# Semidominant Soybean Mutation for Resistance to Sulfonylurea Herbicides

S. A. Sebastian,* G. M. Fader, J. F. Ulrich, D. R. Forney, and R. S. Chaleff

## ABSTRACT

**A soybean [*Glycine max* (L.) Merr.] mutation conferring resistance to a wide range of sulfonylurea (SU) herbicides would greatly enhance the weed control options available to soybean farmers. This report describes the selection, characterization, and potential utility of such mutants. Seed mutagenesis (using N-nitroso-N-methylurea and ethyl methanesulfonate) followed by selection for resistance to chlorsulfuron [2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide] yielded a soybean mutant with a high degree of resistance to both postemergence and preemergence applications of a variety of SU herbicides. Resistance was monogenic, semidominant, and not allelic to any of the previously identified recessive genes *hs1*, *hs2*, or *hs3* that confer tolerance to SU herbicides. Biochemical tests indicate that the mechanism of resistance is reduced sensitivity of acetolactate synthase to SU inhibition. The SU resistance afforded by this mutation (designated *Als1*) can be used to enhance soybean weed control options and can serve as a selectable marker for seed purification.**

THE HIGH HERBICIDAL ACTIVITY and low mammalian toxicity (Beyer et al., 1988) of sulfonylurea (SU) herbicides have prompted commercial development of many SU-based herbicides. Although commercial SU herbicides offer a wide variety of weed control spectra, very few SU herbicides can be used on soybean due to crop sensitivity. A soybean mutation conferring resistance to a wide range of SU herbicides would greatly enhance the weed control options available to soybean farmers.

Although natural crop selectivity is based on differential metabolism of specific SU herbicides (Beyer et al., 1988), dramatic increases in SU resistance can be obtained from semidominant mutations that alter the sensitivity of the target enzyme, acetolactate synthase (ALS), to SU inhibition (Chaleff and Mauvais, 1984). Resistance can result from a specific substitution in the ALS amino acid sequence that renders the ALS enzyme insensitive to SU herbicides without destroying the enzyme's normal catalytic activity (Yadav et al., 1986). This type of mutation is very infrequent and has only been isolated by use of highly efficient selection systems, such as those utilizing cell culture (Chaleff and Ray, 1984). Currently, selection for soybean mutants at the cellular level is precluded by the absence of a totipotent regeneration system. Consequently, seed mutagenesis and subsequent selection among whole plants is a viable alternative. Previous screening (Sebastian and Chaleff, 1987) indicated that recessive mutations for increased SU tolerance could be isolated at fairly high frequency. Mutants 1-184A, 1-166A, and 1-126A were shown to contain nonallelic recessive mutations at the *Hs1*, *Hs2*, and *Hs3* loci, respectively. However, out of 50 000 $M_2$ soybean seedlings screened, no ALS-based dominant mutations conferring a high level of resistance to SU herbicides were found. Finally, after screening much larger $M_2$ populations, soybean mutants with semidominant, ALS-based SU resistance were isolated. This report describes the selection, characterization, and potential utility of such mutants.

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## MATERIALS AND METHODS

### *Mutagenesis*

Eight different $M_2$ populations were employed in the present study. These populations differed in either the parent used as starting material, the chemical mutagen used, or the soaking regimes used in the mutagen treatment. All three parental cultivars (Williams, Williams 82, and A3205) are agronomically acceptable but have SU-sensitive ALS activities. Mutagenic agents included ethyl methane sulfonate (EMS) and N-nitroso-N-methylurea (NMU). A detailed protocol for the generation of one $M_2$ population (A3205-EMS) is outlined to illustrate the general procedure.

