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A second mutation enhances resistance of a tobacco mutant to sulfonylurea herbicides

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Summary. Cultures of *Nicotiana tabacum* cells homozygous for a mutation (*S4*) at the *SuRB* locus that confers resistance to the sulfonylurea herbicides chlorsulfuron and sulfometuron methyl (Chaleff and Ray 1984; Chaleff and Bascomb 1987) were used to isolate a doubly mutant cell line (*S4 Hra/S4* +) resistant to even higher herbicide concentrations. Growth of cells homozygous for both the *S4* and *Hra* mutations (*S4 Hra/S4 Hra*) was uninhibited by a herbicide concentration 500-fold higher than a concentration by which growth of *S4*+/*S4*+ callus was inhibited by 75%. Plants homozygous for both mutations were at least five-fold more resistant to foliar applications of chlorsulfuron than were singly mutant *S4*+/*S4*+ plants. This enhanced resistance was inherited as a single, semidominant, nuclear trait that is genetically linked to the *S4* mutation. Acetolactate synthase (ALS) activity in extracts of leaves of doubly mutant (*S4 Hra/S4 Hra*) plants was approximately 20-fold more resistant to inhibition by chlorsulfuron and sulfometuron methyl than was ALS activity in singly mutant (*S4*+/*S4*+) leaf extracts, which was in turn more resistant to inhibition by these compounds than was the normal enzyme. Extracts prepared from plants of these three genotypes possessed the same ALS specific activities. Therefore, *Hra* represents a second independent mutation at or near the *SuRB* locus that reduces the sensitivity of tobacco ALS activity to inhibition by sulfonylurea herbicides.

Key words: Herbicide resistance – Acetolactate synthase – *Nicotiana tabacum*

Introduction

Resistance to the sulfonylurea herbicides chlorsulfuron and sulfometuron methyl results from single semidominant mutations at either of two loci, *SuRA* and *SuRB*, in the nuclear genome of *Nicotiana tabacum* (Chaleff and Ray 1984; Chaleff and Bascomb 1987). One mutation, which was designated *S4* and which resides at the *SuRB* locus, results in production of an acetolactate synthase (ALS) activity with reduced sensitivity to inhibition by the two herbicides. Cosegregation of the herbicide-resistant phenotype with the altered form of ALS provided definitive evidence that inhibition of ALS is the herbicidal mechanism of the two sulfonylurea compounds (Chaleff and Mauvais 1984). In the present study, cultured cells homozygous for the *S4* mutation were subjected to further selection in the hope of recovering mutants exhibiting an even higher degree of herbicide resistance than that displayed by singly mutant plants. The genetic and physiological characterization of tobacco plants bearing a second mutation (*Hra*) that enhances by approximately five-fold the level of herbicide resistance expressed by plants homozygous for the *S4* mutation is now reported.

Materials and methods

Plant material and origin of mutants

The methods and media compositions employed for establishing and maintaining callus cultures of *Nicotiana tabacum* cv. Xanthi and for plant regeneration have been described previously (Chaleff and Parsons 1978). The isolation and genetic characterization of *S4/S4* mutant plants was described by Chaleff and Ray (1984). Concentrated solutions of chlorsulfuron and sulfometuron methyl (in 50 mM Hepes, pH 7.5) were sterilized by filtration and added to autoclaved medium to the final concentrations indicated. Media were buffered with 1 mM MES

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(pH 5.8). Androgenic haploid plants were recovered from anthers plated on a half strength Murashige and Skoog (1962) medium containing 3% sucrose, 0.1% activated charcoal (Anagnostakis 1974), and 1% agar.

Mutant cell lines with enhanced tolerance for sulfonylurea herbicides were selected by transferring callus cultures derived from *S4/S4* mutant plants to C1 agar medium supplemented with sulfometuron methyl at a concentration of 200 ppb (approximately 5.6×10^{-7} M). Growth of *S4/S4* cells is completely inhibited on this selective medium. Resistant colonies were propagated on selective medium for two additional three-week passages prior to regeneration of plants. One such highly resistant cell line (designated *Hra*) from which fertile plants could be regenerated was chosen for study.

