# Assessment of ALS-Inhibiting Herbicides Tolerance in Pepper Cultivars

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### ALS 저해형 제초제 내성 고추품종 검정 폰프롬 토사폰\* · 변종영\*\*

### ABSTRACT

Selection of pepper (*Capsicum* sp.) cultivars tolerant to acetolactate synthase (ALS)-inhibiting herbicides {imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridine-carboxylic acid, and primisulfuron methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino] carbonyl]amino]sulfonyl]benzoate} was investigated. Pepper cultivars such as Red Top, Happy Dry, Golden Tower, and Hagyeorae showed relatively tolerant response to imazethapyr, while cultivars; Korea, Cheongyang, Oriental Glory, and Hanam were susceptible. Red Horn, Jopoong, Kwangbok, and Wangcho cultivars were tolerant to primisulfuron whereas Korea, Dahhong, Chamjoah, and Poongchon cultivars were susceptible. Determination of growth inhibition by ALS-inhibiting herbicides showed that the I<sub>50</sub> estimates of growth from the susceptible- and tolerant-cultivars were 0.075 and 0.20kg ai/ha for imazethapyr; 0.06 and 0.16kg ai/ha for primisulfuron, respectively. Furthermore, the GR<sub>50</sub> estimates of growth from the susceptible and tolerant cultivars were 0.05 and 0.20kg ai/ha for imazethapyr; 0.07 and 0.16kg ai/ha for primisulfuron, respectively. This result, based on the GR<sub>50</sub> and I<sub>50</sub> values, indicates that responses of pepper to ALS-inhibiting herbicides between tolerant- and susceptible-cultivars were different about 3- to 4-fold to imazethapyr, and 2- to 3-fold to primisulfuron.

Key words: Acetolactate synthase, imazethapyr, primisulfuron, pepper, tolerance

### INTRODUCTION

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Development of crop cultivars tolerant to specific herbicides offers a favorable alternative to the

traditional approach of identifying and evolving new crop herbicides.<sup>2,9)</sup> Response of crop cultivars to herbicides depends on their inheritance within a plant species that enable the crop to escape the toxic effects of the herbicides. Some herbicides

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are not as selective as is desired on particular crops. However, careful germplasm screening within a crop species can potentially identify a genotype with herbicide tolerance. Generally, the presence of differential responses of crops cultivars to herbicides is seen as changes in plant growth or morphology following application of the herbicide.

Nowadays, acetolactate synthase (ALS, E.C. 4.1.3.18)-inhibiting herbicides, imidazolinones, and sulfonylureas, have become a major target of herbicide-tolerant crop plants development. The mode of action of these compounds is inhibition of ALS, also known as acetohydroxyacid synthase (AHAS), the first common enzyme in the biosynthesis of the essential amino acids; leucine, valine, and isoleucine in higher plants. Leucine, valine, and isoleucine in higher plants. Differential tolerance of ALS-inhibiting herbicides in plants is of interest, partly due to the finding that newer herbicides with high specific activity, including imazethapyr and primisulfuron, inhibit the activity of this enzyme.

Pepper cultivar tolerance, previously, the differential tolerance to herbicide within a plant species was recognized only between particular pepper cultivars to bentazon herbicide. 3,5,12,19,20) Although ALS-inhibiting herbicides, imazethapyr and primisulfuron, have not been evaluated for use in the pepper, both of these herbicides have the potential to control some troublesome weeds in pepper. To date, however, there is limited information on imazethapyr- and primisulfuron-tolerance in pepper cultivars. To assess the variability in ALS-inhibiting herbicides tolerance among cultivars, the objective of this study was to evaluate the tolerance of 42 pepper cultivars to postemergence applications of imazethapyr and primisulfuron under greenhouse conditions.

### MATERIALS AND METHODS

### Herbicide treatments

To determine herbicide tolerance to ALS inhibiting-herbicides, the treatment solution of the desired herbicide concentrations as prepared using commercial formulations of imazethapyr and technical grade primisulfuron (purity 97%). All treatments were applied at the postemergence, and included a non-ionic surfactant (X-77) (principal functioning agents-alkylaryl-polyoxy-ethylene, glycols, free fatty acids, isopropanol) at 0.25% by volume.

