Enantioselective phytotoxicity of metolachlor against maize and rice roots

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ABSTRACT

Rac-metolachlor, a widely used chloracetanilide herbicide, is now being replaced by *S*-metolachlor in many countries. The enantioselective effects of *rac*- and *S*-metolachlor on root growth of maize and rice was studied in hydroponics. Visible morphological changes in root growth were observed after treatment with *rac*- or *S*-metolachlor. The main root and lateral roots were shorter in length, and the number of lateral roots was reduced. The half inhibition ($IC_{50,5d}$) values for root length of *rac*- and *S*-metolachlor were 18.86 and 10.61 μM, respectively, for maize, and 7.33 and 5.35 μM, respectively, for rice. The root system activity after treatment with *rac*- or *S*-metolachlor was lower than that of the control, while the root membrane permeability was higher. The activities of superoxide dismutase, peroxidase, and catalase in the roots were lower after *rac*- or *S*-metolachlor treatment compared to those of the control, while the malondialdehyde content was higher. After rice was treated with 3.1 μM *rac*- or *S*-metolachlor, the cell wall separated from the cell membrane, and some destruction of nuclei and organelles was observed. The entire cell was destroyed after treatment with 12.4 μM *rac*- or *S*-metolachlor. The results showed that *S*-metolachlor has stronger effects than *rac*-metolachlor on crop roots.

1. Introduction

More than 30% of currently used pesticides are chiral compounds [1], including synthetic pyrethroids, organophosphorus insecticides, imidazolinones and chloracetanilide herbicides. The percentage of chiral pesticides is increasing with the introduction of more complex structures [2]. The assessment of enantiomer selectivity in both exposure and effects is required for comprehensive risk assessments [3]. An increasing number of studies have investigated the environmental fate and microbial transformation of chiral pesticides [4,5]. The toxicities of chiral pesticides and their metabolites against non-target animals and human cancer cell lines have also been studied [6,7]. However, the enantioselective ecological effects and toxicities of chiral herbicides against plants have not received as much attention as those observed in animals [8]. As important ecological receptors in the ecosystem, plants are immobile and cannot avoid harmful effects. Therefore, the enantioselective phytotoxicity of herbicides against plants merits more attention.

Metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, is one of the most important

herbicides used for the selective weed control of more than 70 crops worldwide [9]. Metolachlor has two chiral elements: a chiral axis and a stereogenic center, leading to four stereoisomers [10]. Metolachlor was introduced into the market in 1976 as a racemic product, which contains two *R*- and *S*-enantiomers that occur in an equal ratio. In 1982, it was determined that about 95% of the herbicidal activity of metolachlor resides in the two (1′*S*) diastereomers [11], and since 1997, metolachlor (*rac*-metolachlor) has been replaced by *S*-metolachlor in a number of countries. A content ratio of about 90% 1′*S* -isomers has the same biological effect at 65% of the racemic dosage [9] has been accomplished (e.g., United States in 1997, Switzerland in 1997, Canada in 1998, South Africa in 1998 and Australia in 1999) [12,13].

Metolachlor inhibits the synthesis of proteins and chlorophyll in plants, but it has a low toxicity in mammals. The sorption and desorption behaviors of metolachlor in the soil has been studied [14], along with its dissipation properties, metabolites and effects on organisms [15–17]. The enantioselectivity of *rac*- and *S*-metolachlor to *Daphnia magna* and *Chlorella pyrenoidosa* have been compared [10,18], and the stereoselective degradation of metolachlor has been studied [19,20]. However, relatively few studies have examined the enantioselectivity of *rac*- and *S*-metolachlor against plants [21], and there is a dearth of information on the effects of *rac*- and *S*-metolachlor on root growth in maize and rice.

In the present study, the enantioselectivity of two commercial products, *rac*- and *S*-metolachlor, on root growth in maize and rice was evaluated under hydroponic conditions. The enantioselective

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effects of *rac*- and *S*-metolachlor on root growth were compared. The half inhibition ($IC_{50,5d}$) values of *rac*- and *S*-metolachlor were calculated. Root system activity and root membrane permeability treated with *rac*- and *S*-metolachlor after 24 h were measured. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of maize and rice roots were examined. The malondialdehyde (MDA) content of maize and rice roots were determined. In addition, the effects of *rac*- and *S*-metolachlor on the ultrastructure of rice root cells were studied by transmission electron microscopy (TEM). An attempt was made to compare the effects of *rac*- and *S*-metolachlor and to reveal possible effects of chiral differences on the root toxicities of these two herbicides.

2. Materials and methods

2.1. Chemicals

Rac-metolachlor (97% chemical purity) was obtained from the Qingfeng Pesticide Company (China), and *S*-metolachlor (96% chemical purity) was obtained from Syngenta (Switzerland). All other reagents used in this study were analytical reagents.