Phenotypic characterization

Growth responses of callus cultures on herbicide-supplemented media were determined as previously described (Chaleff and Ray 1984). Effects of chlorsulfuron at the whole plant level were evaluated by spraying leaves of five week old plants with 5 ml of an aqueous solution containing the indicated concentration of herbicide, 0.2% Tween 20, and 20% (by volume) acetone. Shoot heights were measured after two weeks. Progeny of crosses were scored by germinating surface-sterilized seed on medium (Chaleff and Parsons 1978) containing 1 mM MES (pH 5.8) and 1 ppm sulfometuron methyl. Moderately resistant (*S4+/S4+*) and highly resistant (*S4 Hra/S4–*) individuals were readily distinguished by a comparison of shoot and root growth after an incubation period of two weeks.

ALS extraction and assay

Extracts were prepared from young leaves by the method of Chaleff and Mauvais (1984). However, a different buffer containing 100 mM potassium (pH 7.5), 10 mM DTT, 1 mM EDTA, and 0.1 mM sodium pyruvate was used in most extractions. The original buffer described by Chaleff and Mauvais (1984) was employed in some experiments as indicated. Total protein concentration and ALS activity were assayed as described previously (Chaleff and Mauvais 1984).

Metabolism studies

Petioles of young leaves were sliced under water and placed in 5 ml of Hoagland's solution containing approximately 0.77 μCi of [^{14}C-2-pyrimidine]sulfometuron methyl (specific activity 153 μCi/mg). After two hours petioles were transferred to herbicide-free Hoagland's solution. Radioactivity was extracted from leaves into 80% acetone at 2, 6, and 24 h after transfer to the herbicide-free nutrient solution. The acetone extracts were taken to dryness under a nitrogen stream. Residues were redissolved in 20% acetonitrile and analyzed with a DuPont Model 850 liquid chromatography system, equipped with a DuPont Zorbax-ODS column (6.2 mm × 25 cm). Following injection of 100–200 μl of sample, isocratic elution was performed with a mobile phase consisting of 36% acetonitrile, 64% water, and 0.1% formic acid. Column temperature was maintained at 35 °C and the flow rate at 1.4 ml/min. Radioactivity in the column eluate was measured with a radioactive flow detector (Radio Instruments & Chemical Co.).

Results

Genetic characterization

Plants regenerated from the highly resistant cell line (putative genotype *S4 Hra/S4+*) were backcrossed to the homozygous *S4+/S4+* progenitor for two successive generations to eliminate unrelated mutations that may have occurred in vitro. The putatively doubly mutant plant was used as the male parent to minimize transmission of chromosomal aberrations. The highly resistant phenotype (evaluated by growth responses of derivative callus cultures) was expressed by approximately half of the backcross progeny. Inheritance as a single, semidominant nuclear trait was confirmed by analysis of B_2F_1 progeny obtained from self-fertilization of a heterozygous highly resistant individual produced by the two backcrosses of a regenerated mutant plant. Of twenty B_2F_1 progeny scored by growth tests of derivative callus cultures, five possessed the intermediate level of resistance characteristic of *S4+/S4+* callus cultures, and fifteen clearly exhibited a higher degree of resistance. Testcrosses (data not shown) of the fifteen highly resistant B_2F_1 plants with *S4+/S4+* plants revealed that ten were heterozygous (i.e., both highly resistant and moderately resistant progeny were obtained from the testcross) and five were homozygous (i.e., all testcross progeny were highly resistant) for the *Hra* mutation. These results are in perfect agreement with the Mendelian ratio of 1:2:1 that is expected for segregation of a single, dominant, nuclear mutation.