### General greenhouse procedures

Seeds of 42 different pepper cultivars were sown in plastic tray (surface area: 27×53cm²) containing a potting mixture of soil: vermiculite: peat (2:1:1 by vol). The trays were then planted and maintained in a greenhouse at 28 to 30℃ during the day and night, respectively. At ten days after emergence, seedlings were thinned to one per plot. After the pepper plants reached a 2- to 3-leaf stage, all herbicide treatments were applied with a CO₂-pressurized backpack handheld boom sprayer. The sprayer was calibrated to deliver 1,000 L/ha at 300 kPa.

Phytotoxicity was visually evaluated at 2 weeks after treatment (WAT) based on a 0 to 100 scale, where 0 = no injury, 30 = moderate foliar chlorosis with small necrotic lesions, 70 = severe necrosis, and 100 = death of plants. Subsequently, plant heights from the soil surface to the last fully expanded leaf were determined. At two WAT, the plants were harvested and shoot fresh weights recorded. The shoots were then oven dried at 80 °C for 48 hr and dry weights measured. Plant height, shoot fresh weight, and dry weight reduction was determined for each of the herbicide-treated plants by converting data to percent of

the corresponding control and subtracting from 100%. The study was repeated and the data were combined.

### Screening for tolerance to herbicides

A preliminary greenhouse experiment was conducted to determine the herbicide concentration necessary to cause differential responses among 42 pepper cultivars to imazethapyr and primisulfuron. Herbicides were treated with 0.05 and 0.10kg ai/ha for imazethapyr; 0.02 and 0.04kg ai/ha for primisulfuron, respectively. Plants were treated at one month after germination and harvested 2 weeks later using the previously described scale. Each treatment was replicated two times and untreated control was provided for comparison. The treatments were arranged in a completely randomized design with three replications.

### Growth response of pepper cultivars to herbicides

For the determination of growth inhibition by herbicides, this experiment was conducted to compare the different concentrations of herbicide to the pepper, presenting in the representatives of tolerant and susceptible cultivars from screening experiment. The seeds of two tolerant cultivars, Red Top and Happy Dry and two susceptible cultivars, Korea and Hanam for imazethapyr, and two tolerant cultivars, Red Horn and Kwangbok and two susceptible cultivars, Poongchon and Chamjoah for primisulfuron were planted as previously described procedure. Pepper plants were treated at the 2- to 3-leaf stage with imazethapyr at 0, 0.025, 0.05, 0.10, and 0.20kg ai/ha. Primisulfuron rates were 0, 0.01, 0.02, 0.04, 0.08, and 0.16kg ai/ha. Herbicide rates required to inhibit growth by 50% (I<sub>50</sub> value) for visual injury or to reduce growth by 50% (GR50 value) for plant height, shoot fresh weight, and dry weight were estimated for each replicate. The experiment was randomized completely with four replications.

### RESULTS AND DISCUSSION

### Screening for tolerance to herbicides

ALS-inhibiting herbicides can be very potent. broad spectrum herbicides. Two classes of herbicides, imidazolinones and sulfonylureas, are becoming widely used. So far no information is available on the evaluating tolerance of pepper cultivars to ALS-inhibiting herbicides, imazethapyr and primisulfuron. We attempted to assess the variability in imazethapyr and primisulfuron tolerance among cultivars of pepper. To estimate their susceptible and tolerance to herbicides, 42 pepper cultivars were applied at 0.05 and 0.10kg ai/ha rate of imazethapyr; 0.02 and 0.04kg ai/ha rate of primisulfuron, respectively. The plants were categorized as herbicide tolerant or susceptible based on the range and distribution of visual injury, plant height, shoot fresh biomass, and dry weight reduction data.

