A Study on Tolerance to Pretilachlor in Eight Species of *Echinochloa*

**WANG Qing-ya**¹, **QIAO Li-ya**¹, **WEI Jie-gang**¹, **DONG Li-yao**², **LI Yang-han**¹ (¹College of Life Science, Nanjing Agricultural University, Nanjing 210095, China; ²College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China)

**Abstract:** Height of seedlings, length of leaf and coleoptile cells, and α-amylase activities of eight species or varieties of barnyardgrass (*Echinochloa*) were measured after seeds being treated with pretilachlor. There existed obvious differences in the tested traits among the materials. The inhibition rate of pretilachlor to *Echinochloa crusgalli* (L.) Beauv. var. mitis (Pursh.) Peterm. was the lowest, and that to *E. colonum* (L.) Link was the highest among the eight species or varieties of barnyardgrass. It suggested that the different species or varieties of barnyardgrass possess variable tolerance to pretilachlor.

**Key words:** *Echinochloa*; weed; pretilachlor; herbicide tolerance; herbicide

Barnyardgrass (*Echinochloa* Beauv.), a noxious weed throughout the world [1,2], is the most important one of the 15 noxious field weeds in China [3], especially in rice fields. The major aim of most herbicides used in rice fields is to control barnyardgrass. However, barnyardgrass gradually has attained the resistance stemming from the frequent application of herbicides through selection pressure and inheritance means [4]. In China, *E. crusgalli* (L.) Beauv. could develop the resistance to herbicides like butachlor and benthiocarb if the successive application of the herbicides was over five years in the same field [5-9]. In general, there are several species and varieties/genotypes in *Echinochloa*, and they are often treated as one species in studying herbicides resistance due to their similar morphological characters. However, the specific and varietal distribution of *Echinochloa* in different regions is variable. How does the difference be caused? Does the difference result from herbicide tolerance of *Echinochloa*? The related reports have not been found yet at present.

Pretilachlor is the herbicide frequently used in rice fields, which could restrain the transportation of nutrient from leaves to embryos by the inhibition of α-amylase, causing energy shortage during the weed germination, inhibit the division, growth and differentiation of plant cells and ultimately retard the growth of barnyardgrass [11]. To evaluate the difference in tolerance capability of the species or varieties of barnyardgrass to herbicide pretilachlor, and to provide the theoretical data for the integrated management and the tolerance of barnyardgrass, eight species or varieties of barnyardgrass (*Echinochloa*) grown in the same rice cropping region of Jiangsu Province were chosen as tested materials to evaluate their tolerance to the herbicide pretilachlor.

**MATERIALS AND METHODS**

**Experimental materials**

Eight species/varieties of barnyardgrass used in the experiment were *E. crusgalli* (L.) Beauv. var. *mitis* (Pursh) Peterm, *E. crusgalli* (L.) Beauv. var. *zelayensis* (H.B.K.) Hitche, *E. crusgalli* (L.) Beauv., *E. hispidula* (Retz) Nees, *E. crusgalli* var. *austro-japonensis* Ohwi, *E. colonum* (L.) Link, *E. caudate* Roshev., *E. crus-pavonis* (H.B.K.) Schult. All of them were obtained from the rice fields in the same region of Nanjing in October 2001. The herbicide 30% pretilachlor was a product from Ciba Geigy AG. All agents for α-amylase measurement in vitro are AR level.

**Methods**

*Inhibition of barnyardgrass growth after treated with different concentrations of pretilachlor*

Two pieces of filter paper were put onto the bottom of a Petri dish with 9 cm diameter, and 50
‘seeds’ (i.e. grains) of barnyardgrasses were placed on the filter paper. Later, 5 mL of pretilachlor or water at dropped into the Petri dish, and each treatment was repeated four times. Pretilachlor was diluted to 0.01, 0.1, 1.0, 10.0 mg/L, and water was considered as control. The shoot length was measured every day at 1-5 days after germinating.