2.2. Plant culture

Maize seeds (Yedan 13, *Zea mays* L.) and rice seeds (II You 92, *Oryza sativa* L.) were submersed in tap water for 24 h, sterilized in a 3‰ (v/v) Clorox solution for 5 min, washed several times with sterilized deionized water, and germinated in the dark for 48 h at 25–30 °C. Uniformly germinated seedlings were selected and placed in growth media for 5 days at 25–30 °C with a 16 h light/8 h dark cycle. The growth medium for maize was a nutrient solution containing 0.25 mM KH_2PO_4, 0.1 mM KCl, 0.6 mM $MgSO_4$, 1.0×10^{-3} mM H_3BO_3, 4.0×10^{-3} mM $FeCl_3$, 1.0×10^{-3} mM $ZnSO_4$ and 1.0×10^{-4} mM $CuSO_4$. The growth medium for rice was a modified Hoagland nutrient solution with a pH of 5.0–5.1, and contained 914 mg/L NH_4NO_3, 403 mg/L $NaH_2PO_4 \cdot 2H_2O$, 714 mg/L K_2SO_4, 3240 mg/L $MgSO_4 \cdot 7H_2O$, 886 mg/L $CaCl_2$, 800 mg/L Na_2SiO_4, 3 mg/L $MnCl_2 \cdot 4H_2O$, 0.15 mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.87 mg/L H_3BO_3, 0.07 mg/L $ZnSO_4 \cdot 7H_2O$, 0.06 mg/L $CuSO_4 \cdot 5H_2O$, and 15.40 mg/L $FeCl_3 \cdot 6H_2O$.

2.3. Growth inhibition tests

Rice growth inhibition tests were performed according to the OECD guidelines for chemical testing [22]. Ten seedlings were tested for each replicate. Three replicates were tested for each treatment. *Rac*- and *S*-metolachlor were dissolved in methanol to produce stock solutions of 10,000 mg/L, and the methanol concentration was 0.02% (v/v) or less in the treatment conditions. *Rac*- and *S*-metolachlor were added to attain the concentrations of 0, 6.2, 18.6, 37.2, 74.4, 93, and 124 μM for maize and 0, 1.55, 3.1, 6.2, 12.4, 24.8, and 31 μM for rice. The roots were scanned by a stereomicroscope (LEICA MZ9.5 Germany). The mean root lengths of the seedlings were measured, and the relative inhibition rates of maize and rice root elongation caused by *rac*- or *S*-metolachlor in each concentration were calculated after 5 days of treatment. The relative inhibition rate was calculated as follows:

$$RI(\%) = \frac{(X_0 - X_n) \times 100\%}{X_0} \qquad (1)$$

where X_0 represents the average root length of CK and X_n represents the average root length of each treatment. The concentration of each herbicide that caused a 50% inhibition (IC_{50}) of maize and rice root elongation was determined from the dose–response regression curve using a logarithmic model.

2.4. Root system activity and root membrane permeability

Maize plants were treated with 0, 18.6, 37.2 or 74.4 μM *rac*- or *S*-metolachlor, Rice plants were treated with 0, 1.55, 3.1 or 6.2 μM *rac*- or *S*-metolachlor.

Root system activity was determined using the triphenyl tetrazolium chloride (TTC) method [23]. Maize and rice roots after 24 h of treatment were washed with distilled water, and the root tips of the main roots were cut into small pieces of 0.5 cm in length. A 0.2 g portion of each root tip sample was placed into a beaker, and 5 mL of 0.4% TTC and 0.1 M phosphate buffer solution (pH 7.0) were added and allowed to react for 3 h at 37 °C. The root tips turned red, and 2 mL of 1 M H_2SO_4 was added to stop the reaction. The tips were dried with filter paper and transferred to tubes with stoppers. Then 20 mL of methanol was added to turn the tip color white (approximately 3–5 h). The root activity was expressed as the amount of triphenyl formazan (TPF) that was deoxidized by TTC.

The root membrane permeability was determined by a method modified from Alpaslan and Gunes [24]. Maize and rice roots after 24 h of treatment were washed with distilled water and dried, and then, the roots were cut into small pieces that were 2 cm in size. A portion of fresh material (1 g) was placed into test tubes containing 20 mL of deionized, distilled water. The test tubes were vortexed for 5 s, and the solution was assayed for initial electrical conductivity (EC_0). The test tubes were immersed at 30 °C for 12 h and then assayed for EC_1. After boiling the samples for 10 min, their conductivity was measured, when the solution was cooled to room temperature (EC_2). The percent of root membrane permeability was calculated as

$$EC\ (\%) = \frac{EC_1 - EC_0}{EC_2 - EC_0} \times 100\% \qquad (2)$$

where EC_1 and EC_2 represent the electrolyte conductivities measured before and after boiling, respectively.

2.5. SOD, POD, and CAT activities and MDA content

Maize plants were treated with 0, 18.6, 37.2 or 74.4 μM *rac*- or *S*-metolachlor. Rice plants were treated with 0, 1.55, 3.1 or 6.2 μM *rac*- or *S*-metolachlor.

Roots (7.5 g fresh weight) that were subjected to different treatments were used for enzyme extractions and analyses after 24 h of treatment. Roots were homogenized in a cooled mortar with quartz and 20 mL of 0.1 M phosphate-buffered solution (PBS) (pH 7.5) containing 1% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was filtered through three layers of Miracloth and centrifuged at $12,000 \times g$ for 20 min at 4 °C. The supernatant was collected, and the samples were stored at −80 °C for the determination of antioxidant enzyme activities. The protein content was determined according to the method of Bradford [25] using bovine serum albumin (BSA) as a standard. Three replicates were performed for each treatment. All experiments were conducted out at 4 °C.

The activity of SOD was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), a method that was modified from Giannopolitis and Ries [26]. The assay was performed with illumination for 20–30 min at 25–35 °C in a 5 mL cuvette containing 0.4 mL of 130 mM L-Met, 0.4 mL of 750 μM NBT, 80 μL of 500 μM EDTA-2Na, 0.4 mM of 100 μM vitamin B2, 0.5 mL of protein suspension and 2.22 mL of PBS (pH 7.6) against a blank with no protein suspension. One unit of SOD activity was defined as the amount of enzyme that was required to cause 50% inhibition of the reduction of NBT at 560 nm.