Under greenhouse screening tests, we have obtained ALS-inhibiting herbicides tolerance in pepper cultivars. The results of cultivars as affected by ALS-inhibiting herbicide treatments are presented in Table 1 and 2. Observation of pepper cultivars to imazethapyr showed that 4 cultivars represented the group of tolerance such as Red Top, Happy Dry, Golden Tower, and Hagyeorae, whereas 4 cultivars represented the susceptible group such as Korea, Cheongyang, Oriental Glory, and Hanam (Table 1). The observation of pepper cultivars to primisulfuron indicated that 4 cultivars showed relatively tolerant response to it such as Red Horn, Jopoong, Kwangbok, and Wangcho, whereas 4 cultivars were susceptible ones such as Korea, Dahhong, Chamjoah, and Poongchon (Table 2), respectively.

There is difference in their response to ALS-inhibiting herbicides among pepper cultivars.

Table 1. Visible injury, plant height, fresh weight, and dry weight of pepper cultivars tolerant and susceptible to 0.05 and 0.10kg ai/ha imazethapyr.

		Phytotoxicity <sup>b/</sup>		Plant height		Fresh weight		Dry weight	
Pepper cultivar	Rate (kg/ha)	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
			_		% of	control			
Red Top		20	30	65.42	56.45	64.56	60.64	76.74	65.09
Happy Dry		20	30	63.79	57.66	71.34	60.89	76.76	67.26
Golden Tower		20	30	60.09	50.13	63.29	60.96	77.97	64.11
Hagyeorae		20	30	59.64	50.91	66.96	64.23	78.12	66.22
Korea		45	55	50.70	48.14	47.51	23.07	52.50	38.25
Cheongyang		45	55	50.59	35.85	48.46	31.38	51.33	41.31
Oriental Glory		45	55	50.16	42.03	48.50	37.78	53.95	43.72
Hanam		45	55	51.19	48.96	46.00	28.71	53.77	43.19

a/ Nonionic surfactant (X-77) was included in all treatments at 0.25% by vol.

Table 2. Visible injury, plant height, fresh weight, and dry weight of pepper cultivars tolerant and susceptible to 0.02 and 0.04kg ai/ha primisulfuron.

		Phytoto	oxicity <sup>b/</sup>	Plant	height	Fresh	weight	Dry v	veight
Pepper cultivar	Rate (kg/ha)	0.02	0.04	0.02	0.04	0.02	0.04	0.02	0.04
			-		% o:	f control			
Red Horn		15	25	78.53	62.68	78.72	69.13	81.35	73.04
Jopoong		15	25	76.30	66.07	78.95	63.61	85.16	76.14
Kwangbok		15	25	75.26	66.57	80.38	66.13	82.30	77.50
Wangcho		15	25	78.79	72.73	80.20	64.28	85.34	70.29
Korea		35	45	61.21	54.46	69.32	55.74	66.95	59.46
Dahhong		35	45	63.97	55.79	68.76	56.73	70.75	59.33
Chamjoah		35	45	63.38	56.43	68.34	56.78	79.73	55.51
Poongchon		35	45	59.85	54.31	66.02	55.34	72.08	54.26

a/ Nonionic surfactant (X-77) was included in all treatments at 0.25% by vol.

Growth was inhibited by imazethapyr within a few hour after treatment, but injury symptoms appeared after 10 days or more. Meristematic areas became chlorotic, followed by a slow general foliar chlorosis and necrosis. When the tolerant plants were treated with imazethapyr at 0.05kg ai/ha, they showed 20% inhibition in plant growth and had plant heights, fresh weights, and dry weights equal to or greater than 60% of the untreated control. Whereas, the susceptible plants showed 45% inhibition in plant growth and had plant heights, fresh weights, and dry weights greater than 45% of the untreated control.

Furthermore, when the tolerant plants were treated with higher concentrations at 0.10kg ai/ha rate of imazethapyr, 30% inhibition was shown in plant growth and had plant heights, fresh weights, and dry weights equal to or greater than 50% of the untreated control. The susceptible plants, however, showed 55% inhibition in plant growth and had plant heights, fresh weights, and dry weights greater than 20% of the untreated control (Table 1).

Growth was rapidly inhibited by primisulfuron resulting in stunted plants. Foliar chlorosis began to appear within 5-7 days after treatment, followed by chlorosis of the growing point. At rates of

b/ Visual rating: 0 = no injury, and 100 = death of plants.