Because the *Hra* mutation was isolated and subsequently maintained in a genetic background containing the *S4* mutation, the phenotype conferred by *Hra* in the absence of *S4* is unknown. Without knowledge of the phenotype (viz., *+Hra*), linkage of the two mutations cannot be analyzed by conventional crosses. For example, of fifty progeny tested that were produced by self-fertilization of a *S4 Hra/+ +* plant, 37 were resistant and 13 were sensitive to chlorsulfuron. But this observed segregation ¼ sensitive progeny would be expected either in the case of linkage or in the absence of linkage if *+Hra/+ –* individuals are not resistant to the herbicide. Similarly, a testcross of a plant heterozygous for both mutations yielded 26 resistant and 24 sensitive progeny. However, something other than 1:1 segregation could be expected only if *S4* and *Hra* assort independently and *+Hra/+ +* individuals are resistant.

Fortunately, the phenotype of *+Hra* segregants need not be known to determine the linkage relationship of the *S4* and *Hra* mutations by means of anther culture. In the case of linkage, a doubly heterozygous plant carrying the two mutations in coupling (*S4 Hra/+ +*) would produce gametes of only two genotypes and, therefore, would yield half highly resistant (*S4Hra*) and half sensitive (+ +) androgenic offspring. The appearance of *S4+* individuals, which possess an intermediate degree of herbicide resistance (and of *+Hra* individuals of unknown phenotype), would be expected in the case of independent assortment. Evaluation of the growth responses of callus cultures established from 51 androgenic plants

Table 1. Numbers of sensitive, moderately resistant, and highly resistant haploid plants obtained from cultured anthers of doubly heterozygous (*S4 Hra*/+ +) plants. Callus cultures established from androgenic haploid plants were tested for growth on media supplemented with various concentrations of sulfometuron methyl. Genotypes were assigned on the basis of growth responses of genetically defined diploid callus cultures. Diploid cells homozygous for both the *S4* and *Hra* mutations are capable of growth in the presence of 500 ppb sulfometuron methyl (highly resistant). Therefore, haploid cell cultures resistant to this herbicide concentration were presumed to be of the genotype *S4 Hra*. Similarly, because *S4*+/*S4*+ diploid cells grow in the presence of 5 ppb sulfometuron methyl, but not in the presence of 500 ppb of this herbicide, haploid cells exhibiting this moderately resistant growth response would have been presumed to be singly mutant. Growth of both haploid and diploid nonmutant cell lines is completely inhibited by sulfometuron methyl at 5 ppb

Putative genotype	Numbers of individuals expected if:		Numbers observed
	linked	unlinked	
S4 Hra	25.5	12.75	24
S4+	0	12.75	0
+*Hra* [1]	0	12.75	0
+ +	25.5	12.75	27

[1] Individuals of this genotype were not available because the *Hra* mutation was isolated in the presence of, and is genetically linked to, the *S4* mutation. Therefore, the phenotype of such individuals could not be determined

showed 27 to be sensitive and 24 to be highly resistant to inhibition by sulfometuron methyl (Table 1). The failure to recover individuals of an intermediate resistance phenotype is evidence of linkage between the *S4* and *Hra* mutations.

Phenotypic characterization

Growth responses to sulfometuron methyl of cell lines initiated from six genetically defined B_2F_1 progeny obtained by self-fertilization of a *S4 Hra*/*S4*+ B_2 plant are presented in Fig. 1. Growth of cell line 4, which is homozygous for both the *S4* and *Hra* mutations, was uninhibited by a herbicide concentration 500-fold above that which effected 75% growth inhibition of cell lines 7 and 11, which are homozygous for the *S4* mutation, but do not carry the *Hra* mutation. Cell lines 8 and 10, which are homozygous for the *S4* mutation and heterozygous for the *Hra* mutation, displayed an intermediate growth response. Therefore, *Hra* appears to be semidominant. Although the absolute values of the average final fresh weights of the doubly homozygous (*S4 Hra*/*S4 Hra*) cell line 19 grown in the presence of various concentrations of the herbicide were lower than the corresponding values obtained for cell line 4, the patterns of the responses of the two doubly homozygous mutant cell lines were similar (i.e., the mean final fresh weights of cell lines 4 and 19 were nearly the same in the presence of 1,000 ppb sulfometuron methyl as in the absence of herbicide). The reduced vigor of cell line 19, which is also evident during growth in the absence of herbicide, probably reflects segregation of variation at another locus unrelated to herbicide response.

