Protein Patterns of Rice(*Oryza sativa* L.) Cultivars as Affected by Herbicide Thiobencarb

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제초제 Thiobencarb 처리에 의한 수도품종간 단백질 유형의 변화

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ABSTRACT

This study was conducted to evaluate the susceptibility of rice(*Oryza sativa* L.) cultivars to herbicide thiobencarb through the determination of protein patterns using SDS-PAGE. In both greenhouse and laboratory screening tests, IR 10198-66-2 and IR 9660-50-3-1 showed relatively tolerant response to thiobencarb, while IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 were susceptible to it. The total protein content of susceptible cultivars markedly decreased as the thiobencarb concentrations increased, but tolerant cultivars such as IR 10198-66-2 and IR 9660-50-3-1 showed very slight changes suggesting that protein synthesis of susceptible cultivars may be inhibited by thiobencarb. The protein profiles of the tolerant cultivars were not much affected by thiobencarb treatment. However, the protein spots with molecular weights of 14.4 kD and 55 kD in the susceptible cultivar of IR 22 disappeared with the treatment of 3 ppm thiobencarb, indicating that differential susceptibilities of rice cultivars against thiobencarb can be attributed to their difference in protein metabolism affected by thiobencarb.

Key words : Protein patterns, Rice(*Oryza sativa* L.), Thiobencarb

INTRODUCTION

Thiobencarb [S-(4-chlorobenzyl)-*N*, *N*-diethylthiolcarbamate] is a carbamate herbicide known to be selective for pre-emergence control of grasses and broad-leaved weeds in paddy fields. Mechanism of action of thiobencarb is not clear yet although it has been known to inhibit protein synthesis and biosynthesis of gibberellin-induced α-amylase in aleurone layer of germinating seeds[14,15]. In rice, thiobencarb is absorbed rapidly and translocated to the upper part of the plant[8]. The absorption of this herbicide was higher in the upper part of the plant than in the root and dissipation of the herbicide was rapid in the upper part compared to that in the root[10]. Differential cultivar responses to the herbicide can be attributed to differences in ecogeographic race[13].

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For example, Ichizen[6] reported that Japonica type rice cultivars had higher tolerance to thiobencarb than Indica type and their hybrid cultivars. Kim et al.[9] made the similar report that phytotoxicity of the mixture of thiobencarb and simetryne was higher in Indica×Japonica type cultivars than in Japonica. Shin[13] also reported that there was differential cultivar responses of rice among Indica type cultivars against thiobencarb herbicide.

Many herbicide-resistant plants have been identified both in weeds and in crops[1,4,5,10]. Genetic variation in herbicide tolerance of plants can be used to modify the crop genotype to tolerate herbicides or to raise their tolerance level to herbicides, so that it should be possible to improve herbicide selectivity[3].

Many workers have identified differential cultivar responses of rice to thiobencarb[6,9,13]. However, mechanism of differential cultivar responses has not been precisely understood, especially these based on biochemical aspects. Understanding the mechanism of differential response of rice cultivar to the specific herbicide is necessary to improve herbicide selectivity or to develop a herbicide-tolerant cultivar by introducing genetic variation of herbicide tolerance.

This study was conducted to determine any possibility of difference in protein patterns between thiobencarb-resistant and -susceptible rice cultivars recommended by Shin[13] and further confirmed by the preliminary study.

MATERIALS AND METHODS

Experiment 1a. Greenhouse Screening for Rice Cultivar Tolerant to Thiobencarb

The seeds of five rice cultivars, *i.e.*, IR 22, IR 9660-50-3-1, IR 10198-66-2, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 obtained from the International Rice Research Institute(IRRI) were soaked for 24 h and incubated at 25℃ in darkness for 48 h before seeding. Germinated seeds of each rice cultivar were planted in pots and allowed to grow for 3 days. Four plants having similar height and appearance were transplanted in 5 rows in each plastic trays filled with a clay loam soil which was sieved through a 3mm sieve. Water level was maintained at a depth of 2cm just before herbicide application.

The commercial formulations of thiobencarb (7% G) were applied at rates of 3.0, 4.5, 9.0 and 12.5kg/10a at 4 days after transplanting. All treatments were triplicated and untreated controls were provided for comparison. Plant height, root length and dry weight of whole plant were measured to evaluate the responses of the cultivars to the herbicide. Plant height and root length were taken from the base of the culm to the top of the longest leaf or root at 15 days after treatment(DAT). Dry weight was measured after oven-drying for 48 hours at 80℃.