The activity of POD was determined by the oxidation of guaiacol in the presence of H_2O_2. One unit of POD activity was defined as the amount of enzyme that was required to oxidize guaiacol in 1 min at 470 nm and at 20 °C [27]. The reaction mixture contained 50 mL of
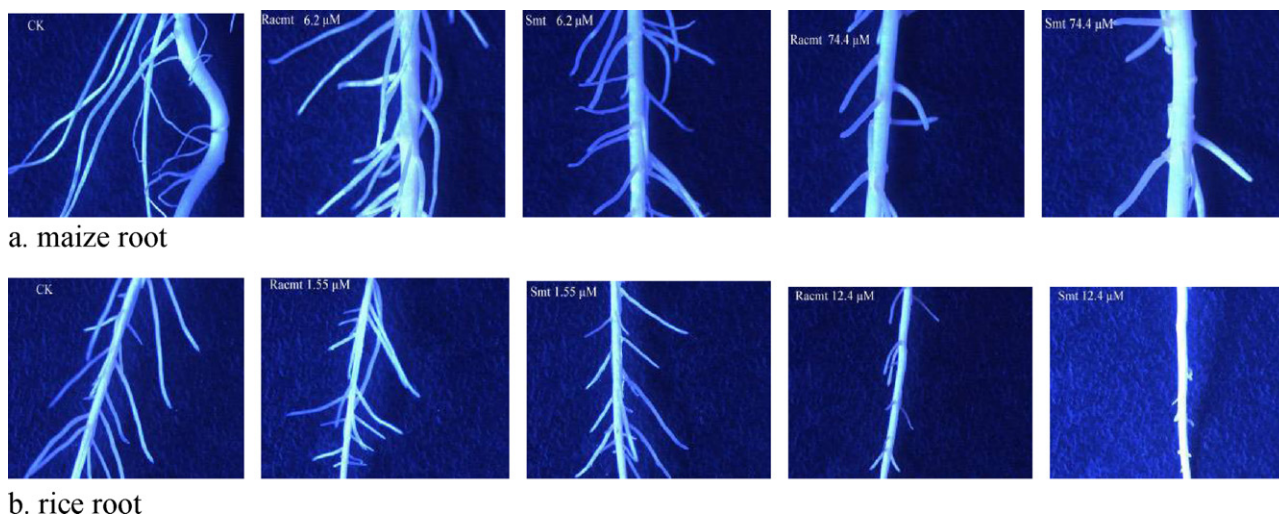
Fig. 1. Effects of *rac-* and *S*-metolachlor on root morphology.

100 mM PBS (pH 6), 19 μL of guaiacol, and 28 μL of 30% H_2O_2 as a substrate. The reaction was initiated by adding 0.5 mL of a protein suspension, 2.5 mL of PBS, and 1 mL of substrate against a blank containing protein suspension but no substrate for 3 min [27].

The activity of CAT was determined by measuring the consumption of H_2O_2. One unit of CAT activity was defined as the amount of enzyme that decomposed H_2O_2 in 1 min at 240 nm at 20 °C [28]. The reaction mixture contained 100 mL of 100 mM PBS (pH 7.0) and 340 μL of 30% H_2O_2 as a substrate. The reaction was initiated by adding 0.5 mL of a protein suspension, 2.5 mL of PBS, and 1 mL of substrate against a blank containing protein suspension but no substrate for 3 min [28].

The MDA content was measured according to Aydin et al. [29]. Frozen maize and rice roots (0.5 g) samples for each treatment were homogenized in 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at $15,000 \times g$ for 5 min. Then, 1.0 mL of supernatant was added to 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at $10,000 \times g$ for 10 min, the absorbance of the supernatant was monitored with a spectrophotometer at 532 nm and 600 nm, respectively, and calculated using the extinction coefficient of 155 mmol^{-1} cm^{-1}. The following formula was used.

$$MAD \ (nmol/mL\,FW) = \frac{(A_{532} - A_{600})}{155,000} \times 10^6 \tag{3}$$

2.6. Transmission electron microscopy

Rice plants were treated with 0, 1.55 or 12.4 μM *rac-* or *S*-metolachlor. Root tips (3 mm in length) from rice seedlings were collected after 3 days for TEM, using a method modified from Liu and Xiong [10]. The root tips were fixed with 2.5% glutaraldehyde overnight at 4 °C and then rinsed with 0.1 M phosphate buffer (pH 7.0) three times for 15 min each. The sample was post-fixed with 1% osmium tetroxide for 2 h and then washed three times in 0.1 M phosphate buffer (pH 7.0). Next, the sample was dehydrated by a series of ethanol solutions (50%, 70%, 80%, 90%, 95% and 100%) for 15–20 min at each step and then transferred to pure acetone for 20 min. The sample was placed in a 1:1 mixture of pure acetone and Spurr resin for 1 h at room temperature, and then transferred to a 1:3 mixture of pure acetone and Spurr resin for 3 h, finally it was placed in pure Spurr resin to set overnight. The sample was placed in capsules containing embedding medium and heated at 70 °C overnight. Ultra-thin sections (70–90 nm) were cut with a

diamond knife and stained with uranyl acetate and lead citrate for 15 min. Later, they were examined using a JEM-1230 electron microscope.

