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Primisulfuron-Tolerant Pepper : A Biochemical Basis of Tolerance

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ABSTRACT

Response of pepper (*Capsicum* sp.) cultivars to primisulfuron {methyl 2-[[[[[4,6-bis (difluoromethoxy)-2-pyrimidinyl] amino] carbonyl] amino] sulfonyl] benzoate} was evaluated at the whole plant and enzyme levels. Tolerant cultivars, Red Horn and Kwangbok, demonstrated 3 fold tolerance to primisulfuron in terms of plant height and dry weight compared to those of susceptible cultivars, Chamjoah and Poongchon. Acetolactate synthase (ALS) levels showed that the concentrations of primisulfuron required for 50% *in vitro* inhibition of ALS activity were 10-15 times greater for the tolerant cultivars compared to the susceptible ones. These data suggest that the differential ALS sensitivity may be considered for an alteration at the target site for primisulfuron tolerance. In addition, the level of tolerance by the ALS enzyme is related to observe whole plant tolerance to primisulfuron.

Key words : acetolactate synthase, pepper, primisulfuron, tolerance

INTRODUCTION

Primisulfuron, a sulfonylurea herbicide, inhibits acetolactate synthase (ALS, E.C. 4.1.3.18), the first enzyme in biosynthesis of branched-chain amino acids valine, leucine, and isoleucine in plants. There are currently three major herbicide chemical classes which target this enzyme: the imidazolinones, triazolopyrimidines, and sulfonylureas. ALS inhibition stops protein synthesis, causing decrease photosynthate translocation to meristems that leads to rapid cessation of cell division and plant growth (Shaner and Conner, 1991; Stetter, 1994).

Herbicide tolerant crop plants have become important factors in current weed management strategies and the implementation of new crop production technologies. Due to the numerous concerns about ALS-tolerant crop plants, much current research focuses on strategies for developing and identifying the mechanisms of herbicide tolerance. Early in the development of ALS-inhibiting herbicides, attempts to assess for a herbicide-tolerant crop and the mechanism of tolerance through various methods were successful in a wide range of species including maize (Newhouse *et al*., 1991; Renner *et al*., 1988; and Sander and Barrett, 1989), rice (Li *et al*., 1992), soybean (Kent *et al*., 1988; Sebastian and Chaleff, 1987; and Wixson and Shaw, 1991), tobacco (Chaleff and Ray, 1984; and Lee *et al*., 1988), wheat (Newhouse *et al*., 1992), as well as in several other crop plants (Hart *et al*., 1992; Hart *et al*., 1993; and Pornprom and Pyon, 1997). In most

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cases, tolerance couributes to an alteration at the target site, the ALS enzyme, preventing ALS-inhibiting herbicides from inhibiting the enzyme.

Crop plants tolerant to ALS-inhibiting herbicides are of great concern for several reasons. Properties of the ALS enzyme are important factors that can affect the differential response of herbicides between species or for differential response of a species between herbicides. Several pepper cultivars investigated in this research were chosen because of wide variation in sensitivity to ALS-inhibiting herbicides. In these whole plant studies, the tolerant pepper cultivars were tolerant to primisulfuron, but it was not known if tolerance was due to a nonsensitive ALS enzyme (Pornprom and Pyon, 1997). Recently, however, the mechanisms of tolerance in ALS-inhibiting herbicides tolerant pepper cultivars have not previously been examined for tolerance at the enzyme level. Therefore, direct comparisons of the affinity of the active site, the ALS enzyme, among pepper cultivars to primisulfuron inhibition are needed.

The objective of the studies is to determine if differences in response of pepper cultivars to primisulfuron could be explained by differences in the target site, the ALS enzyme, of primisulfuron.

MATERIALS AND METHODS

Plant material

Four pepper cultivars, previously identified as being primisulfuron tolerant cultivars, Red Horn and Kwangbok, and susceptible cultivars, Chamjoah and Poongchon were used in this study (Pornprom and Pyon, 1997). All of these pepper cultivars are the production of Korea. The seeds of tolerant and susceptible cultivars were planted in plastic tray (surface area : 27x35 cm^2) containing 2:1:1 (by volume) mixture of soil : vermiculite : peat. All plants were maintained in the greenhouse at 28/30 ± 3 °C day/night with a 12 hr photoperiod. Plants were thinned after emergence to uniform numbers and grown for 1 month.