Approximately 50 000 seeds (8.6 kg) of the cultivar A3205 were poured into a 50 L carboy filled with 45 L of tapwater to pre-soak the seeds. After 8 h of soaking under continuous aeration, the excess tapwater was drained and the swollen seeds were added to a second carboy containing 32 L of 25 mmol $L^{-1}$ EMS in 0.1 mol $L^{-1}$ $K_3PO_4$ buffer (pH 5.6). The seeds were soaked in the presence of the mutagen under continuous aeration for 3 h. Treated seeds were washed of exogenous mutagen by first draining the EMS solution and then filling and draining the carboy twice with 30 L tapwater. A third volume of 30 L tapwater was added to the carboy and retained as a postwash to soak the seeds for 7 h under continuous aeration. Following the postwash treatment, seeds were again rinsed with three batches of 20 L tapwater. After the final rinse, seeds were decanted onto flat cardboard sheets to drain. After drainage, the seeds were field-planted 2 cm deep in rows spaced 76 cm apart with a density of approximately 30 seeds per meter within the row. The resulting $M_1$ plants were allowed to reach maturity and produce $M_2$ seeds. The $M_2$ seeds were harvested in bulk and thoroughly mixed to randomly distribute the progeny of any given $M_1$ plant. Table 1 lists relevant deviations from the above procedure used to generate the other seven $M_2$ populations.

### *Selection of Resistant Lines*

Mutants 1-184A, 1-183A, 1-166A, and 1-126A (all contain single recessive mutations for chlorsulfuron tolerance), and 71 other soybean plants have been selected as SU-tolerant by soaking $M_2$ seeds in a chlorsulfuron [2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide] solution and then planting the seeds in untreated soil (Sebastian and Chaleff, 1987). Although this screen was successful, $M_3$ progeny testing showed that a large majority of such selections proved to be escapes or artifacts of the seed soak technique. Later, it was discovered that growth of wild type soybean plants (including Williams, Williams 82, A3205, and a wide variety of cultivars and plant introductions) could be uniformly inhibited by planting the seeds 2.5 cm deep in an inert, granular planting medium and then irrigating the medium (three to four times daily) with tapwater containing 100 $\mu g$ $L^{-1}$ chlorsulfuron. With this

**Table 1. Mutagenesis regimes and population sizes for eight different soybean $M_2$ populations.**

| Population† code | Mutagen dose | Exposure time | Postwash time | $M_1$ seeds treated | Estimated $M_1$ survivors | Estimated $M_2$ screened |
|---|---|---|---|---|---|---|
| | m*M* | h | | no. | | |
| A3205-EMS | 25 | 3 | 7 | 50,000 | 40,000 | 100,000 |
| Williams-EMS-A | 50 | 9 | 9 | 10,000 | 7,700 | 42,000 |
| Williams-EMS-B | 50 | 9 | 5 | 10,000 | 7,500 | 88,000 |
| Williams-NMU-A | 2.5 | 3 | 9 | 10,000 | 6,200 | 55,000 |
| Williams 82-NMU-A | 2.5 | 5 | 3 | 2,500 | 1,500 | 26,000 |
| Williams 82-NMU-B | 2.5 | 5.5 | 2.5 | 2,500 | 1,250 | 30,000 |
| Williams 82-NMU-C | 2.5 | 6 | 2 | 2,500 | 1,000 | 18,000 |
| Williams 82-NMU-D | 2.5 | 6 | 2 | 2,500 | 750 | 20,000 |

† Population code includes (parental cultivar), (mutagen), and (letter code for designated soaking regime); EMS = ethyl methane sulfonate; NMU = N-nitroso-N-methyl urea.

continuous chlorsulfuron exposure, wild type soybean lines emerged normally and opened cotyledons but failed to develop true leaves. Under the same conditions, SU-tolerant mutants, such as 1-184A, emerged, opened cotyledons, and formed clearly visible but deformed (narrow and crinkled) unifoliolate leaves. Although 1-184A plants could be consistently differentiated from wild type and rescued as early as 5 d after planting, they cold not develop past the unifoliolate stage under continuous exposure to 100 $\mu$m $L^{-1}$ chlorsulfuron. Based on previous experience with SU-resistant tobacco (*Nicotiana tabacum* L.) mutants (Chaleff and Ray, 1984; Chaleff and Mauvais, 1984), it was predicted that highly resistant soybean mutants (having ALS-based SU resistance) would grow normally, despite extended exposure to 100 $\mu$g $L^{-1}$ chlorsulfuron. Following this logic, the hydroponic technique was adopted to screen large $M_2$ populations in search of such mutants.