2.7. Statistical analysis

SPSS.15 was used for the statistical evaluation of the results. The results were designed as completely randomized with three replicates of each parameter. Mean values followed by the same letter were not significantly different, as determined by an analysis of variance (ANOVA), and the differences were compared by a Duncan's range at to a significance level of $p < 0.05$.

3. Results and discussion

3.1. Enantioselective effects of rac- and S-metolachlor on root growth inhibition

After 5 days of treatment, visible morphological changes in root growth were observed (Fig. 1). The main and lateral roots were shorter, and the number of lateral roots was reduced. Morphological toxicity symptoms were observed at high metolachlor concentrations, and *S*-metolachlor was showed to have stronger effects than *rac*-metolachlor.

The concentration–response curves (Fig. 2) of *rac*- and *S*-metolachlor suspensions on root elongation in maize and rice were calculated using the following logarithmic model:

$$y = a \ \ln x + b \tag{4}$$

where x is the concentration of *rac*- or *S*-metolachlor and y is the inhibition rate of each concentration. The regression equation and IC$_{50,5d}$ of root elongation are shown in Table 1. Plant growth was significantly inhibited after *rac*- or *S*-metolachlor treatments ($p < 0.05$). The root lengths were 22.63 cm for maize and 12.27 cm for rice under control after 5 days of cultivation. The maize root lengths were reduced to 15.09 and 13.17 cm after 6.2 μM *rac*- or *S*-metolachlor treatment, respectively, and the rice root lengths were reduced to 8.55 and 7.96 cm after 1.55 μM *rac*- or *S*-metolachlor treatment, respectively. The IC$_{50,5d}$ values of *rac*- and *S*-metolachlor were 18.86 and 10.61 μM for maize roots, and 7.33 and 5.35 μM for rice roots, respectively, showing that rice roots are more sensitive to *rac*- and *S*-metolachlor than maize roots. The field-recommended concentration of metolachlor is approximately 6.2 μM, hinting the
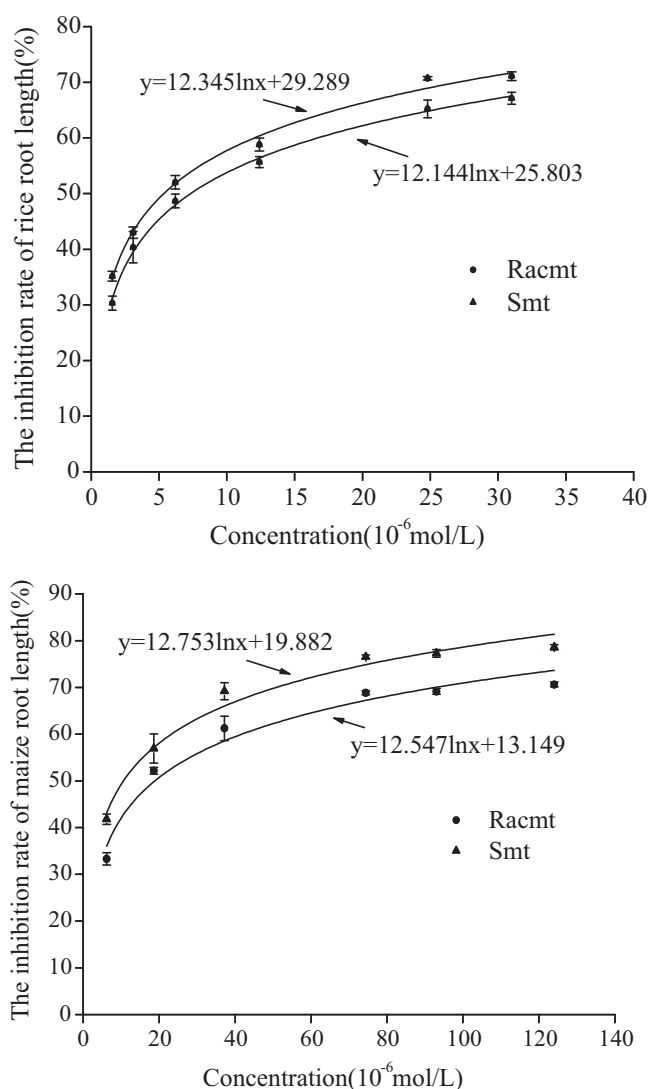
Fig. 2. The concentration–response curves of *rac*- and *S*-metolachlor using the logarithmic model.

presence of the phytotoxic impacts associated with the two herbicides.

The lengths of the taproots treated with *rac*-metolachlor were higher than those of *S*-metolachlor in both maize and rice. The significant differences between *rac*- and *S*-metolachlor treatment were observed in maize taproots when the concentration exceeded 18.6 μM. These results indicated that the inhibitory effects of *S*-metolachlor on maize and rice roots elongation were stronger than those of *rac*-metolachlor. The dissipation of *S*-metolachlor in maize roots was more rapid than that of *rac*-metolachlor [21], and dissipation includes both degradation and uptake by crop plants. Therefore, enantioselective toxicity might be partially related to a difference in uptaken ability.

3.2. Enantioselective effects of rac- and S-metolachlor on root system activity and root membrane permeability

Root activity is used as an important physiological parameter for the evaluation of the uptake of ions, such as Na^+ and K^+, but few studies are available concerning the enantioselective effects of chiral herbicides on root activity. The effects of different *rac*- and *S*-metolachlor concentrations on maize and rice root activity (as demonstrated by TTC-reducing capacity) after 24 h of treatment are shown in Fig. 3. The root activity was decreased after treatment with *S*-metolachlor and higher *rac*-metolachlor concentrations (37.2 and 74.4 μM). The changes in the root activity of rice showed the same trend as that of maize. Root activity after treatment with *rac*-metolachlor was higher than after treatment with *S*-metolachlor for each concentration, and there were significant differences between the two herbicides. The *rac*-/*S*-metolachlor ratios of root activity were 1.34, 1.20 and 1.20 in maize root, and 1.28, 1.70, and 2.41 in rice root, respectively.