The responses to chlorsulfuron of normal, singly mutant, and doubly mutant genotypes at the whole plant level are summarized in Fig. 2. Shoot growth of doubly mutant plants was inhibited 50% by foliar application of a concentration of chlorsulfuron more than 5-fold higher than the concentration that effected the same degree of inhibition of shoot growth of *S4*+/*S4*+ plants. Because shoot growth of normal plants was inhibited 50% by a chlorsulfuron concentration well below the range tested, quantitative comparisons with normal plants cannot be made on the basis of these experiments. Shoot growth of normal and of doubly mutant plants did not differ significantly in the absence of herbicide (Fig. 2). Normal, singly mutant, and doubly mutant plants were also morphologically indistinguishable. Therefore, there is no evidence that the *S4* and *Hra* mutations, either separately or in combination, have any deleterious effect on plant growth or vigor.

Biochemical characterization

ALS specific activities of extracts of leaves of normal plants (336 ± 32 nmol acetoin/mg protein/h), of plants homozygous for the *S4* mutation (368 ± 15 nmol acetoin/mg protein/h), and of plants homozygous for both the *S4* and *Hra* mutations (355 ± 34 nmol acetoin/mg protein/h) did not differ significantly. However, these activities did differ in their sensitivity to chlorsulfuron (Fig. 3). ALS activity in extracts of *S4*+/*S4*+ plants was less sensitive to inhibition by chlorsulfuron than was the activity in extracts of normal plants, as described previously (Chaleff and Mauvais 1984; Chaleff and Bascomb 1987). The activity present in extracts of doubly mutant *S4 Hra*/*S4 Hra* plants was even more resistant to herbicidal inactivation than was the activity in extracts of plants bearing only the *S4* mutation. Approximately a 20-fold higher concentration of chlorsulfuron was required to inhibit ALS acitvity in extracts of doubly mutant plants than to inhibit the activity in extracts of singly mutant plants to the same extent.

Sulfometuron methyl was metabolized at the same rate by leaves from singly and doubly mutant plants. At 24 h after removal of petioles from a solution of radioactive herbicide, the per cent of parent compound converted to an unidentified radioactive HPLC peak by *S4*+/*S4*+ and *S4Hra*/*S4Hra* leaves was 28.3 ± 6.2 and 25 ± 6.2 and 25 ± 6.6, respectively. During the same time period, 16.0 ± 2.1% of the radioactive parent compound

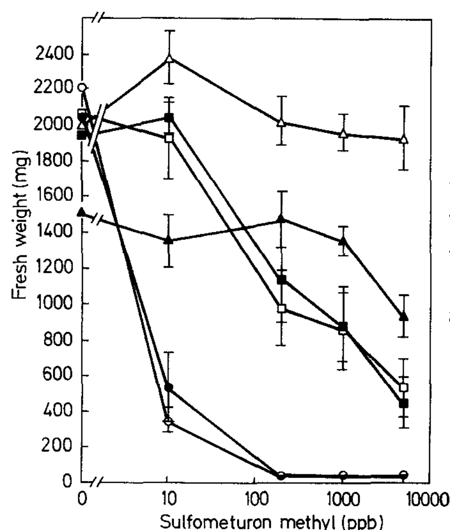

Fig. 1.