Crop injury response

Individual greenhouse experiment was conducted to compare the response of each plant species to primisulfuron, presenting in the representatives of tolerant and susceptible cultivars from screening experiment. The seeds of primisulfuron tolerant and susceptible cultivars were planted as above described procedure (Pornprom and Pyon, 1997). Commercial formulations of primisulfuron were applied with nonionic surfactant (X-77) at a rates of 0.25 % (v/v). Rates evaluated were 0.01, 0.02, 0.04, 0.08 and 0.16 kg ai/ha, applied when plants were at the three-leaf-pair stage. Herbicide treatments were applied with a CO_2-pressurized backpack hand-held boom sprayer delivering 1,000 L/ha at 300 kPa. Visual injury ratings were taken 14 days after treatment (DAT) using a scale from 0 to 100, where 0 = no injury and 100 = complete desiccation. Immediately after rating, the above-ground biomass was harvested, oven-dried, and weighed. The experiment was conducted in a completely randomized design and was repeated twice with four replications per treatment. The results were expressed as a percentage of the untreated controls.

Acetolactate synthase activity

ALS was extracted and enzyme activity levels measured in the presence of primisulfuron with a modification of the methods outlined by Hart *et al*. (1992 and 1993), Ray (1984), and Shaner *et al*. (1984). All extraction, centrification, and column separation procedures were conducted on ice or at 4°C. One-month-seedlings of pepper, samples were a composite of newly formed plant

leaves excised from the apex of several plants. Each 10g sample was homogenized in an homogenization buffer [0.1 M K_2HPO_4, pH 7.5, 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate, 10 mM flavin adenine dinucleotide (FAD), 10% (v/v) glycerol] equivalent to 2.5 times the weight of the plant tissue. The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 27,000 g for 20 min. The supernatant fraction was brought to 50% saturation with cold $(NH_4)_2SO_4$ and allowed to stand 1 hr on ice. The mixture was then centrifuged at 15,000 g for 15 min. The supernatant was discarded and the precipitated pellet dissolved in resuspension buffer (0.1 M K_2HPO_4, 20 mM sodium pyruvate, 0.23 mM $MgCl_2$, pH 7.5). This solution was passed through a Sephadex® G-25M PD-10 column equilibrated with the same buffer. The desalted enzyme preparation was immediately used for enzyme assays.

ALS enzyme assays were carried out in a final volume of 1.5 ml containing the enzyme preparation, reaction buffer (25 mM K_2HPO_4, 0.625 mM $MgCl_2$, 25 mM sodium pyruvate, 0.625 mM thiamine pyrophosphate, 1.25 μM flavin adenine dinucleotide, pH 7.0), and technical grade primisulfuron at 10^{-8} to 10^{-5} M concentrations. Reaction tubes were incubated for 1 hr at 35 °C when the reaction was stopped with the addition of 50 μL of 6 N H_2SO_4, and the solutions tubes were heated for 15 min at 60 °C. Then 0.5 ml of 0.5 % weight by volume α-naphthol freshly prepared in 2.5 N NaOH were added consecutively to each tube. The solutions were heated for an additional 15 min at 60 °C and the acetoin content measured by the method of Westerfield (1945). Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

The experiment was repeated with 3 replications of each herbicide concentration per experiment. Data presented are the means of the 3 experiments. ALS enzyme activity is presented as a percent of control assays.

RESULTS AND DISCUSSION

Whole plant response

Plant species differed in response to the rates of primisulfuron applied in this experiment. The response of pepper cultivars to postemergence applications of primisulfuron was similar to the results of previous studies by Pornprom and Pyon (1997). The data presented here indicate that Red Horn and Kwangbok cultivars show relatively tolerant response to primisulfuron, while Chamjoah and Poongchon cultivars are susceptible to it. In previous studies, primisulfuron tolerance was established by comparing data on visual injury and fresh weight of the tolerant and susceptible cultivars at different herbicide rates. Plant height and dry weight data are presented here, though visual injury and fresh weight showed similar trends. The accumulation of both plant height and dry weight of shoots for tolerant and susceptible cultivars decreased as primisulfuron rate increased (Fig. 1). However, the primisulfuron rate by species or cultivars interacted the response of tolerant and susceptible pepper cultivars to primisulfuron was dissimilar. The tolerant cultivars required 0.16 kg ai/ha primisulfuron to reduce plant growth 50% while the susceptible cultivars required 0.07 kg ai/ha. The tolerant cultivars demonstrated about 3-fold tolerance at the whole plant level compared to susceptible plants (Table 1). This suggested that the degree of herbicide injury to a susceptible crop depends on the plant species and the herbicide concentration that contacts the plants. Additional support can be derived from greenhouse studies demonstrating that crop cultivar response to herbicides may be determined by evaluation of various injury symptoms, crop emergence and development, and possible yield reductions (Kent