A total of approximately 400 000 seedlings from the eight different bulk $M_2$ soybean populations (Table 1) were screened for chlorsulfuron resistance using the described procedure. Using mutant 1-184A as a standard for comparison, putative mutant $M_2$ seedlings were selected based on their ability to form healthy (nondeformed) unifoliolates after approximately 8 d of exposure to chlorsulfuron. Putative mutants were then rescued from chlorsulfuron exposure, transplanted into untreated soil, and allowed to develop to maturity and to produce $M_3$ families. Selected plants and their selfed progeny were named with a code corresponding to their parental cultivar and to the chronological order in which they were selected. For example, mutant W20 was the 20th mutant selected from an $M_2$ population originating from the parental cultivar Williams. Within 1 wk after transplantation, it became obvious that some of the selected plants grew normally while others (including the 1-184A controls) were severely stunted and slow to recover from the chlorsulfuron treatment.

To confirm the phenotype of putative mutants, 30 $M_3$ seeds from selected $M_2$ plants were tested for chlorsulfuron resistance. In the $M_3$ test, seeds were again planted in an inert, granular medium irrigated with 100 $\mu$g $L^{-1}$ chlorsulfuron. However, developing seedlings were grown under continuous exposure to the herbicide to determine whether development would proceed past the stage of unifoliolate formation. It became immediately obvious that some of the $M_3$ families were either homogeneous or segregating for the ability to develop normal trifoliolates in the presence of a chlorsulfuron concentration that completely inhibited such growth of both wild type and the SU-tolerant line 1-184A. Subsequent testing indicated that the ability to develop healthy trifoliolates when grown hydroponically in the continuous presence of 100 $\mu$g $L^{-1}$ chlorsulfuron was a repeatable qualitative characteristic of certain mutant lines. In the context of this paper, the term resistance was used to denote such a striking lack of injury in response to herbicide exposure that was extremely injurious to both wild type and previously isolated SU-tolerant soybean lines (Sebastian and Chaleff, 1987).

Based on the $M_3$ progeny tests, six mutant lines derived from Williams (W4, W17, W19, W20, W23,and W28) were selected as being true breeding for this novel chlorsulfuron resistance phenotype. Because all six mutants were selected from a rather small (55 000 $M_2$ seeds) population (Williams NMU-A), it was suspected that most or all of them originated from the same $M_1$ plant. To help confirm this suspicion. W20 was crossed reciprocally with each of the other five mutants to obtain both $F_1$ and $F_2$ progenies. At least 3 $F_1$ and at least 60 $F_2$ progeny from each of these crosses were tested for chlorsulfuron reaction (using the hydroponic screen) and found to be uniformly resistant. This evidence supports the hypothesis that all six mutants contain allelic or closely linked mutations. Given their common origin, it is highly possible that all six lines trace back to the same mutational event. For this reason, mutant line W20 was used to represent this new class of soybean mutants in the following phenotypic characterizations.

### *Postemergence SU Resistance*

A postemergence treatment test was conducted to study the response of W20 to foliar applications of chlorsulfuron and two other SU herbicides. Mutant W20 was compared to a previously isolated SU-tolerant mutant 1-184A (Sebastian and Chaleff, 1987) and to wild type Williams 82. Williams 82 is a near-isogenic line of Williams (Williams[7] × Kingwa) that carries gene *Rps1*$^k$ for resistance to phytophthora root rot (*Phytophthora megasperma* f. sp. *glycinea*). As indicated previously, Williams 82 is indistinguishable from Williams in terms of reaction to chlorsulfuron (using the hydroponic screening method).

Pots (20-cm diam.) of each soybean line (2 seeds per pot) were planted using sterile peat-based potting mix. When the seedlings reached the second trifoliolate stage, plants were thinned back to one plant per pot. Each pot was then sprayed with a specific herbicide treatment using a conveyor belt spray apparatus to emulate field application. Control pots were sprayed with carrier only (920 mL $L^{-1}$ acetone, 40 mL $L^{-1}$ glycerine, 38 mL $L^{-1}$ water, and 2 mL $L^{-1}$ Tween 20) at a spray volume of 374 L $ha^{-1}$. The five herbicide treatments (delivered through the described carrier) included one rate of chlorsulfuron and two rates each of two other SU herbicides (DPX-M6316, and DPX-L5300). DPX-M6316 is the code name for methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate and DPX-L5300 is the code name for methyl 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-methylamino]carbonyl]amino]sulfonyl]benzoate. In commercial applications, SU herbicides are normally applied at rates of 4 to 35 g $ha^{-1}$ for postemergence weed control. Each treatment was replicated twice.