<sup>\*</sup> Plants were treated<sup>a/</sup> one month after germination and harvested 2 weeks later.

b/ Visual rating: 0 = no injury, and 100 = death of plants.

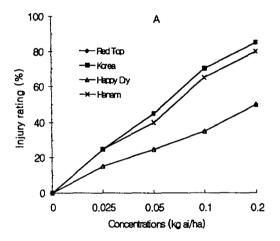
<sup>\*</sup> Plants were treated one month after germination and harvested 2 weeks later.

0.02kg ai/ha in primisulfuron, they showed 15% inhibition in plant growth and had plant heights, fresh weights, and dry weights equal to or greater than 75% of the untreated control. Whereas, the susceptible plants showed 35% inhibition in plant growth and had plant heights, fresh weights, and dry weights equal to or greater than 60% of the untreated control. In addition, when the tolerant plants were treated with higher concentrations at 0.04kg ai/ha rate of primisulfuron, they showed 25% inhibition in plant growth and had plant heights, fresh weights, and dry weights greater than 60% of the untreated control. The susceptible plants, however, showed 45% inhibition in plant growth and had plant heights, fresh weights, and dry weights greater than 50% of the untreated control (Table 2). These data suggest that the levels of tolerance to ALS-inhibiting herbicides differed with depending upon the herbicides and their concentrations. The phytotoxicity, plant height, shoot fresh weight, and dry weight were decreased as ALS-inhibiting herbicides concentrations increased. It is assumed that the effect of ALSinhibiting herbicides agrees with the influences of the herbicide on phytotoxicity, plant height, fresh weight, and dry weight of tolerant and susceptible cultivars.

## Growth response of pepper cultivars to herbicides

This experiment was performed to determine the approximate rate of ALS-inhibiting herbicides in their response to pepper cultivars that would reduce growth in each cultivar by 50% (GR<sub>50</sub> or I<sub>50</sub>) compared to untreated control plants. The pepper cultivars utilized in screening experiments, presenting in the representatives of the herbicideresponse cultivars, were again evaluated using imazethapyr-tolerant cultivars, Red Top and Happy Dry and -susceptible cultivars, Korea and Hanam; primisulfuron-tolerant cultivars, Red Horn and

Kwangbok and -susceptible cultivars, Chamjoah and Poongchon. For the determination of growth inhibition by various concentrations of herbicide, the growth response of pepper cultivars to ALS-inhibiting herbicides, as mentioned earlier, was determined at different concentration at 0, 0.025, 0.05, 0.10, and 0.20kg ai/ha for imazethapyr; 0, 0.01, 0.02, 0.04, 0.08, and 0.16kg ai/ha for primisulfuron, respectively. Based on visual injury, plant height, fresh weight, and dry weight, ALS-inhibiting herbicides tolerance varied considerably within pepper cultivars, suggesting the growth of



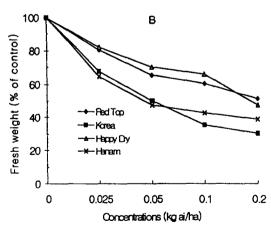


Fig. 1. Effect of imazethapyr on injury (A) and shoot fresh weight (B) of tolerant (Red Top and Happy Dry) and susceptible (Korea and Hanam) cultivars determined 10 days after herbicide application.

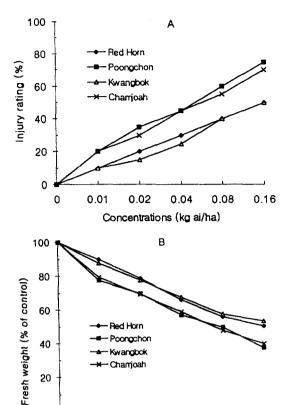


Fig. 2. Effect of primisulfuron on injury (A) and shoot fresh weight (B) of tolerant (Red Horn and Kwangbok) and susceptible (Poong-chon and Chamioah) cultivars determined 10 days after herbicide application.