Membrane permeability is a useful parameter for investigating the effects of stressful conditions [30], because permeability is dependent on the maintenance of the structural integrity of all components. The effects of *rac*- and *S*-metolachlor on the root membrane permeability of excised roots of maize and rice after 24 h treatment are shown in Fig. 4. Both *rac*- and *S*-metolachlor caused an increase in the root membrane permeability. There was a significant difference between the values obtained after treatment and for the control. The root membrane permeability was 1.46 times and 1.94 times that of the control in maize with 74.4 μM *rac*- or *S*-metolachlor, respectively, and 1.43 times and 1.84 times that of the control in rice with 6.2 μM *rac*- or *S*-metolachlor, respectively. The results indicated that *rac*- and *S*-metolachlor might damage the integrity of the plasma membrane. Root membrane permeability values were higher after treatment with *S*-metolachlor than after treatment with *rac*-metolachlor at each concentration, and there were significant differences between the two herbicides. The *rac*-/*S*-metolachlor ratios were 0.754, 0.862 and 0.755 in maize root, and 0.822, 0.789 and 0.778 in rice root, respectively.

3.3. Enantioselective effects of rac- and S-metolachlor on SOD, POD, and CAT activities and MDA content

The SOD, POD, and CAT activities and MDA contents in maize and rice roots after 24 h treatment are shown in Fig. 5. The SOD activities in maize and rice roots were significantly inhibited after *rac*- or *S*-metolachlor treatment, and the level decreased with increasing metolachlor concentrations. The SOD activities were 103.9%, 90.1%, and 75.9% of the control at 18.6, 37.2 and 74.4 μM *rac*-metolachlor, respectively, and 84.8%, 70.0%, 55.3% of the control at 18.6, 37.2, and 74.4 μM *S*-metolachlor, respectively. The SOD activities of rice showed the same trend as those of maize (Fig. 5A). The changes in the POD activities in maize roots showed the same trend as those of the SOD, and there were significant differences observed between the treatments and the controls, but the POD activities in rice roots were not significantly affected by *rac*- or *S*-metolachlor treatment (Fig. 5B). The CAT activities in both maize and rice roots were significantly inhibited by *rac*- or *S*-metolachlor treatment, the
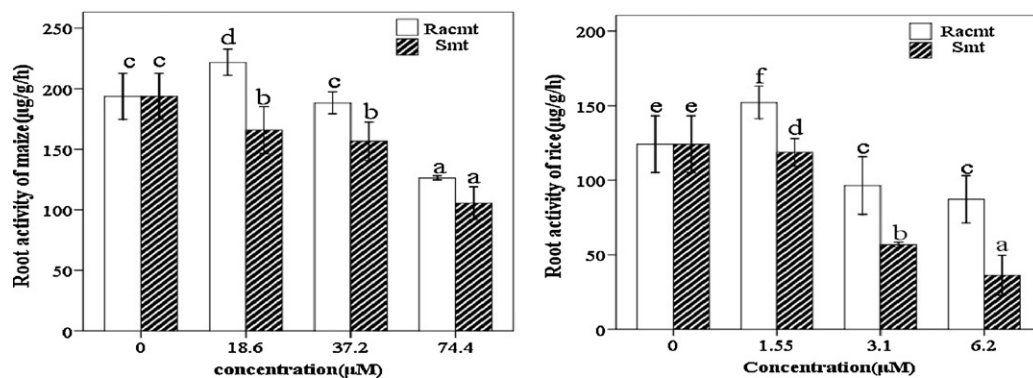
Table 1
IC$_{50,5d}$ of *rac*- and *S*-metolachlor to maize and rice root elongation.

Crop	Herbicide	Regression equation	IC$_{50,96h}$ (μM)	R^2
Maize	Racmt	$y = 12.547 \ln x + 13.149$	18.86 [9.63, 38.66]	0.9688
	Smt	$y = 12.753 \ln x + 19.882$	10.61 [5.90, 20.16]	0.9787
Rice	Racmt	$y = 12.144 \ln x + 25.803$	7.33 [5.99, 8.98]	0.9974
	Smt	$y = 12.345 \ln x + 29.289$	5.35 [3.95, 7.28]	0.9942

Note: Logarithmic model was used for calculating the IC$_{50}$: $y = a \ln C + b$, y is the inhibition rate (%), x is the herbicide concentration. Racmt: *rac*-metolachlor, Smt: *S*-metolachlor.

Fig. 3. Effects of *rac*- and *S*-metolachlor on the root activities of maize and rice.

greatest differences were 14.5% and 51.2% that of the controls for the 74.4 μM *S*-metolachlor treatment in maize and rice, respectively (Fig. 5C).

Enantioselective effects of *rac*- and *S*-metolachlor on the activities of SOD, POD, and CAT were observed in maize and rice roots. The *rac*-/*S*-metolachlor ratios of SOD activity at each level were 1.23, 1.29 and 1.37 in maize roots, and 1.29, 1.13, and 1.36 in rice roots, respectively. Similarly, the *rac*-/*S*-metolachlor ratios of POD activity at each concentration were 1.48, 1.43 and 1.16 in maize roots, and 1.19, 1.12, and 1.06 in rice roots, respectively. The *rac*-/*S*-metolachlor ratios of CAT activity at each concentration were 1.58, 2.43 and 2.93 in maize roots, and 1.15, 1.09, and 1.37 in rice roots, respectively. The results indicated that *S*-metolachlor has stronger inhibitory effects than *rac*-metolachlor on SOD, POD, and CAT activities.