After herbicide treatment, plants were returned to the greenhouse and provided with ample light, moisture and

**Table 2. Injury scale for rating plants treated with postemergence applied sulfonylurea herbicides.**

| Rating | Visual symptoms |
|---|---|
| % injury | |
| 0 | No apparent injury compared to untreated controls. |
| 10 | Slightly stunted. |
| 20 | Noticeable stunting and/or slight reddening of pulvini. |
| 30 | Stunted with reddening of veins in treated leaves. |
| 40 | More stunting, reddening, and chlorosis/reddening of apex. |
| 50 | Stunting which reduces plant to 1/2 of the size of control plants, severe reddening and inhibition of apical bud. |
| 60 | Same as 50 but with partially necrotic apical bud. |
| 70 | Severe stunting and complete necrosis of apical bud. Recovery possible but plant will be very weak. |
| 80 | Severe stunting and necrosis of apex, upper leaves, and stem. Recovery doubtful. |
| 90 | Most of plant tissue is necrotic. |
| 100 | Plant is completely necrotic. |

nutrients to support healthy growth. At 7, 14, and 21 d after treatment, each pot was visually rated for percent herbicide injury according to the scale shown in Table 2. The three ratings per pot were averaged to obtain a single value for each pot. Analysis of variance on injury ratings was performed separately by herbicide. Fisher's least significant difference (LSD) was used to determine the significance of line mean differences at each rate. At 1 to 3 wk after herbicide treatment, 30% injury (according to Table 2) was considered the threshold between agronomically acceptable and unacceptable levels of soybean injury.

### *Preemergence SU Resistance*

At study was conducted to compare development of mutant W20 with that of wild type Williams 82 after preemergence application of chlorsulfuron and chlorimuron ethyl [ethyl-2-[[(4-chloro-6-methoxypyrimidin-2-yl)aminocarbonyl]aminosulfonyl]benzoate]. Williams 82 and W20 seeds were planted 2 cm deep in steam-sterilized Sassafras loamy sand [fine-loamy, siliceous, mesic Typic Hapludults) 0.8% organic matter, pH 6.7] in 18-cm-diam. pots. Six seeds of either line were planted per pot. One day after planting, the pots were sprayed with various herbicide treatments using a conveyer belt spray apparatus. Control pots were sprayed with carrier alone (500 mL $L^{-1}$ acetone, 458 mL $L^{-1}$ water, 40 mL $L^{-1}$ glycerine, and 2 mL $L^{-1}$ Tween 20) at a spray volume of 374 L $ha^{-1}$. Treatments (delivered through the same carrier) included seven rates of each herbicide (Fig. 1). In commercial applications, SU herbicides are generally applied at rates of 8 to 70 g $ha^{-1}$ for preemergence weed control. Each treatment was replicated six times. After spraying, the pots were arranged in randomized-complete blocks on greenhouse benches and each pot received 80 mL of water spread as uniformly as possible over the soil surface. Following this initial watering, pots received an adequate amount of water to sustain healthy plant growth. After viable seedlings had emerged (1 wk after spraying), random seedlings were pulled from each pot so that four plants remained per pot. This was done to equilibrate competition within pots and comparisons among pots. Nineteen days after spraying, visual estimates of percent foliar injury were recorded. The injury caused by the preemergence SU treatments was expressed mainly as stunting; very little vein reddening and leaf necrosis was observed. For example, an injury rating of 50% reflects a plant that was approximately half the size of the control plants. A single Fisher's LSD value ($\alpha = 0.05$) was used to compare line injury means at any given herbicide rate. Stunting of 30% was considered the threshold between agronomically acceptable and unacceptable levels of soybean injury.

