Butachlor에 대한 벼 유묘의 생리적 반응

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Physiological Responses of Rice Seedlings to Butachlor

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ABSTRACT

The herbicide butachlor [N-(butoxymethyl)-2-chloro-N-(2,6-di-methylphenyl) acetamide] is widely used by farmers as a tool for weed management of transplanted rice(*Oryza sativa* L.) in Taiwan. The herbicide did not stop germination of rice and weed seeds, but strongly inhibited the subsequent growth of young shoots and roots. The inhibition was also strong on established seedlings. However, they could recover to normal growth after the herbicide effect disappeared.

Butachlor greatly decreased the endogenous indole-3-acetic acid (IAA) but increased the endogenous abscisic acid (ABA) contents of rice seedlings. Addition of IAA into growth medium (Hoagland's solution) partly relieved growth inhibition. Pretreatment of both gibberellic acid (GA_3) and IAA 24 hours before butachlor treatment almost completely alleviated the butachlor-interfere with GA and/or IAA metabolism or their action resulting in the growth inhibition of rice.

Butachlor was readily absorbed by rice roots. During 24 hours of uptake experriment, 32% of the applied herbicide was absorbed. Pretreatment of the herbicide for 2 days did not affect the absorption. Of the absorbed herbicide, 80% remained in roots, only 20% transported into shoots, and more than 50% was metabolized to water soluble substances. Thin-layer chromatographic (TLC) analysis indicated that the Rf value of the most abundant metabolite was butachlor-glutathione conjugate. Rice, barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), and monochoria (*Monochoria vaginalis* Presl) seedlings contained relatively high level of non-protein thiols, while the glutathione S-transferase (GST) activity was found highest in rice, barnyardgrass the next, monochoria the lowest. The difference in GST activity among these species might be related to their sensitivity to butachlor.

Key words : Rice, Butachlor, physiological response

INTRODUCTION

The herbicide butachlor is widely used by farmers as a tool for weed management in transplanted rice in Taiwan since 1971(Hu 1986) The herbicide is applied in granular formulation 2-4 days after transplanting at the rate of 1.5kg

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ai/ha. It controls many annual weeds such as barnyardgrass, monochoria, *Cyperus difformis* L., *Lindernia pyxidaria* All., *Cyperu miliacea* L., etc. in rice field.

The successful introduction of butachlor for rice weed management in Taiwan is indeed an outstanding achievement. One may ask why butachlor can be used successfully in Taiwan(and now also in some Asian countries)? The answer is simple that is based on the technique of proper time of application in combination with good water management. The concept of water management in relation to weed management has been extended to other herbicides. Therefore, weed management of rice to farmers is not a problem now in Taiwan except in areas where perennial weeds are predominant.

Several studies on the physiological responses of plants to butachlor have been conducted in my laboratory with graduate students. The present paper is a summary of the results which have published elsewhere(Liu and Tasi 1986, Wang et al 1992, 1995).

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Effect on seed germination and seedling growth

Butachlor at the concentration up to 20ppm (6.4×10^{-5}M) could not completely stop the germination process of rice and weed seeds. Percentage of germination was nearly the same in butachlor treated seeds and non-treated control after 9 days of incubation. However, the subsequent growth of young buds and roots was strongly inhibited during the incubation period. As shown in Fig. 1, butachlor at 0.1ppm(3.2×10^{-7}M) significantly inhibited shoot and root growth of *Cyperus difformis* and monochoria