Previous studies have shown that antioxidant enzymes are an important for the mechanism of herbicide tolerance [31]. SOD is part of a group of metalloenzymes that catalyze the conversion of reactive $O_2^{\bullet-}$ to produce H_2O_2, H_2O_2 is subsequently detoxified by two types of enzymes: CAT and POD [32]. The enzyme activities of maize and rice roots decreased after treatment with both *rac*- and *S*-metolachlor, which was likely to be the result of the defensive mechanism of the cells that prevents subsequent damage due to metolachlor [33].

Membrane integrity during conditions of oxygen deprivation is one of the key factors in plant survival. Under anoxic conditions, a decrease in membrane integrity is a symptom of injury [34]. In our study, both *rac*- and *S*-metolachlor caused an increase in the root membrane permeability of maize and rice, which may due to *rac*- and *S*-metolachlor induced plant cell anoxia, leading to compromised membrane integrity.

MDA is the final product of lipid peroxidation, and MDA content can reflect a plant's stress tolerance. The autotoxic effects of *rac*- and *S*-metolachlor were observed in maize and rice roots by way of their MDA contents (Fig. 5D). The MDA content increased significantly after *rac*- and *S*-metolachlor treatments, with the greatest difference being 2.02 times that of the control after treating maize with 74.4 μM *S*-metolachlor, and 1.88 times that of the control after treating rice with 6.2 μM *S*-metolachlor. The MDA content was higher after *S*-metolachlor treatment than after *rac*-metolachlor treatment. The *rac*-/*S*-metolachlor ratios of MDA contents at each concentration were 0.847, 0.907, and 0.851 for maize roots, and 0.821, 0.949, and 0.900 for rice roots, respectively.

The results of the antioxidant enzyme and MDA content analyses showed that *rac*- and *S*-metolachlor leads to the production of oxygen radicals, which results in increased lipid peroxidation and oxidative stress in roots. MDA is regarded as a biomarker for the evaluation of lipid peroxidation or damage to the plasmalemma and organelle membranes that increases with environmental stress. Lipid peroxidation is linked to antioxidant enzyme activity, such as increases in SOD, APX, GPX and CAT activities [35]. In this study, the MDA contents in maize and rice roots increased with increases in the concentrations of *rac*- and *S*-metolachlor, which may be an indication that activities of SOD, POD and CAT were critical factors in the damage due to oxidative stress.
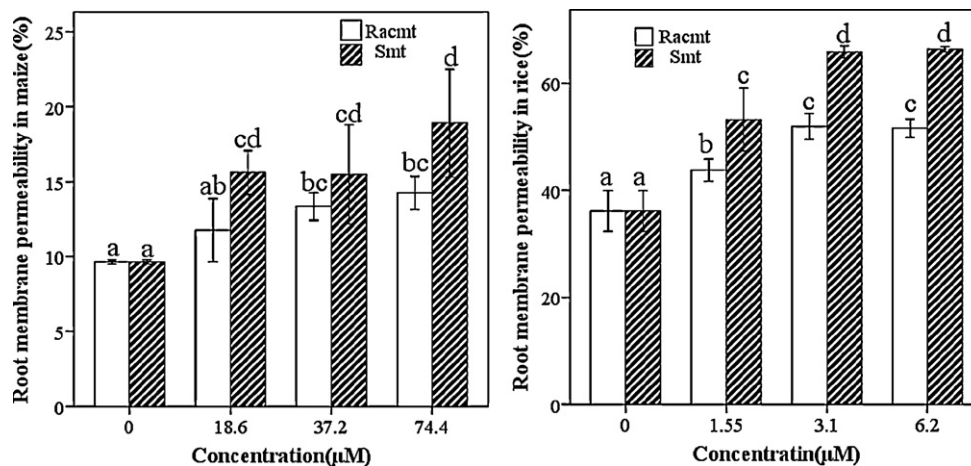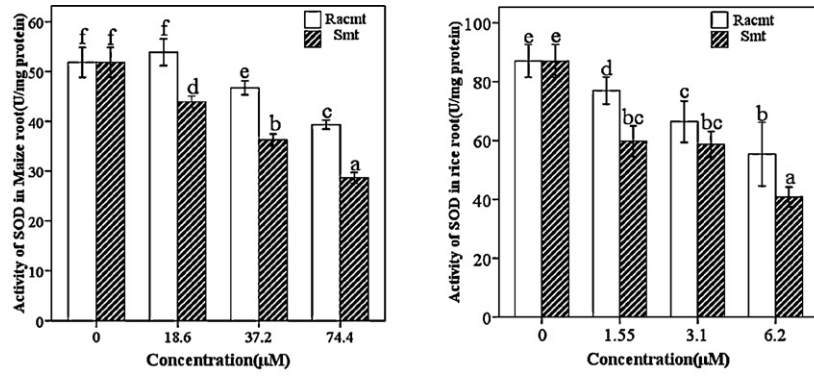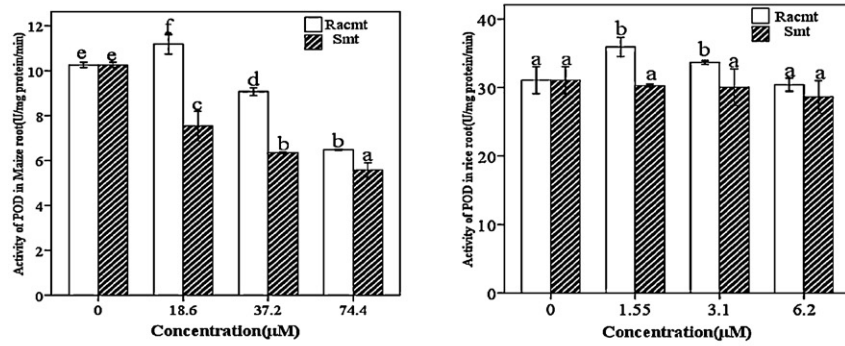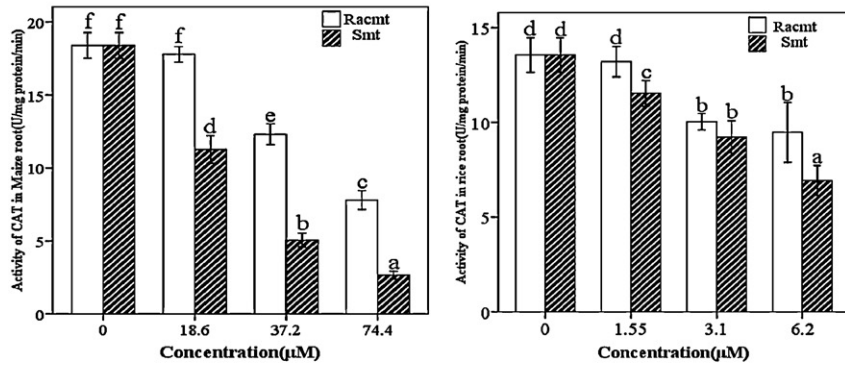