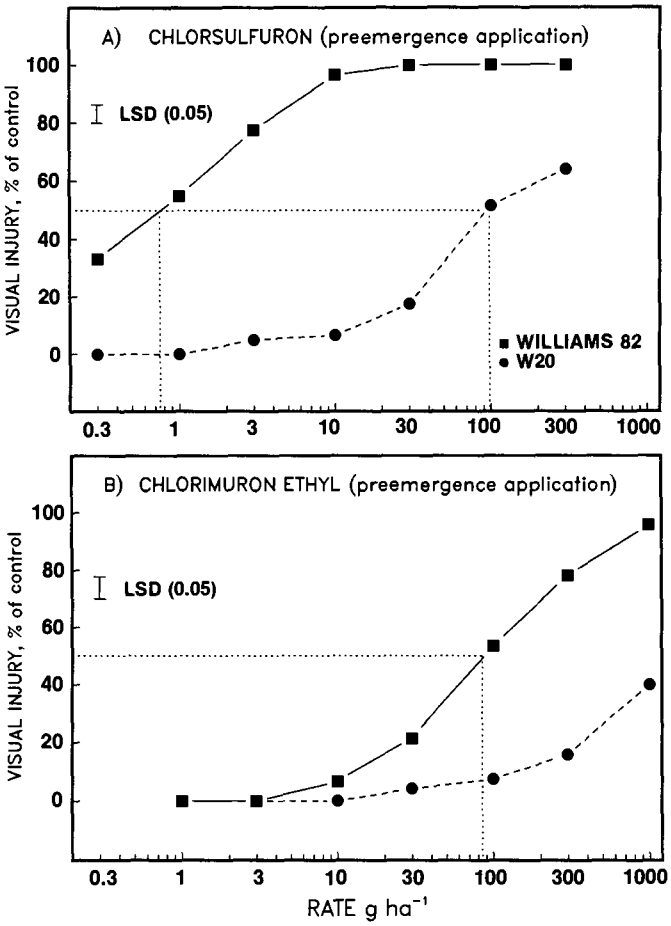


Fig. 1. Mean injury of Williams 82 and W20 soybean plants at 19 d after preemergence treatment with two SU herbicides (See Table 2 for description of % visual injury).

### *Genetics*

Mutant W20 was crossed with the SU-sensitive wild type line Williams 82 and with previously isolated mutants with recessive non-ALS based SU-tolerance at the *Hs1*, *Hs2*, and *Hs3* loci (Sebastian and Chaleff, 1987). To study the inheritance of W20's SU resistance, $F_1$ and $F_2$ plants, and $F_3$ families from these crosses were tested for reaction to 100 $\mu$g $L^{-1}$ chlorsulfuron using the described hydroponic procedure. After 11 d of chlorsulfuron exposure, each plant was classified as either resistant, tolerant, or sensitive to chlorsulfuron. Resistance was defined as the ability to form normal trifoliolate leaves. Tolerance was defined as the ability to develop unifoliolate leaves but no further shoot development. Sensitivity was defined as the inability to develop true leaves of any kind following emergence and cotyledon expansion.

### *SU Resistance at ALS Level*

The ALS assays were conducted to determine if W20 displays SU-resistance at the ALS enzyme level. ALS was extracted from leaves of W20 and Williams 82 soybean plants growing vegetatively. Two to three of the youngest fully expanded leaves from the upper nodes of plants (4–6 wk old) were harvested to obtain 2 g of tissue for homogenization. Leaf tissue was homogenized with a Polytron for 60 s in four volumes of buffer containing 100 mmol $L^{-1}$ HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid pH 7.5), 1 mmol $L^{-1}$ sodium pyruvate, 0.5 mmol $L^{-1}$ MgCl, 0.05

mmol $L^{-1}$ thiamine pyrophosphate, 10 $\mu$mol $L^{-1}$ FAD, 100 g $L^{-1}$ glycerol, 50 g $L^{-1}$ PVPP, and 2 g $L^{-1}$ mercaptoethanol. The homogenate was filtered through cheese cloth and centrifuged at 20 000 *g* for 20 min. The ALS was precipitated from the supernatant with ammonium sulfate. The enzyme was collected between 20 and 60% saturation by centrifugation at 20 000 *g* for 20 min. The pellet was resuspended in buffer containing 100 mmol $L^{-1}$ Hepes, 1 mmol $L^{-1}$ sodiumpyruvate, and 5 mmol $L^{-1}$ MgCl, and desalted on PD-10 columns (Pharmacia) equilibrated with the same buffer.

