toxicity of rice straw is an important factor, the addition of organic materials into soil may help to suppress *Echinochloa* weeds selectively.

**Key words:** *Echinochloa* weed, Ferrous iron, Growth suppression, Organic material, Paddy soil, Rice, Weed.

One of the most difficult procedures in rice production is weeding. Recently consumers are concerned about food safety and the present trend in weed control is to reduce dependency on herbicides by using natural products. Utilization of rice residues in paddy fields has long been recognized as an important source for improvement of the organic matter status of soil and has also been reported to suppress the growth of weeds. For example, addition of the by-products of rice reduced the emergence of *Echinochloa oryzicola* Vasing. and improved rice yield (Xuan et al., 2003). One mechanism of this suppression is thought to be an allelopathic effect of organic materials. Although many allelochemicals such as phenolic acids, cytokinins, fatty acids, indoles and terpenes have been found in rice root exudates and decomposing residues, it is not clear which compounds play major roles in allelopathy (Khanh et al., 2007). In our previous paper, we suggested that the accumulation of Fe(II), the concentration of which increases with the increase in the amount of organic materials, in soil solution was one of the factors related to the growth suppression of paddy weeds (Nozoe et al., 2009).

Two *Echinochloa* weeds are commonly observed in Japanese paddy fields (Shibayama 2001). One is *E. oryzicola* Vasing. (early watergrass) an obligate weed with an

elaborative survival strategy in flooded rice fields in Japan (Yamasue 2001). The weed is the most dominant, is distributed only in flooded rice fields, and has mimicry of rice plants throughout its development from seedling to heading. The other is *E. crus-galli* (L.) Beauv. var. *crus-galli* (barnyard grass), which is highly polymorphic and is widely distributed in various habitats from water ditches and rice paddy fields to dry open land.

Although the incorporation of rice straw into soil is known to suppress the growth of rice, its effects on the growth of *Echinochloa* weeds have not been elucidated. If rice has a stronger tolerance for growth suppression than *Echinochloa* weeds, the difference in tolerance might help to suppress the growth of weeds selectively. In this study, therefore, laboratory and pot experiments were conducted to investigate the difference in growth suppression between rice and *Echinochloa* weeds in the presence of rice straw and to investigate the relationship between growth suppression and Fe toxicity.

## Materials and Methods

### 1. Plant materials

Seeds of rice (*Oryza sativa* L. cv. Hoshinoyume) were collected from the field of National Agricultural Research Center (NARC) for Hokkaido Region in Sapporo City,

Japan (43° 0' N, 141° 24' E). This variety is widely cultivated in Hokkaido, the northernmost island of Japan (Fujino 2003). Seeds of *E. oryzicola* Vasing. and *E. crus-galli* (L.) Beauv. var. *crus-galli* were sampled from plants grown in concrete pots (50 cm × 50 cm × 15 cm in height) in October 2006 at NARC for Tohoku Region, Daisen City, Japan (39° 29' N, 140° 29' E). To break dormancy, they were placed in moist soil in a refrigerator at 5°C for more than two months. Germination rates in rice, *E. oryzicola* and *E. crus-galli* were 100%, about 60% and about 30%, respectively. These data were obtained after incubation of seeds in distilled water at 30°C for 7 d.

## 2. Soil

Volcanic-ash soil was sampled from the plow layer (0 to 15 cm in depth) of a paddy field at NARC for Hokkaido Region. Bulk density of the soil sample was 0.89 g cm<sup>-3</sup>, which was measured by the method of Blake and Hartge 1986. The soil sample was air-dried and passed through a 2-mm mesh sieve. Value of pH was measured using a mixture of soil and distilled water (soil: water(w/v)=1:2.5) (Thomas 1996). Amount of total nitrogen (N) was analyzed using Kjeldahl's method (Bremner 1996), and that of free iron oxide was analyzed by the method of Asami and Kumada (1959). The pH value was 5.8, and amounts of total N and free iron oxide were 3.4 g kg<sup>-1</sup> and 4.6 g kg<sup>-1</sup> (as Fe), respectively.