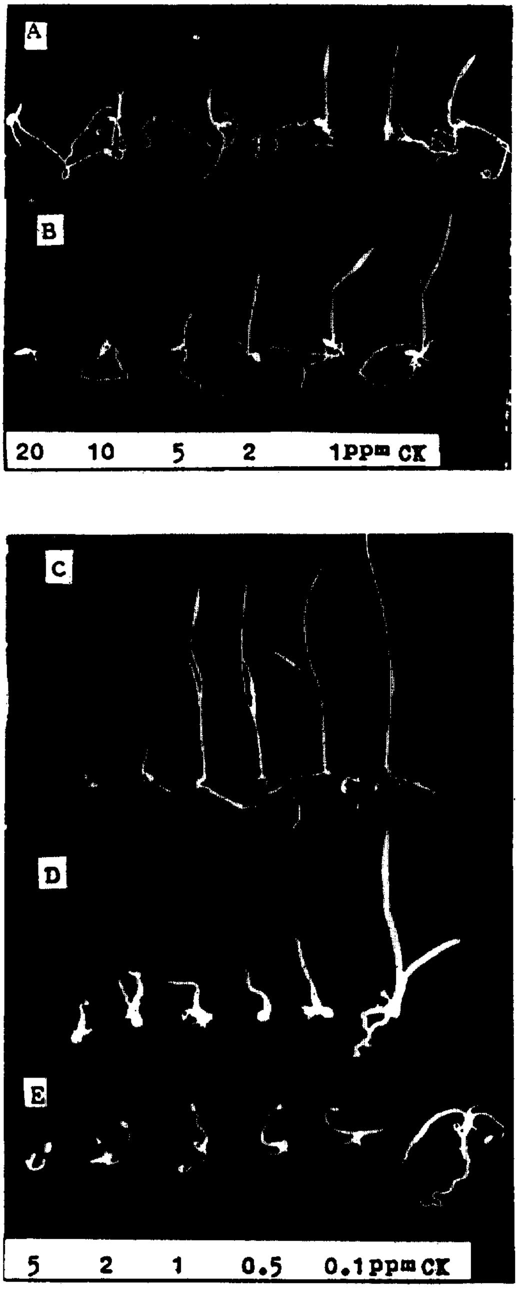

Fig. 1. Effect of various concentrations of butachlor on seed germination and subsequent growth of rice, cv. Tainan 5(A), cv. Taichung Native 1(B), barnyardgrass (C), *Cyperus difformis* (D) and *Monochoria vaginalis* (E).

while barnyardgrass required 2ppm(6.4×10^{-6}M) for the same degree of inhibition. Though rice was more resistant to butachlor, the growth of young seedling was also inhibited at the concentration above 5ppm. These results imply that the poor seedling growth after germination would result in the failure of subsequent emergence of the seedling form the soil particularly under

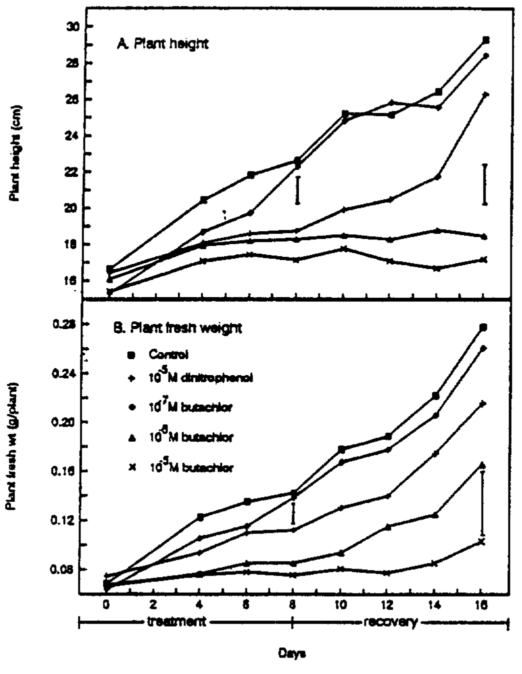

Fig. 2. Growth inhibition of plant heigth (A) and plant fresh weight (B) of rice to butachlor seedlings. 3-leaf age rice seedings were grown in nutrient solution containing various concentrations of butachlor for 8 days. The herbicide was then removed and seedlings continued to grow for another 8 days. The vertical bars indicate the LSD at 5% level.

water. For established rice seedlings, butachlor at 10^{-5}M and 10^{-6}M almost completely stop the growth of plant height and fresh weight accumulation(Fig. 2). Removal of the herbicide from the nutrient solution 8 days after treatment, the seedlings gradually recovered to growth. None was found to be killed by the herbicide. In case of transplanted rice, the application of commercical formulated butachlor 3 day after transplanting, seedling growth was initially retarded but was nearly recovered to normal growth at 34 days after application(Table 1). In Taiwan, butachlor is recommended to be applied 2-4 days after transplanting. At this time of application, the herbicide is very effective to inhibit the emergence of weeds and only temporarily retards the growth of transplanted rice.

Table 1. The growth of rice as affected by the application of butachlor 3 days after transplanting

Cultivar	Rate (kg/ha)	Plant height (cm)		Shoot fr. wt. (g/hill)
		13*	34*	34*
Tainan 5	0	52.6a	64.5a	39.1a
	1.5	51.5a	63.4a	38.0a
	3.0	50.4a	63.8a	37.2a
Taichung Native 1	0	52.6a	63.1a	38.1a
	1.5	48.7a	63.7a	35.9a
	3.0	42.7b	34.1a	35.7a

* Days after butachlor application

Table 2. Effect of butachlor on endogenous IAA amd ABA content of rice seedlings.