Assays were carried out in a final volume of 0.5 mL at 30 °C. The final reaction mixture contained 100 mmol $L^{-1}$ HEPES (pH 7.5), 1 mmol $L^{-1}$ MgCl, 60 mmol $L^{-1}$ sodium pyruvate, 0.4 mmol $L^{-1}$ thiamine-pyrophosphate, 40 mmol $L^{-1}$ FAD, and various concentrations of chlorsulfuron (Fig. 2). The assays were initiated with the addition of 100 $\mu$ L enzyme and terminated with the addition of 50 $\mu$L of 3 mol $L^{-1}$ $H_2SO_4$. The acidified reaction mix was heated at 60 °C for 15 min, after which 0.5 mL of creatine solution (5 g $L^{-1}$) and 0.5 mL of napthol solution (5.0 g $L^{-1}$ napthol in 2.5 mol $L^{-1}$ NaOH) were added. The reaction mixtures were heated for an additional 15 min at 60 °C, and acetoin was measured by reading the absorbance at 525 nm (Chaleff and Mauvais, 1984). All absorbances were converted to a percentage of control (no chlorsulfuron) basis to correct for any differences among extracts in the amount of enzyme present. All analyses were performed in duplicate. Data are expressed as the average of four individual plants per soybean line. A single Fisher's LSD value ($\alpha$ = 0.05) was used to compare line mean differences for ALS activity at any given herbicide concentration.

To confirm ALS resistance as the basis for whole plant SU resistance, cosegregation of enzyme and whole plant SU response was studied. Using the described ALS assay procedure, leaf extracts from 26 individual $F_2$ plants from the cross W20 × Williams 82 were assayed for ALS activity in the presence of 200 $\mu$g $L^{-1}$ chlorsulfuron. Data were expressed as percent of control (reaction mixture with no chlorsulfuron added) activity and were the average of two extracts from each $F_2$ plant. Approximately 30 $F_3$ progeny from each of the same $F_2$ plants were then screened hydroponically (as described previously) for chlorsulfuron resistance to determine the genotype of each $F_2$ plant. The $F_2$ plant genotype (based on $F_3$ progeny testing) was expressed as the number of resistant alleles (homozygous sensitive = 0, heterozygous = 1, homozygous resistant = 2). Using data from 26 random $F_2$ plants, ALS enzyme activity in the presence of chlorsulfuron vs. number of resistant alleles was plotted (Fig. 3) to determine if ALS resistance cosegregated with whole plant resistance. The mean ALS activity of four Williams 82 and four W20 plants were also plotted as parental controls.

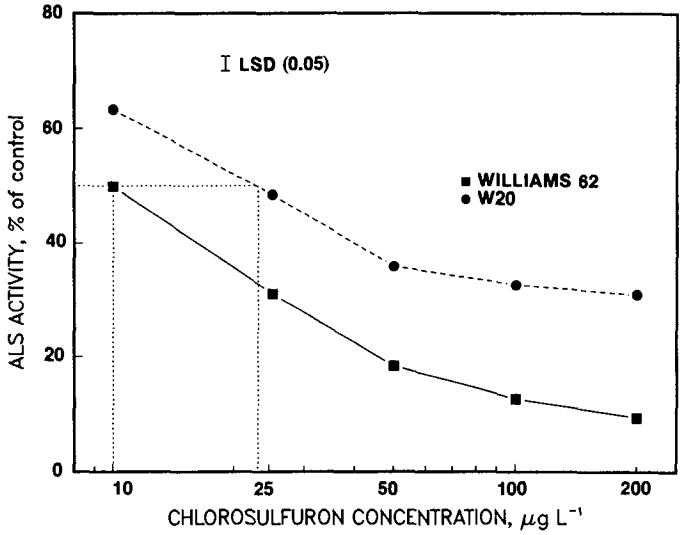


Fig. 2. Percentage of ALS activity remaining in wild type (Williams 82) and mutant (W20) leaf extracts at increasing concentrations of chlorsulfuron.

## RESULTS

In the postemergence treatment test, the relative injury (as compared to controls) remained fairly constant over the three rating intervals (7, 14, and 21 d after treatment). Hence, the mean of the three ratings (Table 3) was used to reflect the average injury observed during the 3-wk period following postemergence treatment with the SU herbicides. Experimental error was low (see LSD's in Table 3); two replicates were sufficient to document the striking difference between W20 and the other two lines. The mutant W20 (representative of the new class of soybean mutants) resisted postemergence rates of chlorsulfuron, DPX-M6316, and DPX-L5300 that severely injured or killed both Williams 82 and 1-184A. This dramatic resistance phenotype was suggestive of a mutation that changed the sensitivity of the ALS enzyme to SU herbicides (Chaleff and Mauvais, 1984; Yadav et al., 1986). Previously isolated mutant 1-184A was not significantly ($P \leq 0.05$) different from Williams 82 in terms of response to postemergence treatment of the tested SU herbicides (Table 3). Sebastian and Chaleff (1987) observed that 1-184A showed slight postemer-