Fig. 4. Effects of *rac*- and *S*-metolachlor on the root membrane permeability of maize and rice.
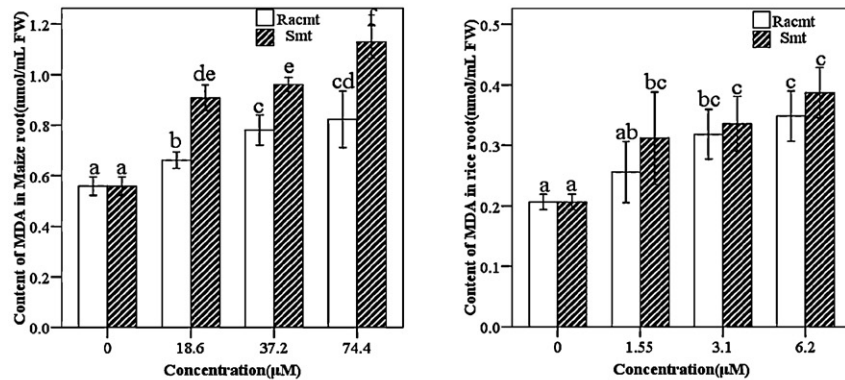
A. Superoxide dismutase (SOD) activities of maize and rice root.

B. Peroxidase (POD) activities of maize and rice root.

C. Catalase (CAT) activities of maize and rice root.

D. Malondialdehyde (MDA) content of maize and rice root.

Fig. 5. Effects of *rac*- and *S*-metolachlor on SOD, POD, and CAT activities and the MDA content in maize and rice roots.
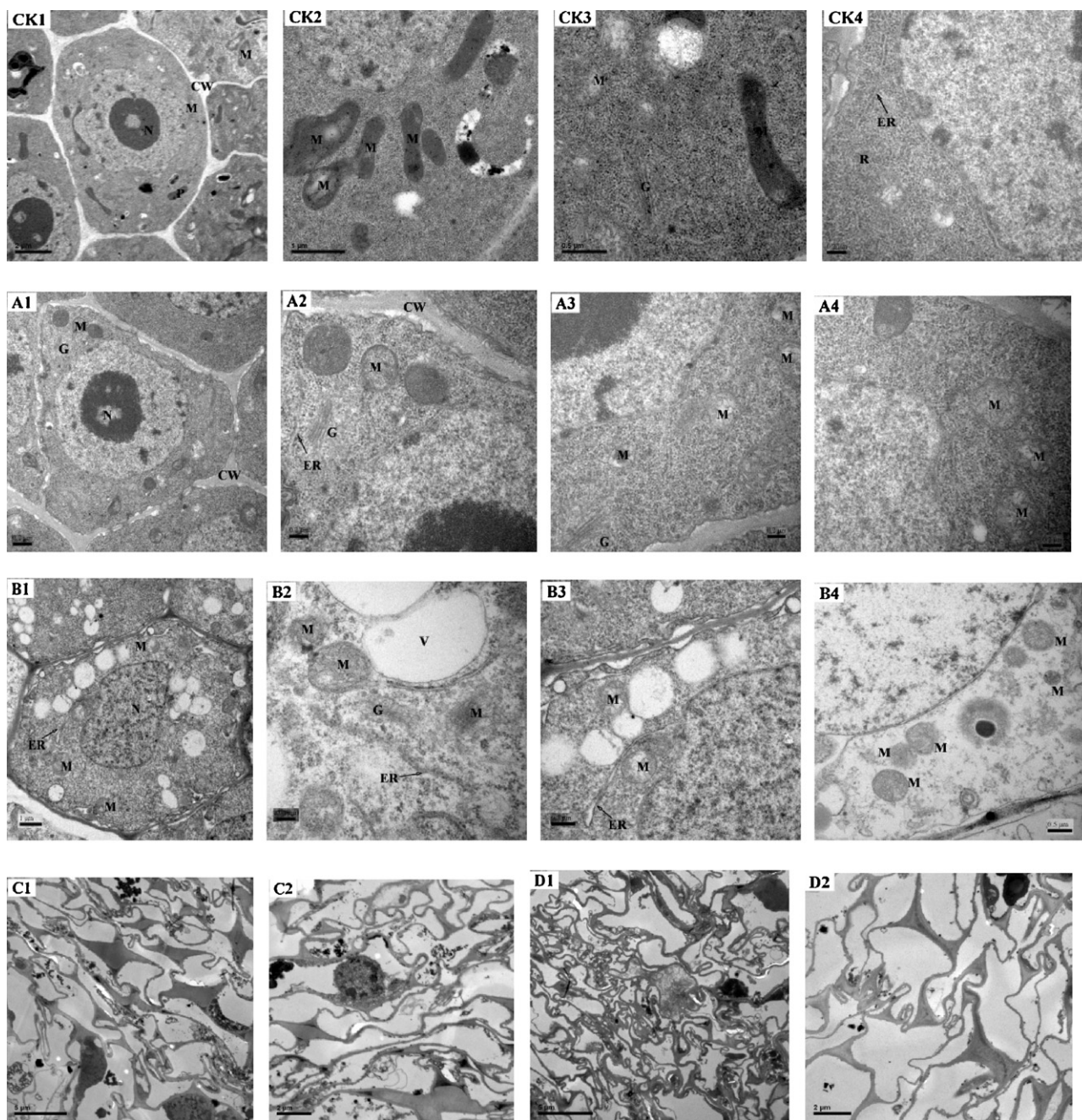
Fig. 6. Transmission electron micrographs of rice root cells grown in *rac-* and *S*-metolachlor.