## 3. Emergence in flooded soil with rice straw added

Rice straw (cv. Hoshinoyume) was obtained after rice cultivation in the field of NARC for Hokkaido Region in 2005. The straw was dried, powdered, and placed in a refrigerator at 5°C. It was passed through a 2-mm mesh sieve before the experiment. One hundred grams of soil with powdered rice straw was put into a polyethylene vessel (60 mm × 60 mm × 85 mm in height). The ratios of straw to soil were 0, 0.3, 0.6 or 0.9% (w/w). Distilled water was added to the soil, followed by thorough stirring to remove air in the soil. Nine seeds of rice and 16 seeds of *E. oryzicola* were sown in the soil at a depth of 2 cm depth. Sixteen seeds of *E. crus-galli* were sown at a depth of 0.5 cm because of poor germination at a depth of 2 cm. The depth of flooded water was maintained at 3–4 cm throughout the incubation. The seeds were incubated at 30°C for 21 days under a 12-hr light/12-hr dark condition. The emergence rates of rice and weeds was recorded on the 7th, 14th and 21st d. A completely randomized design was used and each treatment had 4 replications.

## 4. Growth of seedlings transplanted into flooded soil with rice straw added

To make seedlings, seeds of rice, *E. oryzicola* and *E. crus-galli* were incubated in a tall Petri dish (9 cm in diameter and 6 cm in height) with 10 ml of distilled water at 30°C

under a 12-hr light/12-hr dark condition. The incubation period was 4 d for rice and *E. oryzicola* and 7 d for *E. crus-galli*. Two seedlings each of rice, *E. oryzicola* and *E. crus-galli* were transplanted to vessels prepared in the same manner as described above: 100 g of soil with 0, 0.3, 0.6 or 0.9% (w/w) of powdered rice straw was put into the polyethylene vessel and the vessel was flooded. The seedlings were transplanted on 17 August 2007 and grown in a greenhouse of NARC for Hokkaido Region. The seedlings were thinned to one on the 7th d of cultivation. The period for cultivation was 21 d. At harvest, the shoot was separated from the root, and shoot samples were oven-dried at 70°C for 2 d and weighed. Another plot without seedlings was prepared to analyze Fe(II) contents in soil solution. A small hole was made in the side of the vessel at 2 cm below the soil surface to collect the soil solution. A porous cup (3 mm in diameter and 9 cm in length) was inserted into the hole and set horizontally to the soil surface. The space between the vessel and the porous cup was sealed with adhesive tape. The porous cup was connected to a flexible plastic tube, and the soil solution obtained was introduced into a 10 ml evacuated test tube. After a given period of incubation, the amount of Fe(II) in the soil solution was determined. A completely randomized design was used and each experiment was conducted with 4 replications.

## 5. Growth of seedlings transplanted to ferrous solution

The seedlings were grown hydroponically in a plastic container with 5 L nutrient solution. Seedlings of rice, *E. oryzicola* and *E. crus-galli* were produced in the same manner as that described above: seeds of rice, *E. oryzicola* and *E. crus-galli* were incubated in a tall Petri dish with 10 ml of distilled water for 4 or 7 d in an incubator at 30°C under a 12-hr light/12-hr dark condition. Individual seedlings were planted in a perforated sheet made of styrene foam and floated on the solution. Standard solution for rice nutrition (Mae and Ohira 1981) was used. The nutrient composition of the solution except for Fe was as follows (mM): NH<sub>4</sub>NO<sub>3</sub>, 1.0; NaH<sub>2</sub>PO<sub>4</sub>•2H<sub>2</sub>O, 0.6; K<sub>2</sub>SO<sub>4</sub>, 0.3; CaCl<sub>2</sub>•2H<sub>2</sub>O, 0.3; MgCl<sub>2</sub>•6H<sub>2</sub>O, 0.6; H<sub>3</sub>BO<sub>3</sub>, 5 × 10<sup>-2</sup>; MnSO<sub>4</sub>•2H<sub>2</sub>O, 9 × 10<sup>-3</sup>; CuSO<sub>4</sub>•5H<sub>2</sub>O, 3 × 10<sup>-4</sup>; ZnSO<sub>4</sub>•7H<sub>2</sub>O, 7 × 10<sup>-4</sup>; Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O, 1 × 10<sup>-4</sup>. The amounts of Fe, which were added as Fe(III)-EDTA (ethylenediaminetetraacetic acid), under normal and toxic conditions were 2.5 and 50 mg L<sup>-1</sup>, respectively. These elements were dissolved in distilled water. The solution was adjusted to pH 4.5 using HCl or NaOH, and the solution was replaced every two or three days. The period for cultivation was 14 d. At harvest, the shoot was separated from the root. The shoot sample was oven-dried at 70°C for 2 d, and Fe content in the sample was determined. There were 15 plants per container, and there was no replication of container. Data are expressed as means of 15