Treatment	IAA(n moles/g fw)		ABA((n moles/g fw)	
	8 days after treatment	8 days after butachlor removal	8 days after treatment	8 days after butachlor removal
Control	3.07a	0.98a	0.15a	0.16a
10^{-5}M butachlor	1.05b	0.48b	0.28b	0.20a
Butachlor/control(%)	34	50	190	125

* : Means with a column followed by the same letter are not signiticantly different at 5% level.

Effect on growth interaction with IAA and GA_3

Butachlor treatment significantly decreased the endogenous IAA but greatly decreased endogenous ABA content of rice seedlings(Table 2). Addition of IAA or GA_3 into nutrient at the same time as butachlor treatment could not relieved the growth inhibition induced by butachlor. However, IAA treatment could promote the seedlings to recover their growth more fast after herbicide removal(Fig 3 and Fig 4). Pretreatment of GA_3 and/or GA_3 + IAA 24 hours before butachlor treatment almost completely alleviated the growth inhibition(Table 3, Fig 5). It was concluded that butachlor might interfere with GA and/or IAA metabolism or their action resulting in the growth inhibition of rice seedlings.

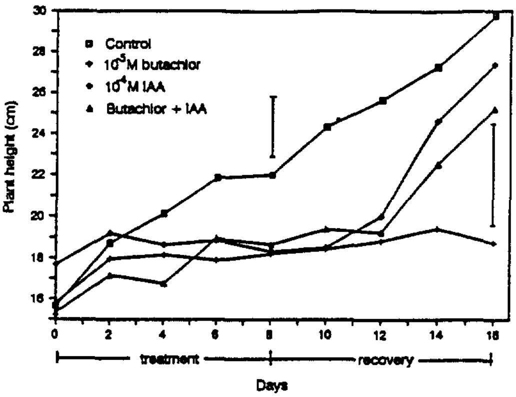

Fig. 3. Effect of IAA on butachlor induced growth inhibition of rice seedlings. IAA was added to the nutrient solution at the same time as butachlor treatment. The vertical bars indicate the LSD at 5% level.

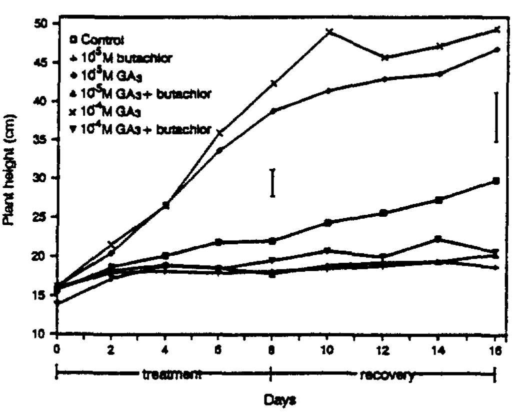

Fig. 4. Effect of GA_3 on butachlor induced growth inhibition of rice seedlings. GA_3 was added to the nutrient solution at the same time as butachlor treatment. The vertical bars indicate the LSD at 5% level.

Absorption and translocation

Butachlor was readily absorbed by rice roots. About 32% of the applied ^{14}C ring labeled butachlor was absorbed during 24 hours of uptake(Table 4). The pretreatment of butachlor 2 days before uptake experiment did not affect the absorption. Of the total absorbed radioactivity, 80% still remained in roots and only 20% transported into shoots(Table 5). This indicates that acropetal movement of butachlor in plants is possible but limited. The autoradiograph developed form treated seedlings also showed that most

Table 3. Growth response of rice seedlings to IAA, GA_3 and butachlor

Treatment	Growth response*
Control	+++
IAA, 10^{-4} M	++
GA_3, 10^{-4} M	+++++
Butachlor, 10^{-5} M	0
GA_3 + butachlor	0
IAA + butachlor	+
Pretreatment of GA_3 and/or IAA 24 hours before butachlor treatment	
GA_3→butachlor	++ ~ ++++
IAA→butachlor	++
GA_3 + IAA→butachlor	+++ ~ ++++

* : Number of + signs less than 3 means growth inhibition, more than 3 means growth promotion, 0 means complete inhibition.