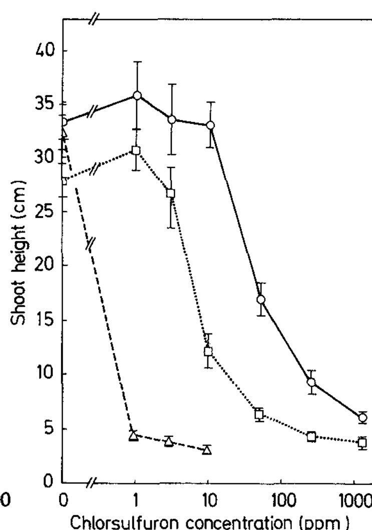

Fig. 2.

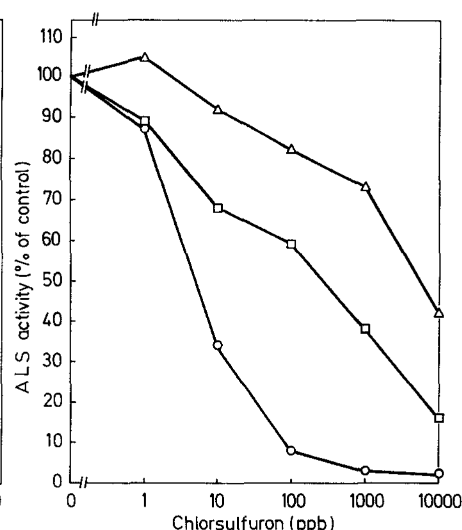

Fig. 3.

Fig. 1. Growth responses to chlorsulfuron of cell lines derived from progeny obtained by self-fertilization of an *S4 Hra*/*S4*+plant. Genotypes of the six cell lines (determined by progeny analysis of the plants from which they were established) were: cell lines o 7, • 11, *S4*+/*S4*+; cell lines □ 8, and ■ 10 *S4 Hra*/*S4*+; cell lines △ 4, and ▲ 19, *S4 Hra*/*S4 Hra;* symbols represent the means of at least 8 determinations, and bars indicate the standard errors of those means

Fig. 2. Shoot heights of normal (+ +/+ +; △——△), singly mutant (*S4*+/*S4*+; □·····□), and doubly mutant (*S4 Hra*/*S4 Hra;* o——o) plants 14 days after foliar application of chlorsulfuron at the indicated concentrations; each treatment consisted of seven plants; means and standard errors of the means are shown

Fig. 3. Responses to chlorsulfuron of ALS activities in extracts of leaves of normal (+ +/+ +; o), singly mutant (*S4*+/*S4*+; □), and doubly mutant (*S4Hra*/*S4Hra;* △) plants

Table 2. Inhibition of normal and mutant ALS activities by valine and leucine; ALS activities are expressed as mean per cent of uninhibited activity ± the standard error of the mean for three independent determinations

Additions	% of uninhibited activity	
	Normal (+ +/+ +)	*S4 Hra*/*S4 Hra*
0.1 mM leu+ 0.1 mM val	67.0±2.8	75.0±3.2
0.5 mM leu+ 0.5 mM val	43.6±3.0	49.5±3.7
5 mM leu+ 5 mM val	28.4±1.8	32.0±2.6
2 mM leu	80.0±6.2	74.5±3.0
5 mM leu	66.1±1.8	65.4±2.9

was metabolized by normal leaves. Extracts of all three genotypes contained only one peak of radioactive material other than the parent compound. This peak of unidentified material appeared at the same elution time in fractionations of all extracts.

ALS activities in leaf extracts of normal and mutant plants were assayed in the presence of leucine and valine to determine if the *S4* and *Hra* mutations affected the sensitivity of ALS to feedback inhibition by these amino acids. No significant differences was detected between the responses of ALS activities in normal and doubly mutant extracts to leucine and valine either separately or in combination (Table 2).