0.02

0.04

Concentrations (kg ai/ha)

0.08

0.16

Kwangbok Chamioah

20

0

0

0.01

plants as inhibited according to the concentration of herbicide (Fig. 1 and 2). Moreover, there was greater difference in response between tolerantand susceptible-cultivars.

The rate of ALS-inhibiting herbicides that caused a 50% inhibition of growth (I<sub>50</sub>) was determined for each cultivar. The tolerant cultivars appeared to be clearly or more tolerant than the susceptible ones at imazethapyr over 0.075kg ai/ha. The susceptible cultivars were injured moderately (injury ratings greater than 30%) by imazethapyr at rate of 0.04kg ai/ha, while the tolerant cultivars were moderately injured by imazethapyr at rate of 0.08kg ai/ha. Furthermore, the susceptible cultivars were injured severely (injury ratings greater than 70%) by imazethapyr at rate of 0.15kg ai/ha and higher, while the tolerant cultivars were severely injured by imazethapyr at rates over 0.20kg ai/ha (Fig. 1A). This injury was expressed as severe foliar injury with subsequent deformity of new growth and greatly reduced growth of plants. These data suggest that two tolerant cultivars, designated as tolerant such as Red Top and Happy Dry, showed high tolerance to imazethapyr. However, the rate of decrease in plant height, fresh weight, and dry weight was greater with susceptible ones such as Korea and Hanam.

The tolerant cultivars appeared to be clearly or more tolerant than the susceptible ones at primisulfuron over 0.06kg ai/ha. The susceptible cultivars were moderately injured(injury ratings, greater than 30%) by primisulfuron at 0.02kg ai/ha, while the tolerant cultivars were moderately injured by primisulfuron at 0.04kg ai/ha. Moreover, the susceptible cultivars were severely injured(injury ratings, greater than 70%) by primisulfuron at 0.14 kg ai/ha and higher, while the tolerant cultivars were severely injured by primisulfuron at rates over 0.16kg ai/ha (Fig. 2A). These data suggest that two tolerant cultivars, designated as tolerant such as Red Horn and Kwangbok, showed high tolerance to primisulfuron. However, the rate of decrease in plant growth was greater with susceptible ones such as Chamjoah and Poongchon.

Since a considerable difference in tolerance to ALS-inhibiting herbicides exists between tolerant and susceptible of peppers, the rate required to reduce growth by 50% (GR<sub>50</sub>) or to inhibit growth by 50% (I<sub>50</sub>) was observed. At imazethapyr application (Table 3), the I<sub>50</sub> evaluates was 0.20kg ai/ha for tolerant cultivars and 0.075kg ai/ha for susceptible ones (Fig. 1A). In addition, the GR<sub>50</sub> estimates was 0.20kg ai/ha for tolerant cultivars, and 0.05kg ai/ha for susceptible ones, respectively (Fig. 1B). This indicates that the rate required to

Table 3.  $GR_{50}$  and  $I_{50}$  evaluates for the response of 4 pepper cultivars to imazethapyr.

Pepper cultivar	GR <sub>50</sub>	I <sub>50</sub>			
_	kg ai/ha				
Red Top	$0.20 \pm 0.01^{\text{a/}}$	$0.20 \pm 0.01$			
Happy Dry	$0.20\pm0.02$	$0.20 \pm 0.02$			
Korea	$0.05 \pm 0.03$	$0.075 \pm 0.01$			
Hanam	$0.05\pm0.02$	$0.075 \pm 0.01$			

a/ Presented data are mean estimates  $\pm$  the standard error of the mean.

**Table 4.**  $GR_{50}$  and  $I_{50}$  evaluates for the response of 4 pepper cultivars to primisulfuron.

Pepper cultivar	GR <sub>50</sub>	I <sub>50</sub>			
_	kg ai/ha —				
Red Horn	$0.16 \pm 0.005^{a/}$	$0.16 \pm 0.003$			
Kwangbok	$0.16 \pm 0.004$	$0.16 \pm 0.005$			
Chamjoah	$0.07 \pm 0.005$	$0.06 \pm 0.005$			
Poongchon	$0.07 \pm 0.010$	$0.06 \pm 0.003$			

a/ Presented data are mean estimates  $\pm$  the standard error of the mean.