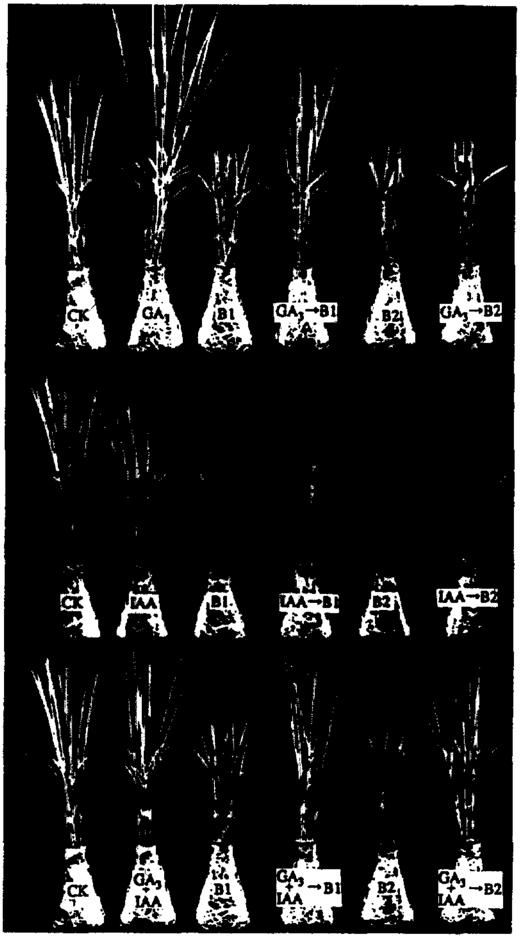

Fig. 5. Effect of GA_3 and/or IAA pretreatment on butachlor induced growth inhibition of rice seedlings. GA_3 and/or IAA were treated 24 hours begore butachlor treatment. Photographs were taken 4 days after butachlor treatment. Ck, control ; B1, 10^{-6} M butachlor 4 days ; B2, 10^{-5} M butachlor 4 days ; GA_3, 5×10^{-4} M GA_3 1 days ; GA_3→B1, 5×10^{-4} M GA_3 1 day then transferred to 10^{-6} M butachlor 4 days ; GA_3→B2, 5×10^{-4} M GA_3 1 day then transferred to 10^{-5} M butachlor 4 days ; IAA, 5×10^{-4} M IAA 1 day ; IAA→B1, 5×10^{-4} M IAA 1 day then transferred to 10^{-6} M butachlor 4 days ; IAA→B2, 5×10^{-4} M IAA 1 day then transferred to 10^{-5} M butachlor 4 days ; GA_3+IAA, 10^{-4} M GA_3+5×10^{-4} M IAA 1 day, GA_3+IAA→B1, 10^{-4} M GA_3+5×10^{-4} M IAA 1 day then transferred to 10^{-6} M butachlor 4 days ; GA_3+IAA→B2, 10^{-4} M GA_3+5×10^{-4} M IAA 1 day then transferred to 10^{-5} M butachlor 4 days.

of the radioactivity distributed in roots and the base of shoot, and least in old leaves(Fig 6). This is in accord with the report that some alachlor in yellow nutsedge(*Cyperus rotundus* L.) moved acropetally(Armstrong et al. 1973).

Glutathione conjugation

After 24 hours of uptake experiment, butachlor and its metabolites were extracted with 80% methanol and partitioned against petroleum ether. It was found that about 50% of radioactivity in roots and 60% in shoots was present in water layer(Table 5). In other words, more than half of the absorbed herbicide was metabolized into water soluble substances within the initial 24 hours. Since root metabolites might move along with butachlor to shoots, the higher proportion of metabolites in shoots than roots can not be attributed to the higher metabolic ability of shoots than roots. There were several metabolites separated from the TLC plate of the root and shoot extracts and their Rf's are given in Table 6. The Rf value of the most abundant metabolite was 0.6, same as that of butachlor-glutathione conjugate. The TLC patterns of root and shoot metabolites differ only slightly indicating that glutathione conjugation was the major metabolic pathway of butachlor in rice roots and shoots.

The non-protein thiol content and GST activity in relation to butachlor sensitivity

The non-protein thiol in Gramineae is composed mainly by glutathione(Breaux et al. 1987). In this study, it was used to indicate the glutathione content in plants. The non-protein thiol concentration of rice seedlings decreased gradually, while its total amount in shoots increased significantly in 3 weeks after germination(Table 7). Barnyardgrass and monochoria also contained

Table 4. Root uptake of butachlor by rice seedlings[1)]

Plant part	Without butachlor pretreatment		With butachlor pretreatment	
	% of total radioactivity applied	dpm/g fw	% of total radioactivity applied	dpm/g fw
Total uptake (roots+shoots)	32.2	-	37.8	-
Roots	25.7	98,300	30.2	98,400
Shoots	6.5	18,200	7.6	22,100
Remaining in nutrient solution	62.6	-	54.6	-

1) Rice seedlings were grown in 1/10 strength Hoagland nutrient solution and pretreated with or without 5 $\times 10^{-5}$ M butachlor for 2 days. The pretreated seedlings were then immersed for 24 hours in the same nutrient solution containing ring labeled ^{14}C-butachlor. The final concentration of the herbicide was adjusted to 5×10^{-5} M with cold butachlor.