**Influence of pretilachlor on cell elongation of barnyardgrass plant**

Germinating seeds of barnyardgrass were transferred into the Petri dishes, and 5 mL pretilachlor at different concentrations (0.01, 0.1, 1.0, 10.0 mg/L) or water (check) was dropped, respectively. At 48 h after treatment, tested materials were fixed in FAA solution and extracts were made with a vacuum pump. The longitudinal sections with 8-μm thickness were obtained by the paraffin method, and stained with safranin-fast green. The sections were observed with a microscope (Olympus-BH-2), and the cell length of young leaves and coleoptile was measured with micrometers.

**Inhibition of α -amylase activities in barnyardgrass treated with pretilachlor at different concentrations**

α-amylase activities in the germinating seeds of barnyardgrass treated as above were determined according to Zhou [10] at the 1st, 3rd, 5th day after germination.

**Statistical analysis**

Data obtained were analyzed with DPS software, and the comparison among means was carried out by the Duncan’s Multiple Range Test.

### Table 1. Inhibition rate of pretilachlor at different concentrations to the shoot growth of barnyardgrass.

<table>
<thead>
<tr>
<th>Species of Echinochloa</th>
<th>0.01 mg/L*</th>
<th>0.10 mg/L</th>
<th>1.00 mg/L</th>
<th>10.00 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. crusgalli</em> var. <em>mitis</em></td>
<td>3.24 a</td>
<td>29.46 a</td>
<td>79.98 a</td>
<td>84.81 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em> var. <em>zelayensis</em></td>
<td>15.72 bc</td>
<td>66.52 b</td>
<td>81.83 a</td>
<td>87.36 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em></td>
<td>14.36 bc</td>
<td>56.99 b</td>
<td>83.74 a</td>
<td>88.13 a</td>
</tr>
<tr>
<td><em>E. hispidula</em></td>
<td>8.06 b</td>
<td>48.26 ab</td>
<td>73.28 a</td>
<td>79.36 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em> var. austro-japonensis</td>
<td>31.60 d</td>
<td>77.22 d</td>
<td>84.67 a</td>
<td>89.95 a</td>
</tr>
<tr>
<td><em>E. colonum</em></td>
<td>44.70 d</td>
<td>76.46 d</td>
<td>89.80 a</td>
<td>93.48 a</td>
</tr>
<tr>
<td><em>E. caudate</em></td>
<td>27.20 bc</td>
<td>66.25 bc</td>
<td>88.25 a</td>
<td>91.45 a</td>
</tr>
<tr>
<td><em>E. crus-pavonis</em></td>
<td>51.97 cd</td>
<td>77.88 cd</td>
<td>87.97 a</td>
<td>92.40 a</td>
</tr>
</tbody>
</table>

*Values followed by different small letters within a column indicated significant difference at the level of α =0.05 among various species;

*Different concentrations of the herbicide.*

**RESULTS AND DISCUSSION**

Comparisons on growth inhibition among species of barnyardgrass treated with pretilachlor at different concentrations

The results revealed that pretilachlor at different concentrations had no inhibitory effect on seed germination, but inhibitory on growth of barnyardgrass (Table 1). Under low concentration (0.01 mg/L) treatment, the inhibition rates of shoot growth for *E. crusgalli* var. *mitis*, *E. crusgalli* var. *zelayensis*, *E. crusgalli*, *E. hispidula* were lower than those for *E. crusgalli* var. *austro-japonensis*, *E. colonum*, *E. caudate*, and *E. crus-pavonis*. The barnyardgrass exhibited different morphological traits at low concentration of pretilachlor. The initial leaves of *E. crusgalli* var. *mitis*, *E. crusgalli* var. *zelayensis*, *E. crusgalli*, and *E. hispidula* expanded and grew normally, however, the initial leaves of the others sprouted and expanded with very apparent shortening. Under 0.1 mg/L pretilachlor treatment, there existed significant differences in the growth inhibition rate among the barnyardgrass, *E. crusgalli* var. *mitis* displayed the lowest value of the growth inhibition rate. The first leaves of *E. crusgalli* var. *mitis* and *E. hispidula* were shortened, but grew normally, while the other barnyardgrasses had different kinds of abnormality. The inhibition rates of growth have no significant differences in the barnyardgrass when treated with 1.0 and 10.0 mg/L pretilachlor. The initial leaves of *E. crusgalli* var. *mitis*, *E. crusgalli*, and *E. hispidula* sprouted with a little abnormality,
while the first leaves of the other barnyardgrass were enveloped by the coleoptiles and could not be able to sprout. When treated with the higher concentrations of pretilachlor, the growth of all barnyardgrass was drastically inhibited. From the results, it could be inferred that the pretilachlor tolerance in *E. crusgalli* var. *mitis*, *E. crusgalli* var. *zelayensis*, *E. crusgalli*, *E. hispidula* was stronger than that in the others.