Experiment 1b. Laboratory Screening for Rice Cultivar Tolerant to Thiobencarb

This experiment was conducted to compare responses of rice cultivars to thiobencarb with laboratory screening method. Five rice cultivars used in Experiment 1a were again evaluated in this experiment. Ten seeds sterilized for 15min. with 1% sodium hypochlorite were germinated on filter paper in petri dishes of 9cm diameter. Ten ml of thiobencarb solution having the rates of 3.0, 4.5, 9.0 and 12.5kg/10a were applied to the petri dishes which were then placed in a chamber maintained at constant temperature of 25℃ under dark condition. All treatment were triplicated. Data on shoot and root length were taken at 7 DAT.

Experiment 2. Protein Patterns of Rice Cultivars

Plant Materials

Rice seeds sterilized with 1% sodium hypochlorite were germinated at 25℃ in an incubator under dark condition. Ten ml of thiobencarb solution having 1, 3 and 5ppm were added to the petri dishes.

Protein Assay

One hundred mg of fresh shoots were ground in a mortar and pestle, and resuspended in 1ml of extraction buffer(62.5mM Tris-HCl, pH 6.8, 5% mercaptoethanol, 4% glycerol, 3% SDS). The homogenate was centrifuged for 10 min at 12,000 rpm. The supernatant was taken in order to measure protein content. Protein content was determined by the Bradford's method[2] with lypophilized bovine gamma globulin used for a standard.

Protein Pattern Analysis

One hundred mg of etiolated shoots in each rice cultivar treated with various concentrations of thiobencarb for 3 days were ground and resuspended in 1ml of sample buffer(62.5mM Tris-HCl, pH 6.8, 5% mercaptoethanol, 4% glycerol and 3% SDS). The homogenate was heated in a water bath at 95℃ for 4 min. The supernatant was then stored at 4℃ until electrophoresis.

To separate proteins in one dimensional gel electrophoresis, the SDS-polyacrylamide gel system[12] was employed using slab gels. The separating gel was prepared by adding 6.5ml of 30% acrylamide/0.3% bisacrylamide, 3.75ml of 4×1.5M Tris-HCl/0.4% SDS(pH 6.8), 4.75ml of distilled water, 50$\mu\ell$ of 10% ammonium persulfate and 10$\mu\ell$ of TEMED. The stacking gel was prepared 0.65ml of 30% acrylamide/0.8% bisacrylamide, 1.25ml of 4×0.5M Tris-HCl/0.4% SDS(pH 6.8), 3.05ml of distilled water, 25$\mu\ell$ of 10% ammonium persulfate and 5$\mu\ell$ of TEMED. Electrode buffer consisted of 25mM Tris-HCl(pH 8.3), 0.195M glycine and 0.1% SDS. Fifteen $\mu\ell$ of extracted proteins were loaded onto the slab gel. Electrophoresis was carried out at 25mA for the stacking gel and then at 40mA for the separating gel. After the electrophoresis, the gels were fixed in the solution of 50%(v/v) methanol, 10%(v/v) acetic acid and 40%(v/v) distilled water and then stained with the solution of 50%(v/v) methanol, 0.05%(w/v) Coomassie brilliant blue R and 10% (v/v) acetic acid. The stained gel were subjected to destaining in the solution of 5%(v/v) methanol and 7%(v/v) acetic acid.

For two-dimensional gel electrophoresis of proteins, rice leaves were ground in a mortar and extracted in two volumes of lysis buffer. The lysis buffer consisted of 8.5M urea, 2% triton X100, 1.6% ampholine(pH 5-7), 0.4% ampholine (pH 3.5-10.0), 5% 2-mercaptoethanol and 5% polyvinylpyrrolidone K30. The extracted proteins were loaded onto isoelectric focusing(IEF) rod gels. IEF and two-dimensional polyacrylamide gel electrophoresis(2D-PAGE) were performed by a modified procedure of the O'Farrell's system[12] with 12.5% polyacrylamide in the separation gel and 4% in the stacking gel. After the electrophoresis, the gel was stained with silver for detecting protein spots.