et al., 1988; Newhouse *et al*., 1992; Renner *et al*., 1988; Sander and Barrett, 1989; and Wixson and Shaw 1991). These results are in agreement with other researches showing the efficacy of ALS-inhibiting herbicides under field and greenhouse conditions varied among different species (Hart *et al*., 1992; Newhouse *et al*., 1991; and Shaner and Conner, 1991). However, this study does not serve as a prediction of injury risk in the field, but does provide an indication of relative cultivar sensitivity if injury does occur.

Acetolactate synthase activity

The effect of target site for primisulfuron tolerance on ALS activity was evaluated in the primisulfuron-tolerant and -susceptible cultivars by an *in vitro* assay. The specific ALS activity average 215.45 and 170.10 mmol acetoin/mg protein/hr in the absence of herbicide for the tolerant and susceptible cultivars, respectively (data not shown).

A complete understanding of the mechanism of action of the primisulfuron-tolerant pepper cultivars begins at the level of ALS activity. The ALS extracts from tolerant and susceptible cultivars show distinct differences in ALS activity at imazethapyr concentrations >10^{-8} M (Figure 2).

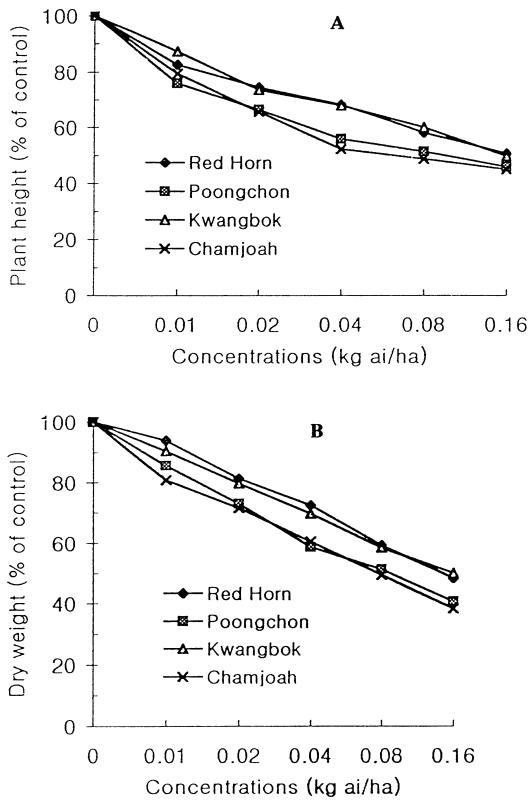

Figure 1 Effect of primisulfuron on plant height (A) and dry weight (B) of shoots for tolerant (Red Horn and Kwangbok) and susceptible (Poongchon and Chamjoah) cultivars determined 10 days after herbicide application.

Table 1 GR_{50} and I_{50} for primisulfuron determined from plant height, dry weight, and acetolactate synthase activity, respectively, of primisulfuron susceptible (S, Chamjoah and Poongchon) and tolerant (T, Red Horn and Kwangbok) pepper cultivars.

Pepper cultivar	GR_{50} (kg ai/ha)	I_{50} (M)
Red Horn	0.16	5×10^{-6}
Kwangbok	0.16	8×10^{-6}
Chamjoah	0.07	3×10^{-7}
Poongchon	0.07	5×10^{-7}
T/S[a/]	2-3	10-15

a/ T/S = tolerance ratio, GR_{50} tolerant/GR_{50} susceptible or I_{50} tolerant/I_{50} susceptible

At primisulfuron concentrations greater than 10^{-8} M, the extracts from the tolerant cultivars maintained ALS activity greater than the extracts from the susceptible cultivars. Activity of ALS from the susceptible cultivars was nearly 50% inhibited by 5×10^{-7} M primisulfuron while approximately 50% of the ALS from tolerant cultivars remained active at 5×10^{-6} M primisulfuron. This indicates that sensitivity of the pepper cultivars to primisulfuron is a result of sensitivity of ALS from that plants to the primisulfuron.