**Morphological and structural changes in barnyardgrass treated with Pretilachlor**

*Morphological and structural changes in barnyardgrass at bud stage before and after treated with pretilachlor*

Pretilachlor inhibited the growth of the buds and shoots of the barnyardgrass. In control (no herbicide treated) plants, leaf blades and coleoptiles expanded normally, and the color of the leaves and coleoptiles was flourishing. In the treated plants, the leaves and coleoptiles were dark green, and the first leaves sprouted from coleoptiles were abnormal and thickening, even could not sprout from coleoptiles. The coleoptiles did not expand, which restrain the expansion of young blades, resulting in the latter leaves to be abnormal and died. The cells of coleoptiles and blades in untreated plants were usually round and arranged uniformly, while those in the herbicide treated plants were arranged loosely with broadened width and shortened length, and some of them began to degrade.

**Changes in cell length of leaf blades and coleoptiles of barnyardgrass during sprouting period before and after treated with different concentrations of pretilachlor**

The cell length of leaf blades and coleoptiles decreased correspondingly with the increasing pretilachlor concentration. *E. crusgalli* var. *mitis* and *E. hispidula* displayed small differences in cell length of leaf blades under different pretilachlor treatments among the tested barnyardgrass, while *E. crusgalli* var. *austro-japonensis* and *E. colonum* exhibited obvious decrease in cell length of leaf blades under 0.01 mg/L pretilachlor treatment (Fig. 1). It indicated that pretilachlor inhibited the cell elongation of *E. crusgalli* var. *austro-japonensis* and *E. colonum* apparently. As shown in Fig. 1-B, cell length of coleoptile of *E. crusgalli* decreased slightly, while that of *E. colonum* reduced very apparently after treated with 0.01 mg/L pretilachlor. With the increasing of the concentration of pretilachlor, the decreasing value in the cell length of coleoptile of *E. crusgalli* var. *mitis* was minor, while the decreasing value of the cell length of *E. crusgalli* increased.

From the results, it was found that cell length of leaf blade and coleoptile of the barnyardgrass decreased drastically, suggesting that *E. crusgalli* var. *mitis*, *E. crusgalli* and *E. hispidula* had strong tolerance to pretilachlor.