RESULTS AND DISCUSSION

Rice Cultivar Response to Thiobencarb under Greenhouse and Laboratory Conditions

Plant height and root length were decreased as the thiobencarb concentration increased irrespective of the cultivars used in greenhouse conditions. The greater decreases in plant height and root length were observed in IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 than those of IR 10198-66-2 and IR 9660-50-3-1(Table 1). A similar trend was observed in dry weight of rice cultivar treated with thiobencarb(Fig. 1). In fact, there are no definite basis to classify tolerant and susceptible cultivars against herbicide. However, in many herbicide screening experiments, the cultivars screened have been categorized for convenience into the two groups, *i.e.* tolerant and susceptible

Effect of different concentrations of thiobencarb on the growth of several rice cultivars treated in pot under greenhouse condition[1)]

Cultivar	Rate (kg prod./ 10a)	Shoot length				Root length			
		3.0	4.5	9.0	12.5	3.0	4.5	9.0	12.5
					% of control				
IR 10198-66-2		99.7a	98.3a	91.3a	86.2a	99.3a	98.2a	92.5a	88.7a
IR 9660-50-3-1		98.8a	97.5a	89.5a	84.9a	98.4a	94.7a	89.3a	86.5a
IR 22		87.3b	83.7c	71.6c	63.9b	89.9b	86.7ab	75.1b	70.3b
IR 31802-48-2-2-2		91.3ab	87.6b	80.6b	68.8b	92.1ab	88.3ab	74.8b	69.3b
IR 20656-R-R-R-6-1		87.4b	85.2bc	69.2c	60.8b	88.3b	82.7b	78.5b	69.5b

[1)] Average of 5 plants with 3 replications, determined at 15 days after treatment. In a column, means followed a common letter are not significantly different at the 5% level by DMRT.

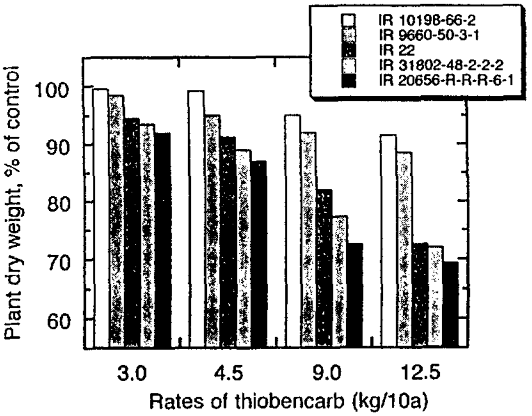

Fig. 1. Dry weight changes of rice cultivars as affected by various rates of thiobencarb.

cultivar. Shin[13)] reported that some cultivars which when treated with 2×10^{-5}M thiobencarb, had plant heights and dry weights equal to or greater than 60% of the untreated check were regarded to be tolerant to the herbicide. According to the criterion, IR 10198-66-2 and IR 9660-50-3-1 can be classified as the tolerant cultivars to thiobencarb while IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 were the susceptible ones to thiobencarb.

In this study, regardless of the herbicide rates used, two cultivars namely IR 10198-66-2 and IR 9660-50-3-1 showed more than 80% of untreated control in both plant height and dry weight, showing relatively tolerant to thiobencarb. The results obtained in this experiment showed a similar trend to those of Shin[13)] although different screening systems were employed.

Under laboratory screening, shoot and root length of rice cultivars as affected by different concentrations of thiobencarb are shown in Table 2. In general, shoot and root length of IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 decreased significantly as the thiobencarb concentration increased.

Increased concentration from 1 to 5ppm of thiobencarb greatly inhibited the growth of shoot and root in both IR 10198-66-2 and IR 9660-50-3-1, but the degree of the inhibition was much lower than that of the three other cultivars.

Since the results observed in the greenhouse condition were well coincided with those of the laboratory screening, IR 10198-66-2 and IR 9660-50-3-1 can be categorized as the tolerant cultivars to thiobencarb while IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 as the susceptible ones to it. Hassan et al.[5)] made the similar observation on evaluating responses of rice cultivars to alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide] and metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide], that the laboratory screening results were well correlated with those from the greenhouse experiment.