Discussion

In these studies a doubly mutant cell line expressing a very high degree of resistance to sulfonylurea herbicides was isolated from cultured tobacco cells bearing the previously isolated and characterized *S4* mutation (Chaleff and Ray 1984), which confers a moderate degree of herbicide resistance. Before being characterized, plants regenerated from the highly resistant cell line were backcrossed twice to eliminate unrelated genetic variation that may have accumulated during cell culture. Independently segregating mutations that moderate a primary mutant phenotype (Chaleff and Parsons 1978) and that alter both the relative and absolute amounts of two ALS isozymes (Chaleff and Bascomb 1987) have been described previously. Such extraneous genetic variation must be minimized through a backcross program before meaningful quantitative evaluation of the mutation of interest can be undertaken.

Because the parental singly mutant cell line was already fairly resistant to chlorsulfuron, sulfometuron methyl, to which the *S4+/S4+* mutant is more sensitive (Chaleff and Ray 1984), was employed in callus growth response tests to determine the degree to which resistance at the cell level was enhanced by the second mutation. Even with this more potent compound, inhibition of growth of one of two cell lines homozygous for both mutations was not observed at the highest herbicide concentration tested (5,000 ppb). The different behavior of the second doubly homozygous mutant cell line, which was inhibited at this highest herbicide concentration and which grew more slowly in the absence of herbicide, indicates that residual heterozygosity influencing both the rate of cell growth and the degree of herbicide resistance remained at other loci even after two backcrosses.

Like all other mutations that have been recovered by selection for resistance to sulfonylurea herbicides at the cell level (Chaleff and Ray 1984; Chaleff and Bascomb 1987), the *Hra* mutation is expressed by the whole plant. However, because the *Hra* mutation is genetically linked to, and has not yet been separated from, the *S4* mutation, the phenotype of *Hra* alone remains unknown. An attempt to measure the contribution of *Hra* to the phenotype of the double mutant was made by comparing the degree of resistance expressed by doubly mutant (*S4 Hra/S4 Hra*) plants with that of *S4+/S4+* plants. In these studies, shoot growth of the double mutant was inhibited 50% by more than a five-fold higher concentration of chlorsulfuron than that which affected the single mutant to the same degree. Unfortunately, a meaningful quantitative comparison between the herbicide responses of doubly mutant and normal nonmutant plants cannot be extrapolated from the available data. In the present studies, shoot growth of normal plants was inhibited almost completely by the lowest herbicide concentration applied. Although a 100-fold difference in chlorsulfuron sensitivities of normal and *S4+/S4+* plants was observed in earlier studies (Chaleff and Ray 1984), the dependence of plant response on growth conditions and developmental stage at the time of treatment makes unreliable any comparison between independent experiments. It is probably for these reasons that in those earlier investigations shoot growth of the *S4+/S4+* mutant was unaffected by treatment with chlorsulfuron at 100 ppm but, in the present studies, plants of this genotype were inhibited significantly by lower concentrations.

Sulfometuron methyl was metabolized at the same rate by leaves of singly mutant (*S4+/S4+*) and doubly mutant *S4 Hra/S4 Hra*) plants. Leaves of normal tobacco plants metabolized sulfometuron methyl at a slightly lower (less than two-fold) rate than did leaves of either mutant. However, it seems more likely that this difference was a result, rather than a cause, of resistance. In this study, sulfometuron methyl was metabolized so slowly by all three genotypes that a 24 h incubation period was required to measure a significant amount of conversion. Furthermore, the specific activity of the radioactive herbicide necessitated use of a very high herbicide concentration (ca. 1 ppm) in pulse-chase experiments. Therefore, the slightly lower amount of herbicide metabolized by nonmutant leaves could very well reflect a general decline in the metabolic activity of sensitive tissue as a result of herbicide toxicity. In plant species, such as wheat, that are naturally tolerant of chlorsulfuron, the herbicide is degraded with a half-life of only 2–3 h (Sweetser et al. 1982).

