Effect of Cytochrome P-450 Monooxygenase Inhibitors on the Rice Tolerance of Azimsulfuron and Bensulfuron-methyl*

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Oxidation by cytochrome P-450 is thought to be one of the important processes for the detoxification of pesticides in plants. The possible involvement of cytochrome P-450 monooxygenases in tolerance of plants to herbicides has been demonstrated through metabolism studies with isolated microsomes from several crops^{3-5,8}. The use of cytochrome P-450 monooxygenase inhibitors effectively supported the tests to certify that the metabolism catalyzed by P-450 monooxygenases played an important role in herbicide detoxification in plants. ABT (1-aminobenzotriazole) is a suicide substrate for the plant cytochrome P-450 monooxygenase responsible for cinnamate hydroxylation⁹, and has been utilized to examine the role of cytochrome P-450 monooxygenases in the metab-

olism of various herbicides^{1,6,7}. Piperonyl butoxide is a well-known animal cytochrome P-450 monooxygenase inhibitor which synergizes the actions of insecticides, and piperonyl butoxide also synergizes the activity of some herbicides¹¹.

O-Demethylation at pyrimidine ring is a key process of the metabolism of bensulfuron-methyl (BSM) (methyl = α -(4, 6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl)-*o*-toluate) in rice¹⁰. Azimsulfuron (1-(4, 6-dimethoxypyrimidin-2-yl)-3-[2-methyl-4-(2-methyl-2*H*-tetrazol-5-yl)-2*H*-pyrazole-3-sulfonyl]-urea) is a sulfonylurea herbicide, and includes dimethoxy pyrimidine ring in its chemical structure, although its metabolic pathway in rice plants has not been explained.

In this paper, the contribution of cytochrome P-450 monooxygenases to the detoxification of BSM and azimsulfuron in rice plants was examined using the two cytochrome P-450 monooxygenase inhibitors, ABT and piperonyl butoxide.

Materials and Methods

A water solution of BSM, azimsulfuron and the cytochrome P-450 monooxygenase inhibitors, ABT and piperonyl butoxide, at each concentration including 1% acetone was prepared. Rice seeds (*Oryza sativa* L. cv. Nipponbare) were incubated in the dark at 30°C for three days to germinate. Six germinated rice seeds sown on a filter paper in a petri dish (9 cm in diameter) containing the

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Table 1. Combination effect of BSM/azimsulfuron and cytochrome P450-monooxygenase inhibitors (ABT/PB) on rice root growth.

Herbicide	(M)	Root Length (mm per plant)					
		ABT (ppm)			PB (ppm)		
		0	5	10	0	10	20
BSM	0	77a	81a	64b	77a	80a	65b
	10^{-7}	64b	48d (s)	48d (s)	64b	57c (s)	49de (s)
	2×10^{-7}	56c	45d (s)	38e (s)	56cd	42e (s)	34f (s)
Azimsulfuron	0	77a	81a	64b	77a	80a	65b
	10^{-7}	62b	53c (s)	46d (s)	62b	50c (s)	44c (s)
	2×10^{-7}	46cd	42de (s)	36e (s)	46c	36d (s)	32d (s)

For each combination, means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

(S)=Synergistic interaction using Colby's equation

water solution of BSM, azimsulfuron or cytochrome P-450 monooxygenase inhibitors were grown in a growth chamber controlled at 25°C and 50,000lux, with a 12 hr photo period. The petri dishes were covered during the test period to prevent drying. Ten days after sowing, the length of the roots was measured. The means of their length were compared using Duncan's multiple range test for the analysis of variance, and interactions between herbicides and cytochrome P-450 monooxygenase inhibitors were analyzed by the method of Colby²⁾.

Results and Discussion

Table 1 presents the combination effects of BSM or azimsulfuron with the cytochrome P-450 monooxygenase inhibitors. Both herbicides significantly inhibited rice root growth at 10^{-7} and 2×10^{-7} M. The two cytochrome P-450 monooxygenase inhibitors, ABT and piperonyl butoxide, affected root growth at their highest rates, 10 and 20 ppm, respectively. Each herbicide inhibited rice root growth more strongly in combination with ABT or piperonyl butoxide, except for 2×10^{-7} M azimsulfuron with 5 ppm ABT. ABT and piperonyl butoxide synergized the

toxicity of the two sulfonylurea herbicides at every rate tested. Their interactions may result from inhibition of metabolism of the sulfonylurea herbicides in rice plants. This suggests that cytochrome P-450 monooxygenase oxidatively metabolize BSM and azimsulfuron making them inactive in rice plants. Furthermore, *O*-demethylation of BSM in rice is probably due to cytochrome P-450 monooxygenases and this process of metabolism may function for azimsulfuron.

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