Changes in Protein Content

Total protein contents of shoots of rice cultivars were affected by various concentrations of thio-

Table 2. Effect of different concentrations of thiobencarb on the growth of several rice cultivars treated in petri dishes.[1)]

Cultivar	Shoot length				Root length			
Conc.(ppm)	0	1	3	5	0	1	3	5
	cm							
IR 10198-66-2	3.7bc	3.9a	3.1a	2.8a	5.6b	6.1b	5.2a	4.3a
IR 9660-50-3-1	3.5c	3.6b	2.6b	2.2b	4.8c	5.2c	4.4b	4.2a
IR 22	3.6c	1.8d	1.1d	1.0c	5.5b	3.9c	3.2d	3.2c
IR 31802-48-2-2-2	3.9b	2.3c	1.8c	0.9c	6.7a	6.3a	5.4a	3.3c
IR 20656-R-R-R-6-1	4.0a	2.1d	0.9d	0.8c	5.5b	4.3d	3.9c	3.7c

[1)] Herbicide treated at 3 days after germination in petri dishes and determined at 48 hours after treatment. Average of 10 seedlings with 3 replications in petri dishes. In a column, means followed a common letter are not significantly different at the 5% level by DMRT.

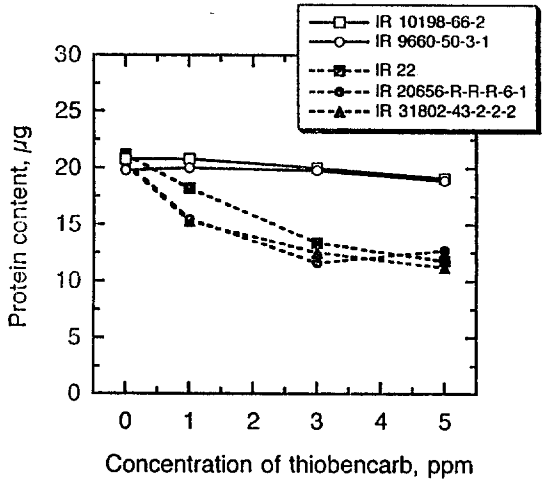

Fig. 2. Changes in protein content of rice cultivars as affected by various concentrations of thiobencarb.

bencarb. As thiobencarb concentration increased from 1 to 5ppm, the total protein content was slightly declined in tolerant cultivars such as IR 9660-50-3-1 and IR 10198-66-2(Fig. 2). However, the total protein contents of susceptible cultivars such as IR 22, IR 31802-48-2-2-2 and IR 20656 -R-R-R-6-1 were markedly decreased as the thiobencarb concentrations increased from 1 to 5ppm (Fig. 2). These data strongly suggest that protein synthesis of susceptible cultivars was greatly inhibited by thiobencarb treatment, resulting in the decreased protein content. Actually, thiobencarb is known to inhibit protein synthesis in treated plants[7,14,15)]. It is assumed that the effect of thiobencarb on protein content was coincident with the influences of the herbicide on plant height and dry weight of susceptible and tolerant cultivars.

Changes in Protein Patterns

When the leaf proteins of various rice cultivars treated with 5ppm thiobencarb was separated on SDS gels and stained with Coomassie blue, banding patterns of proteins were different depending upon rice cultivars. The protein profiles of tolerant group such as IR 9660-50-3-1 and IR 10198-66-2, and susceptible group such as IR 22, IR 31802-48-2-2-2 and IR 20656-R-R-R-6-1 revealed shifts in protein composition in susceptible group, but no shifts in tolerant group(Fig. 3). The bands with molecular weights from about 94 to 30 kD and about 14.4 kD were not observed in susceptible cultivars(lane 1, 4 and 5). These results that tolerance of rice cultivar to thiobencarb may be closely related to the protein having molecular weights between 94kD and 30kD, and of about 14.4kD which disappeared in susceptible cultivar when 5ppm thiobencarb was applied.