Table 5. The distribution of radioactivity in aqueous and petroleum ether fractions of rice seedlings extracts after root uptake of ring labeled ^{14}C-butachlor for 24 hours.

Plant part and fraction	dpm	%
Root		
Aqueous	228,660	40.3
Petroleum ether	224,734	39.6
Shoot		
Aqueous	69,118	12.2
Petroleum ether	44,949	7.9
Total	567,461	100.0

Table 6. TLC separation of the metabolite extract of rice seedlings after root uptake of ring labeled ^{14}C-butachlor for 24 hours.

Shoot			Root		
Rf	dpm	%	Rf	dpm	%
0.35	7.7	0.6	0.41	89	0.5
0.39	13.0	1.0	0.44	138	0.8
0.43	4.6	0.4	0.47	383	2.3
0.46	47.9	3.8	0.50	611	3.7
0.52	297.1	23.4	0.53	1,352	8.3
0.60#	468.9	37.0	0.60	10,999	67.4
0.71	53.3	4.2	0.65	835	5.1
0.77	75.5	5.9	0.73	635	3.9
0.84	99.6	7.8	0.81	724	4.4
0.92	97.4	7.8	0.88	194	1.9
total	1,165.0	91.8	-	15,960	98.3

relatively high level of non-protein thiols as compared to rice.

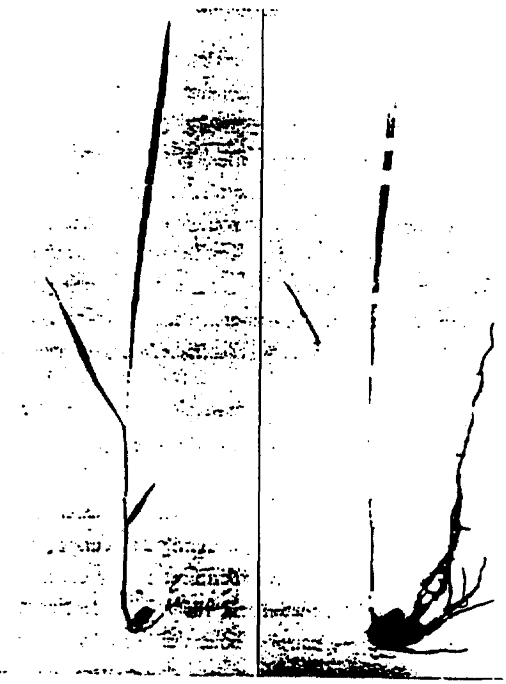

Fig. 6. Autoradiograph showing the distribution of radioactivity in rice seedling. Roots of the seedling were immersed, in Hoagland nutrient solution containing ring labeled ^{14}C-butachlor for 24 hours. Left, plant ; right, autoradiograph.

Rice, barnyardgrass, and monochoria contained different levels of GST activity. The GST activity of 14-15 days old rice seedlings was 0.48 and 0.31 unit/mg protein, respectively(Table 8). In 14-day old barnyardgrass, the GST activity was

Table 7. The non-protein thiol content of rice, barnyardgrass, and monochoria seedlings

Seedling age(days after germination)	nmol/g fw	nmon/shoot
Rice		
4	478.5	6.7
8	497.5	18.2
12	419.7	22.1
18	390.1	28.6
22	390.6	33.7
Barnyardgrass		
18	523.2	67.0
Monochoria		
30	217.8	90.0

Table 8. The GST activity of rice, barnyardgrass, and monochoria

Plant species and seedling age	unit#/mg protein
Rice	
14 days	0.48
30 days	0.31
Barnyardgrass	
14 days	0.20
Monochoria	
30 days	0.05

: unit : The change of A_{340} nm by 1.0 per minute under the assay conditions.

0.20 unit/mg protein, about half of rice. In monochoria the activity was 0.05 unit/mg protein, only about 16% of rice.

Conjugation of herbicide with glutathione was directly affected by the level of glutathione and GST activity in plants(Ekler and Stephenson 1989). The highest GST activity in rice than the two paddy weeds probably is the major factor affecting the sensitivity of different plant species to butachlor.

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