In *N. tabacum*, mutations conferring resistance to sulfonylurea herbicides and resulting in production of herbicide-insensitive forms of ALS arise at two unlinked loci, which presumably represent the structural genes of two ALS isozymes (Chaleff and Bascomb 1987). Therefore, it was anticipated that selection for enhanced resistance in an *S4/S4* genetic background would lead to recovery either of cells carrying multiple copies of the mutant *SuRB* allele as a result of gene amplification or of cells carrying a second mutation at the *SuRA* locus. Demonstration of genetic linkage between *Hra* and *S4* eliminated the latter of these possibilities as an explanation for the origin of the highly resistant phenotype. Gene amplification also seems an unlikely explanation because, although highly resistant plants apparently contain an increased proportion of resistant ALS activity, the specific activity of ALS in these plants is the same as the ALS specific activities of both *S4/S4* and normal plants. Moreover, the stability with which the *Hra* mutation is transmitted through crosses is not characteristic of gene amplification. Thus, it appears that *Hra* is a mutation residing either within or close to the *SuRB* locus. If within the *SuRB* gene, *Hra* could be a mutation that causes a further increase in the degree of herbicide resistance of a partially insensitive form of the enzyme encoded by the *S4* allele. If close by the *SuRB* gene, *Hra* could identify yet a third ALS structural gene, or a regulatory site controlling the relative amount of *SuRB* gene product made.

The *Hra* mutation causes a twenty-fold increase in the resistance of in vitro ALS activity to inhibition by chlorsulfuron (Fig. 3) and sulfometuron methyl (data not shown). Nevertheless, this mutation has no apparent effect on the response of ALS activity to leucine and valine, which are feedback inhibitors of the enzyme (Miflin and Cave 1972). However, for several reasons the validity of such measurements are at best uncertain. First, because the *Hra* mutation is linked to the *SuRB* locus, extracts of doubly mutant *S4 Hra/S4 Hra* plants must contain a normal form of the *SuRA*-encoded isozyme (unless expression of that isozyme is somehow suppressed by the *Hra* mutation), which, presumably, has different kinetic properties. Second, feedback inhibition

of ALS activity (at least as measured in vitro) is not absolute (Miflin 1971; Miflin and Cave 1972). Therefore, assays of crude extracts are attempting to measure an effect of either the *S4* or *Hra* mutation on a partial inhibition of only a fraction of the activity. Thus, although the results suggest that neither *S4* or Hra affects the feedback sensitivity of ALS, and, therefore, that sulfonylureas and allosteric effectors bind the enzyme at distinct sites, it seems most likely that anything less than the complete elimination of feedback sensitivity would not have been detected in these studies.

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References

Anagnostakis SL (1974) Haploid plants from anthers of tobacco: enhancement with charcoal. Planta 115:281–283

Chaleff RS, Bascomb NF (1987) Genetic and biochemical evidence for multiple forms of acetolactate synthase in *Nicotiana tabacum.* Mol Gen Genet 210:33–38

Chaleff RS, Mauvais CJ (1984) Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science 224:1443–1445

Chaleff RS; Parsons MF (1978) Direct selection in vitro for herbicide-resistant mutants of *Nicotiana tabacum.* Proc Natl Acad Sci USA 75:5104–5107

Chaleff RS, Ray TB (1984) Herbicide-resistant mutants from tobacco cell cultures. Science 223:1148–1151

Keil RL, Chaleff RS (1983) Genetic characterization of hydroxyurearesistant mutants obtained from cell cultures of *Nicotiana tabacum.* Mol Gen Genet 192:218–224

Miflin BJ (1971) Cooperative feedback control of barley acetohydroxyacid synthetase by leucine, isoleucine, and valine. Arch Biochem Biophys 146:542–550

Miflin BJ, Cave PR (1972) The control of leucine, isoleucine, and valine biosynthesis in a range of higher plants. J Exp Bot 23:511–516

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Sweetser PB, Schow GS, Hutchison JM (1982) Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. Pestic Biochem Physiol 17:18–23