3.4. Ultrastructural observation of rice roots

Ultrastructural morphology differences between control cells and cells grown in the two herbicides are shown in Fig. 6. A typical cell in the rice control culture had an integrated cell wall, nucleus and organelles such as the mitochondria, Golgi apparatus, and endoplasmic reticulum. After treatment with 3.1 μM of *rac-* or *S*-metolachlor (Fig. 6A and B), the cell walls separated from the cell membranes, and the nuclei and organelles were destroyed. After treatment with 12.4 μM of *rac-* or *S*-metolachlor (Fig. 6C and D), the cell walls were degraded, the nuclear membranes were ruptured, and the nucleoli and cell organelles disappeared. Some starch grains and remnants of the nucleoli and organelles were observed after treatment with 12.4 μM *rac-* metolachlor (Fig. 6C2), whereas

the cells were completely empty after treatment with 12.4 μM *S*-metolachlor (Fig. 6D2).

The nuclei located in the cell center, and nucleoli and nucleolar vacuoles were observed within the nucleus in the control cells and cells treated with 3.1 μM *rac-*metolachlor treatment (Fig. 6CK1 and A1), after treatment with 3.1 μM *S*-metolachlor, the nucleoli had disappeared. For a typical root tip cell grown in 3.1 μM *rac-* or *S*-metolachlor, the mitochondria swelled, elementary particles were reduced, and distribution disordered (Fig. 6A4 and B4). In addition, some mitochondrial membranes were digested (Fig. 6B4). Rough endoplasmic reticulum was clearly observed in the control cells (Fig. 6CK4), but a large number of ribosomes were broken from the surface of the endoplasmic reticulum (Fig. 6A2 and B3), and both ends of the endoplasmic reticulum were swollen (Fig. 6B3).

The Golgi apparatus was swollen, and both ends of the small bubble had collapsed (Fig. 6A3 and B2). These results showed that *S*-metolachlor has a stronger effect than *rac*-metolachlor on root cells.

The results on membrane permeability and MDA content analyses in plant roots were stronger with *S*-metolachlor than with *rac*-metolachlor, which might be due to the stronger effect of *S*-metolachlor on cell walls. Metolachlor is classified as an inhibitor of very long chain fatty acid (VLCFA) formation, and it interferes with normal cell development and inhibits both cell division and cell enlargement [13,36]. The inhibitor binds tightly to the enzyme required for the elongation of C16 and C18–C20 fatty acids [37]. In the present study, the cell walls were separated from the cell membranes after treatment with both *rac*- and *S*-metolachlor, and this effect is probably due to the herbicide binding to the enzyme responsible for VLCFAs synthesis. This interference would lead to an imbalance in the fatty acid composition of cellular plasma membranes, resulting in a loss of cell rigidity and permeability.

4. Conclusions

The present study demonstrated differences in the enantioselective behaviors of *rac*- and *S*-metolachlor on maize and rice roots. The results showed that *S*-metolachlor has more toxic effects than *rac*-metolachlor on maize and rice roots in terms of the root morphology, the $IC_{50,5d}$ values for root length, root system activity, root membrane permeability, SOD, POD, and CAT activities, and MDA content. In addition, the cellular ultrastructural morphology is affected by both herbicides, but *S*-metolachlor has a stronger effect than *rac*-metolachlor. Racemic metolachlor is being replaced by *S*-metolachlor in various applications worldwide. The results of the present study show that the appropriate application of *S*-metolachlor is required.

Acknowledgments

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