Similar to this whole plant response, primisulfuron herbicide inhibited at the level of ALS enzyme. The I_{50} values for ALS inhibition by primisulfuron was determined for tolerant and susceptible cultivars. The ALS I_{50} concentrations were 5×10^{-6} to 8×10^{-6} and 3×10^{-7} to 5×10^{-7} M primisulfuron for tolerant and susceptible cultivars, respectively (Table 1). This indicates that ALS sensitivity of the tolerant and the susceptible cultivars differed by 10-15 fold. ALS from the tolerant cultivars was less sensitive to primisulfuron than that from the susceptible cultivars. This differential ALS enzyme activity contributes to an alteration in the target site in primisulfuron-tolerant pepper cultivars. These effects may be directly related to inhibition of ALS activity by primisulfuron. These results are consistent with the reports of ALS inhibitor-tolerant crops including maize, (Newhouse *et al*., 1991; Renner *et al*., 1988; Sander and Barrett, 1989), rice (Li *et al*., 1992), soybean (Kent *et al*., 1988; Sebastian and Chaleff, 1987; and Wixson and Shaw, 1991), tobacco (Chaleff and Ray, 1984; and Lee *et al*., 1988), wheat (Newhouse *et al*., 1992. In all of these cases, they found that the tolerance apparently was due to an alteration in the target site, insensitive ALS enzymes to ALS-inhibitor herbicides.

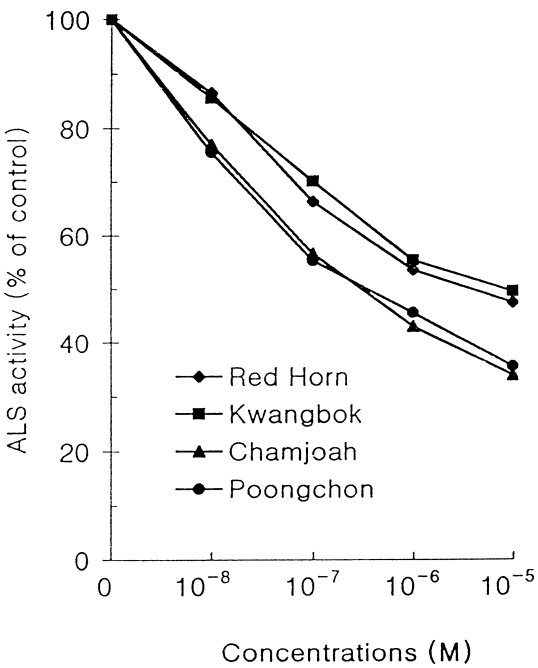

Figure 2 Inhibition of ALS activity by concentrations of primisulfuron ranging from 10^{-8} to 10^{-5} M in tolerant (Red Horn and Kwangbok) and susceptible (Poong chon and Chamjoah) cultivars.

Taken together, the data reported herein and from other published reports (Lee *et al*., 1988; Li *et al*., 1992; Newhouse *et al*., 1991; and Newhouse *et al*., 1992) suggested that the determination of ALS activity from the tolerant cultivars was less sensitive to ALS-inhibiting herbicides than ALS from the susceptible cultivars. Our experiments showed that tolerance to primisulfuron was considered by the high tolerance ratios for whole plant and ALS responses to the herbicide. With regard to tolerance ratios, GR_{50} tolerant/GR_{50} susceptible for plant height and dry weight, or I_{50} tolerant/I_{50} susceptible for ALS inhibition, respectively, were calculated to indicate the degree of tolerance. The tolerant cultivars, Red Horn and Kwangbok, were 3-fold more tolerant at the whole plant level than the susceptible cultivars, Chamjoah and Poongchon. Further more, concentrations of primisulfuron required for 50% in vitro inhibition of ALS activity

were 10-15 times greater for tolerant cultivasrs, compared to susceptible plants. It is reasonable to suggest that a less sensitive ALS enzyme confers this tolerance to these plants, particularly in the tolerant cultivars. Also, tolerance at the whole plant level is related to tolerance at the level of ALS enzyme to primisulfuron.

CONCLUSION

Response of pepper cultivars to primisulfuron was described at the whole plant and enzyme levels. The tolerant cultivars, Red Horn and Kwangbok, were 3-fold more tolerant at the whole plant level than the susceptible cultivars, Chamjoah and Poongchon. At the enzyme levels, ALS sensitivity differed by 10-15 fold between the tolerant and the susceptible cultivars. This suggests that the tolerance is due to an alteration at the target site insensitive ALS enzymes to primisulfuron, the ALS-inhibitor herbicide.

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