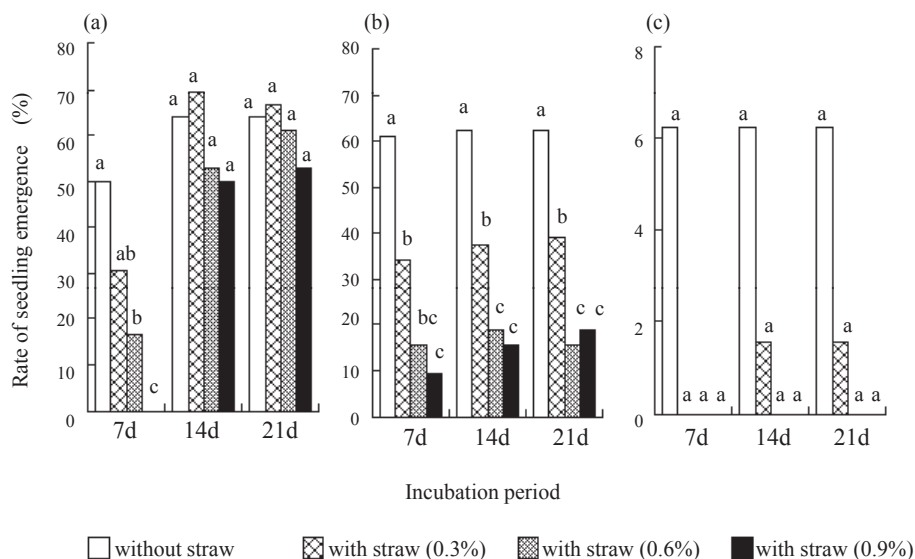


Fig. 1. Effects of addition of rice straw on the emergence of *O. sativa* (a), *E. oryzae* (b) and *E. crus-galli* (c). Sowing depths of *O. sativa*, *E. oryzae* and *E. crus-galli* were 2, 2 and 0.5 cm, respectively. On each recording date, symbols with different letters denote a significant difference at the 5% level by Tukey's multiple range test ( $n=4$ ).

plant samples.

## 6. Chemical analysis of Fe

The amounts of Fe in the soil solution and shoot sample were determined by using a colorimetric method with  $\alpha$ -phenanthroline, which makes an orange compound in the presence of Fe(II) (Loeppert and Inskeep 1996). A spectrophotometer (HITACHI, U-1000) at absorbance of 510 nm was used for determination. The amount of Fe in the shoot was determined by digesting the powdered samples by the method of wet ashing with  $H_2SO_4$  and  $H_2O_2$  (Mingorance 2002). Prior to color development,  $HONH_3Cl$  (hydroxylammonium chloride) was added to the digested solution to reduce Fe(III) to Fe(II), because Fe(III) does not form an orange compound with  $\alpha$ -phenanthroline (Stucki and Anderson 1981).

## 7. Statistical analysis

Data analysis was conducted using statistical software (SPSS16.0J for Windows, SPSS Inc., Tokyo, Japan).

## Results and Discussion

### 1. Emergence in flooded soil with rice straw added

The addition of rice straw suppressed the emergence of rice and *E. oryzae* on the 7th d of incubation (Figs. 1a, 1b). The degree of suppression increased with the increase in straw content. Although the emergence of rice increased with the increase in the incubation period, that of *E. oryzae* hardly changed during the subsequent incubation periods. Even when the sowing depth was 0.5 cm, *E. crus-galli* barely emerged regardless of the addition of straw (Fig. 1c). These findings indicate that the degree

Table 1. Growth suppression of rice and *Echinochloa* weeds in the presence of rice straw.

	Straw/Soil (%)	Dry weight* (mg)	Relative dry weight
<i>O. sativa</i>	0	108 ab	100
	0.3	125 a	116
	0.6	111 a	103
	0.9	93 b	86
<i>E. oryzae</i>	0	129 a	100
	0.3	123 a	95
	0.6	76 b	59
	0.9	71 b	55
<i>E. crus-galli</i>	0	97 a	100
	0.3	79 b	81
	0.6	57 c	59
	0.9	24 d	25

\* Symbols with different letters denote a significant difference at the 5% level by Tukey's multiple range test ( $n=4$ ).

of the suppression by the addition of rice straw was highest in *E. crus-galli* followed by *E. oryzae* and rice in this order.