Fig. 4 showed that the protein profiles of susceptible cultivars as affected by different concentrations of thiobencarb. The bands which were not observed at the concentration of 5ppm were detectable at 1ppm concentration. This confirmed that varied protein content of tolerant and susceptible cultivars might be closely related to thioben-

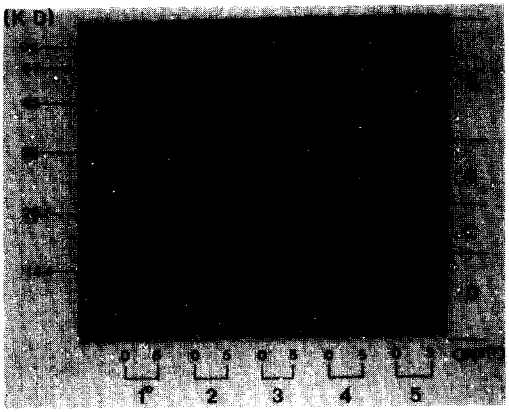

Fig. 3. Difference in protein banding patterns of SDS-PAGE from rice cultivars as affected by thiobencarb.

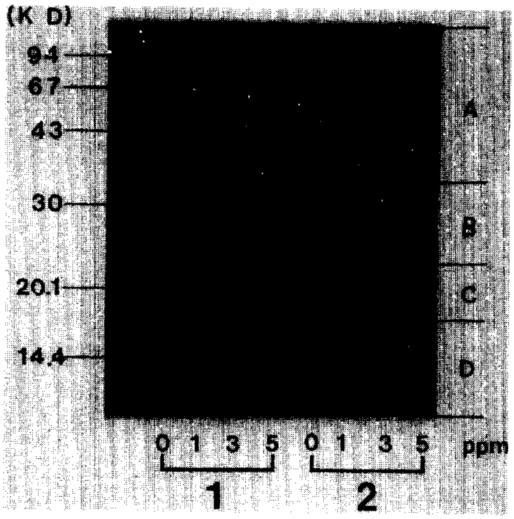

Fig. 4. Changes in banding patterns of SDS-PAGE from IR 22 and IR 31802-48-2-2-2 as affected by various concentrations of thiobencarb.

carb's selectivity.

The patterns obtained by 2D-PAGE for proteins extracted from the leaves of tolerant(IR 10198-66-2) and susceptible(IR 22) rice cultivars with or without the treatment of 3ppm thiobencarb are shown in Fig. 6. The analysis was restricted to a few prevalent spots which showed changes in the relative intensity after the herbicide treatment. Analysis of the proteins by 2D-PAGE revealed similar patterns for the proteins extracted from the tolerant and susceptible cultivars. However, in the treatment of 3ppm thiobencarb, different patterns of proteins between tolerant and susceptible cultivar were found compared to those of their respective control. The spots indicated by solid arrows particularly in the 14.4 and 55 kD region or spots disappeared or decreased in the intensity with the susceptible cultivar of IR 22.

In the investigation on the protein patterns of the two cultivars having differential responses to thiobencarb, we observed that the protein patterns changed in the susceptible cultivar followed by thiobencarb treatment. However, the changes in the tolerant cultivar treated with 3ppm thiobencarb were negligible compared to those of the control, indicating that the treatment of thiobencarb induced the higher inhibition of specific protein synthesis in the susceptible cultivar than tolerant cultivar. Our results indicate that the specific protein spots might be related to the herbicide tolerance and the tolerance would be governed by a gene(s). They also suggests that the gene(s) responsible for the tolerance could be transferred into a target plant by a gene recombination if the tolerant gene(s) are existed in rice.

摘 要

Thiobencarb 처리에 의한 수도품종의 내성 정도를 조사하고 내성 메카니즘을 연구하기 위하여 내성 및 감수성 품종간의 단백질 함량, SDS-PAGE에 의한 단백질 유형의 변화를 조사하였다. 본 시험에 공시한 5개 품종 중 IR 10198-66-2와 IR 9660-50-3-1은 온실 및 실내실험에서 공히 thiobencarb에 대하여 내성을 보였으며 IR 22, IR 31802-48-2-2, IR 20656-R-R-R-6-1은 제초제 농도가 증가함에 따라 생장이 크게 억제되어 감수성을 나타내었다. Thiobencarb 처리에 의한 단백질의 함량은 내성 및 감수성 품종간에 뚜렷한 차이를 보였는데, 감수성 품종