**Changes of α-amylase activities in barnyardgrass under different pretilachlor treatments**

During the course of germination, α-amylase

![Fig. 1. Changes in the cell length of leaf blade and coleoptile treated with different concentrations of pretilachlor.](image-url)
activities of seven species and varieties of barnyardgrass except for *E. hispidula* increased firstly and then decreased under no pretilachlor treatment. The seven species or varieties of barnyardgrass showed the maximum value of $\alpha$-amylase activities at the 3rd day after germination, while *E. hispidula* at the 5th day after germination. The possible reason might be that *E. hispidula* has big sized seeds and higher starch content than the others. When treated with different concentrations of pretilachlor, $\alpha$-amylase activity was inhibited at the 3rd day after germinating in the *E. crusgalli* var. *mitis*, *E. crusgalli* var. *zelayensis*, *E. crusgalli*, *E. crusgalli* var. *austro-japonensis*, *E. colonum*, *E. caudate*, *E. crus-pavonis*, while at the 5th day in *E. hispidula* (Table 2). The difference in the inhibition rate of $\alpha$-amylase activities of *E. crusgalli* var. *mitis* was not significant treated with 0.01 mg/L and 0.1 mg/L pretilachlor, with the inhibition rates being 1.01% and 4.41% respectively, while significant under treatment with high concentration (e.g. 1.00 mg/L) of pretilachlor, with the inhibition rate being 25.62%. The other species showed high inhibition rates treated with low concentration of pretilachlor, and the difference of $\alpha$-amylase activities was significant among different concentrations of pretilachlor (Fig. 2).

The embryo growth depends on degradation of the starch and protein in endosperm during the period of seed germination. We found that $\alpha$-amylase activities of barnyardgrass treated with pretilachlor were inhibited with different degrees. The inhibition degree of the $\alpha$-amylase activities was coincided with growth status of the barnyardgrass. It was inferred that pretilachlor impeded the transportation of nutrients from leaves to embryos resulted from the inhibition of $\alpha$-amylase activities. Therefore, energy was scarce when seeds germinated, and division, growth and differentiation processes of plant cells were inhibited, resulting in the retarded growth of barnyardgrass. The low inhibition degree of $\alpha$-amylase activities of barnyardgrass implied the strong tolerance to pretilachlor. On basis of the above results, *E. crusgalli* var. *mitis* showed the strongest tolerance to pretilachlor among the eight barnyardgrass.

The above experimental results suggested that there existed significant variation in the tolerance to pretilachlor among species or varieties of barnyardgrass collected from the same rice field in Nanjing. The difference embodied with the shoot length, cell length of blades and coleoptiles and $\alpha$-amylase activities etc. *E. crusgalli* var. *mitis* had strong tolerance to pretilachlor in the every aspect, followed by *E. crusgalli* var. *zelayensis*, *E. crusgalli* and *E. hispidula*, and *E. colonum* showed the weakest tolerance. According to our investigation (published in another paper), among the eight species/varieties of barnyardgrasses distributed in rice fields of Jiangsu Province, the species with extensive distribution was *E. crusgalli* var. *mitis*, which had the highest importance value, and caused the most serious damage in rice production. The distribution was coincided with the inter-specific tolerance of barnyardgrasses to pretilachlor. Therefore, we should

### Table 2. Activities of $\alpha$-amylase after treatment of the herbicide.

<table>
<thead>
<tr>
<th>Species of <em>Echinochloa</em></th>
<th>Activity of $\alpha$-amylase (U/g) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mg/L $^b$</td>
</tr>
<tr>
<td><em>E. crusgalli</em> var. <em>mitis</em></td>
<td>0.91 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em> var. <em>zelayensis</em></td>
<td>1.17 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em></td>
<td>0.96 a</td>
</tr>
<tr>
<td><em>E. hispidula</em></td>
<td>0.96 a</td>
</tr>
<tr>
<td><em>E. crusgalli</em> var. <em>austro-japonensis</em></td>
<td>1.40 a</td>
</tr>
<tr>
<td><em>E. colonum</em></td>
<td>1.48 a</td>
</tr>
<tr>
<td><em>E. caudate</em></td>
<td>1.30 a</td>
</tr>
<tr>
<td><em>E. crus-pavonis</em></td>
<td>2.45 a</td>
</tr>
</tbody>
</table>

$^a$Values followed by different small letters in a column indicated the significant difference at the level of $\alpha=0.05$ among different species; $^b$Different concentrations of the herbicide.
rationally choose herbicides and concentration according to different species or varieties of barnyardgrasses distributed in different regions for controlling the grasses in rice production.

**ACKNOWLEDGEMENT**

This work was supported by The Tenth Five-Year Major Project of Jiangsu Province (BE2001346).

**REFERENCES**