### 2. Growth of seedlings transplanted to flooded soil with addition of rice straw

The addition of rice straw did not affect the shoot dry weight of rice (Table 1). On the other hand, the shoot dry weights of *E. oryzae* and *E. crus-galli* decreased with the increase in the amount of rice straw. The growth of *E. crus-galli* in the presence of 0.9% rice straw was greatly suppressed. According to the relative dry weight, growth

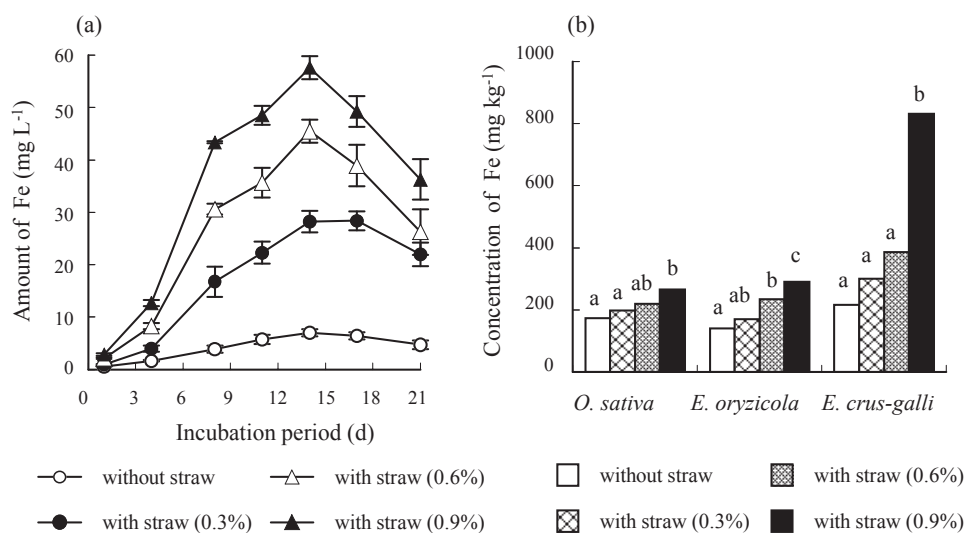


Fig. 2. Effects of addition of rice straw on the changes in amount of Fe(II) in soil solution (a) and the concentration of Fe in shoots of rice and *Echinochloa* weeds (b) under greenhouse conditions. Symbols with different letters denote a significant difference at the 5% level by Tukey's multiple range test ( $n=4$ ).

suppression by the addition of rice straw was in the order of *E. crus-galli* > *E. oryzicola* > rice. The amounts of Fe(II) in soil solution increased with the increase in the amount of rice straw and were in the range of 0 to 60 mg L<sup>-1</sup> (Fig. 2a). The amount of Fe in the shoot increased with the increase in the amount of rice straw (Fig. 2b). In the presence of 0.9% rice straw, the Fe content was higher in *E. crus-galli* than in either rice or *E. oryzicola*.

### 3. Growth of seedlings transplanted into ferrous solution

The presence of 50 mg L<sup>-1</sup> Fe suppressed the growth of rice and *Echinochloa* weeds (Fig. 3). Based on the values shown in Fig. 3, the ratio of shoot dry weight under Fe toxic conditions to that under normal conditions was calculated. The ratios for rice, *E. oryzicola* and *E. crus-galli* were 74, 63 and 27%, respectively. According to these values, Fe suppressed the growth of plants in the following order: *E. crus-galli* > *E. oryzicola* > rice. The Fe concentration in shoots of rice in the solution with 50 mg L<sup>-1</sup> Fe was greater than the concentration in weeds. In other words, rice showed the strongest tolerance to Fe toxicity despite the fact that the Fe content was the highest in rice.

### 4. Growth suppression by rice straw

As the bulk density of the soil was 0.89 g cm<sup>-3</sup>, the soil weight of a field with a plow layer of 10 cm in depth is calculated to be 890 t ha<sup>-1</sup>. If 0.89 t of rice straw is applied to such a field, the ratio of straw to soil by weight would be 0.1%. Therefore, rice straw was added to the soil at the rate of 0.3%, 0.6% or 0.9%. Those values were calculated on the basis of the assumption that 2.7, 5.3 or 8.0 t of straw was applied to a field of 1 ha × 10 cm.