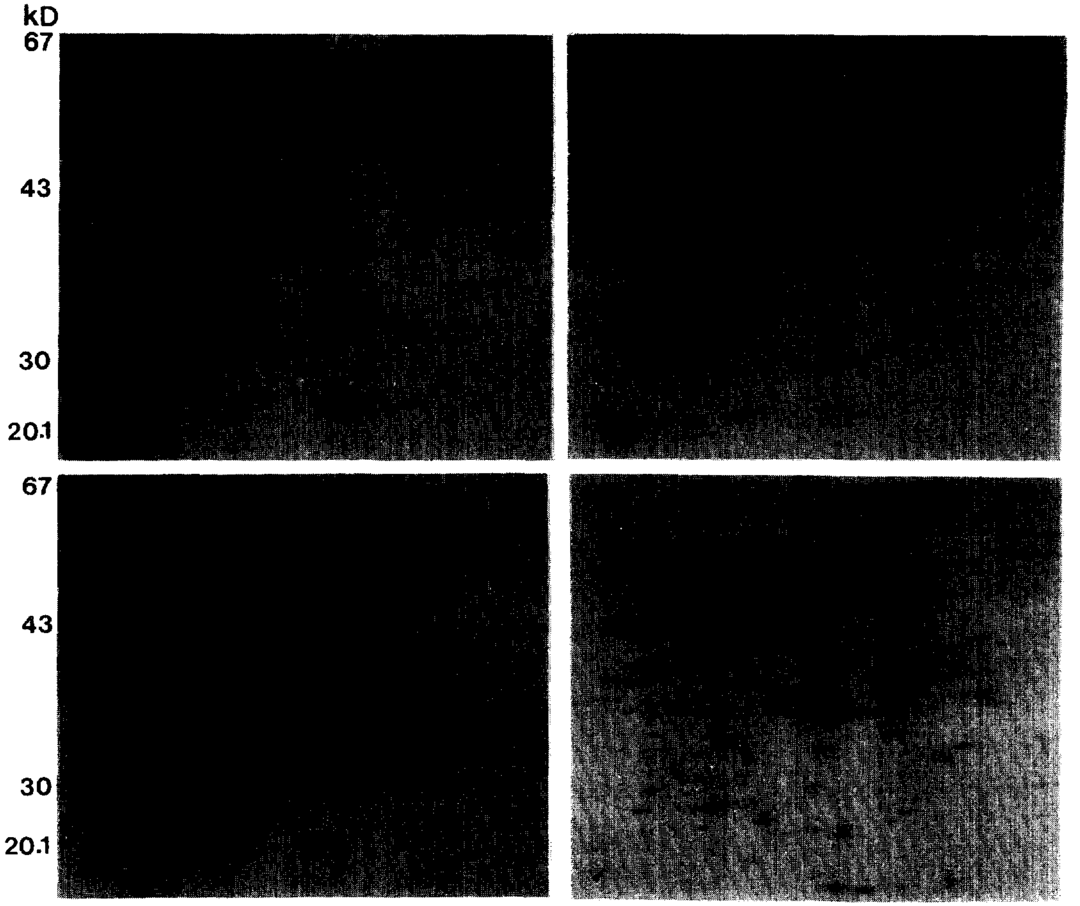

Fig. 5. Changes in protein patterns separated by two-dimensional gel electrophoresis in IR 20656-R-R-R-6-1 and IR 22 as affected by 3 ppm thiobencarb. (A) IR 20656-R-R-R-6-1 without treatment, (B) IR 20656-R-R-R-6-1 with treatment of 3 ppm thiobencarb, (C) IR 22 without treatment, (D) IR 22 with treatment of 3 ppm thiobencarb.

의 단백질 함량은 제초제 농도가 증가함에 따라 크게 감소하는 경향을 나타내었으나 내성 품종의 경우 감소가 거의 없었다. SDS-PAGE에 의한 단백질 밴드는 내성 품종의 경우 5 ppm 처리에서도 변화가 없었으나 감수성 품종은 5ppm 처리에서 94-30 kD 사이와 14.4kD 부근의 단백질 밴드가 사라지는 경향을 보였다. Thiobencarb 3ppm을 처리한 감수성 품종인 IR 22의 2차원 전기영동에 의한 단백질 패턴에서는 14.4kD와 55kD 부근의 spot가 사라지거나 density가 감소하는 경향을 보였다. 이상의 단백질 함량과 유형의 변화로 미루어 보아 thiobencarb 처리는 감수성 품종의 특정 단백질 합성을 억제하는 것으로 나타났으며, 벼의 thiobencarb에 대한 내성은 이 부위의 단백질과 밀접한 관계가 있는 것으로 사료된다.

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