reduce growth by 50% in the tolerant cultivars was 3- to 4-fold higher than that for susceptible ones. Cultivars Red Top and Happy Dry appeared to be equally tolerant to imazethapyr. Of the susceptible cultivars, Hanam was the most susceptible while Korea the least susceptible one. At primisulfuron (Table 4), the I<sub>50</sub> evaluates was 0.16kg ai/ha for tolerant cultivars and 0.06kg ai/ha for susceptible ones (Fig. 2A). Furthermore, the GR<sub>50</sub> evaluates was 0.16kg ai/ha for tolerant cultivars, and 0.07kg ai/ha for susceptible ones, respectively (Fig. 2B). This indicates that the tolerant cultivars were 2- to 3-fold more tolerant to primisulfuron than its susceptible ones. On the I50 or GR50 estimates of growth, similar results were obtained with bentazon suggested that the Iso values generally corresponded with the GR50 values. 5,12) In general, our results confirm previous studies indicating a degree of differential tolerance between susceptible and tolerant cultivars.

Newer herbicides with high specific activity are the imidazolinones and sulfonylureas. The

mode of action of these compounds is inhibition of ALS, the first common enzyme in the biosynthesis of the essential amino acids in higher plants. 2,9,10,11,16) In all of these cases, studies with plants selected for tolerance to both classes of herbicides have clearly demonstrated that ALS is the sole site of action. 1,4,7,8,10,11,16) In this study, growth of pepper cultivars from both tolerant and susceptible groups was inhibited as the ALSinhibiting herbicides concentrations increased. However, the response to herbicide treatment was depended on the cultivars treated. These effects may be directly or indirectly related to inhibition of ALS and/or may be entirely separated sites of action for ALS-inhibiting herbicides.

Early in the development of ALS-inhibiting herbicides, attempts to assess for tolerance through various selection protocols were successful in a wide range of species including maize, 1,10,13,14) rice, 8) soybean, 6,15,16,18) tobacco, 4,7) and wheat. 11) The results of this study confirm the findings of the previous paper, indicating the pepper cultivars vary greatly in tolerance to ALS-inhibiting herbicides, imazethapyr, and primisulfuron, and that selected cultivars tolerate to high ALS-inhibiting herbicide rates. This indicates that the differential response of pepper cultivars may be correlated with tolerance to the herbicide. Preliminary evidence shows that the selection of pepper cultivars tolerant to ALS-inhibiting herbicides was achieved. These data provide fundamental information of pepper tolerance to ALS-inhibiting herbicides, however, future investigation is needed to study the precise selection mechanism of the tolerant plants and the site of action of ALS-inhibiting herbicides.

### 적 요

ALS저해형 제초제 imazethapyr와 primisulfuron에 내성인 고추품종을 선발하기 위하여 42종

고추품종을 공시하여 고추의 약해, 초장, 생체 중 및 건물중을 측정하여 품종 반응을 조사하 였다.

- 1. 레드탑, 해피드라이, 골든 타워, 하교레 품종은 imazethapyr에 내성을, 코리아, 조양, 오리엔탈 글로리, 하남 품종은 감수성을 나타냈다. 레드흔, 조풍, 광복, 왕조는 primisulfuron에 내성을, 코리아, 다홍, 참조아, 풍천 품종은 감수성을 나타냈다.
- Imazethapyr에 대한 I<sub>50</sub>농도는 감수성 품종은 0.075kg/ha, 내성품종은 0.20kg/ha이며, primisulfuron의 경우, 감수성 품종은 0.06kg/ha, 내성품종은 0.16kg/ha이었다.
- Imazethapyr 처리에 따른 GR<sub>50</sub>을 감수성 품 종에서 0.05kg/ha, 내성품종에서 0.20kg/ha이 며 primisulfuron처리의 경우 감수성 품종은 0.07kg/ha, 내성품종은 0.16kg/ha이었다.
- 4. 따라서 ALS저해형 제초제에 대한 내수성품 종과 감수성 품종간 내성 정도는 imazethapyr 에서 3~4배, primisulfuron에서는 2~3배 차 이를 나타냈다.

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