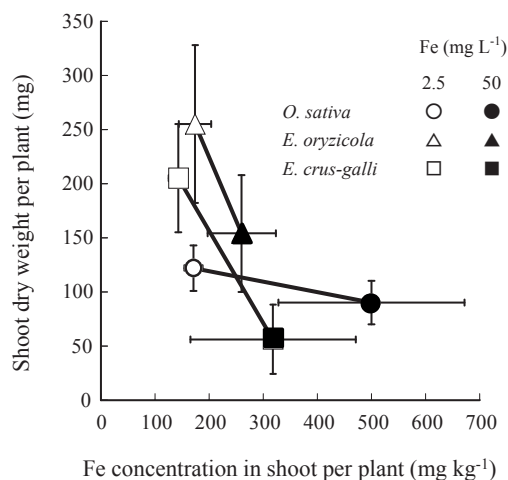


Fig. 3. Relationship between the concentration of Fe in the shoot and dry weight of rice and *Echinochloa* weeds. Bars indicate standard deviation ( $n=15$ ).

While *E. crus-galli* is distributed in various habitats from water ditches and rice paddies to dry open land in Japan, *E. oryzicola* is mainly distributed in flooded paddy fields (Yamasue 2001). Even from the germinated seeds of *E. oryzicola* placed under a submerged condition of 10 cm in depth, coleoptiles elongate to the surface of the water (Yamasue 2001). Rumpho and Kennedy (1981) and Kennedy et al. (1983) reported that *E. oryzicola* as well as rice have characters metabolically adaptive to submerged conditions in seed germination and seedling growth. The results shown in Fig. 1 and Table 1 suggest that adaptability to a reductive condition by the addition of rice straw was in the order of rice > *E. oryzicola* > *E. crus-galli*.

Thus, the application of rice straw can selectively suppress the emergence (Fig. 1) as well as the early growth (Table 1) of *E. oryzicola* and *E. crus-galli*. Field experiments in Japan have shown that the application of rice bran to a rice field suppresses the growth of *Echinochloa* weeds. For example, Fukushima and Uchikawa (2002) showed that the application of 2 t ha<sup>-1</sup> of rice bran suppressed the growth of *Echinochloa* weeds without reduction in the yield of transplanted rice. Although they suggested that this suppression was associated with physical resistance of weed emergence and/or soil reduction, the mechanism has not been elucidated.

In this study, the amounts of Fe(II) in soil solution increased with the increase in the amount of rice straw and were in the range of 0 to 60 mg L<sup>-1</sup> (Fig. 2a). Although the presence of Fe(II) of more than 100 mg L<sup>-1</sup> with serious toxicity for rice has been reported in some fields (Nozoe et al., 2008), the Fe(II) concentration used in this experiment is a common value under normal conditions (Dobermann and Fairhurst 2000).

To investigate the growth suppression by Fe, we conducted hydroponic culture using solutions with Fe concentrations of 2.5 and 50 mg L<sup>-1</sup>. It is difficult to maintain such high concentrations of Fe(II) in hydroponic culture because soluble Fe(II) is easily oxidized to insoluble Fe(III) oxides. Generally, rice plants can absorb chelated Fe(III) as well as Fe(II) ions (Ishimaru et al. 2006), and Fe(III)-EDTA is therefore sometimes used as an Fe source in hydroponics (e.g., Fagelia and Rabelo 1987; Zhang et al., 1999; Shimizu et al., 2005). In this study, therefore, we added Fe as Fe(III)-EDTA to prevent a decrease in Fe content.

Dobermann and Fairhurst (2000) reported that the critical level of Fe to show toxicity in rice leaves was over 300 mg kg<sup>-1</sup> from tillering to panicle initiation stage. However, there has been no report, to our knowledge, in which the critical levels for *Echinochloa* weeds are discussed. In this study, we found that Fe-tolerance of *E. crus-galli* was smaller than that of rice (Fig. 3). When the Fe concentration of *E. crus-galli* was about 300 mg kg<sup>-1</sup>, the growth was severely suppressed. In the pot experiment, the Fe concentration of *E. crus-galli* in the presence of 0.9% rice straw was over 800 mg kg<sup>-1</sup> (Fig. 2b). Thus, the small shoot dry weight of *E. crus-galli* in the presence of 0.9% rice straw (Table 1) was thought to be due to the high Fe content of the shoot.

In conclusion, the application of rice straw suppresses both the emergence and the growth of transplanted plants depending on the amount of rice straw added in the order of *E. crus-galli* > *E. oryzicola* > rice. In the presence of 0.9% rice straw, the smallest tolerance of transplanted *E. crus-galli* is attributed to poor tolerance for Fe toxicity. The difference in tolerance for the toxicity of rice straw can be used to suppress the growth of *Echinochloa* weeds selectively because the rice tolerance is the strongest, and its difference is likely to be a part of the basis of a weed control system by application of organic materials.

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