**Table 3. Comparison of Williams 82, mutant 1-184A, and mutant W20 response to postemergence application of three sulfonylurea herbicides.**

| Herbicide | Rate | Williams 82 | 1-184A | W20 | LSD (0.05) |
|---|---|---|---|---|---|
| | g $ha^{-1}$ | % visual injury† | | | |
| Chlorsulfuron | 8 | 97 | 97 | 13 | 5 |
| DPX-M6316 | 8 | 13 | 13 | 0 | 11 |
| | 32 | 63 | 58 | 6 | 11 |
| DPX-L5300 | 8 | 92 | 90 | 8 | 6 |
| | 32 | 95 | 91 | 63 | 6 |

† See Table 2 for description. The % visual injury is the mean of three ratings.

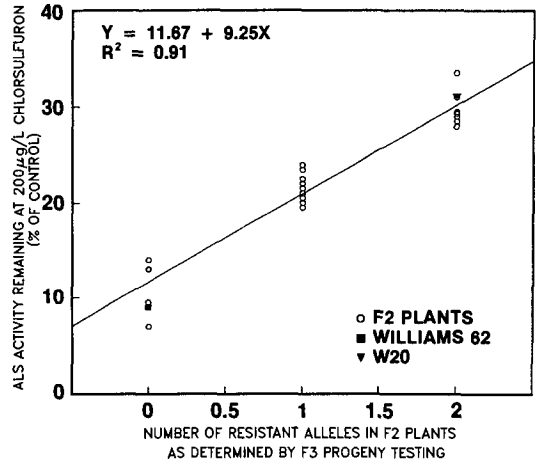


Fig. 3. Cosegregation of ALS resistance with genotype of $F_2$ plants from the cross W20 × Williams 82. Two data points at x:y coordinates 0:9.5, 1:21.5, 1:24.0, and 2:28.5; three data points at x:y coordinates 2:29.5.

gence SU tolerance but only at concentrations that were sublethal to wild type. At higher postemergence concentrations, the responses of Williams 82 and 1-184A converged and both showed serious injury.

Mutant W20 plants were resistant to preemergence applications of chlorimuron ethyl and chlorsulfuron that were extremely injurious to wild type Williams 82 (Fig. 1). By comparing the treatment required to cause 50% stunting of W20 as opposed to Williams 82, rough estimates of the safety margin provided by the W20 mutation could be calculated. For example, when compared with Williams 82, approximately 100 times as much chlorsulfuron was required to cause 50% stunting of W20. With chlorimuron ethyl, stunting of W20 never reached 50% even at the highest rate tested (1000 g $ha^{-1}$); however, 50% stunting of Williams 82 occurred at approximately 100 g $ha^{-1}$ chlorimuron ethyl. Hence, when treated preemergence, W20 withstood 100 times as much chlorsulfuron and at least 10 times as much chlorimuron ethyl as the wild type line.

Parents, $F_1$, $F_2$, and $F_3$ progeny from crosses involving W20 (Table 4) could be scored qualitatively in terms of their ability to grow hydroponically in the presence of 100 $\mu g\ L^{-1}$ chlorsulfuron. Although minor plant to plant variation was observed within parental lines, leaf formation within each line was consistent and served as a good indication of a plant's genotype. All W20 plants were able to form normal unifoliolate and trifoliolate leaves while Williams 82 did not form any leaf tissue. Only two distinct classes of $F_2$ segregants were observed; those similar to the W20 parent (resistant), and those similar to the Williams 82 parent (sensitive). Intermediate reactions were not observed at the tested concentration of chlorsulfuron. The $F_2$ segregation from the W20 × Williams 82 cross (Table 4) was not significantly different than the 3 resistant: 1 sensitive ratio expected for segregation of a single dominant allele conferring resistance. Segregation of $F_3$ families from the same cross was consistent with a 1 resistant: 2 segregating: 1 sensitive ratio expected for monogenic inheritance.

Previously isolated (Sebastian and Chaleff, 1987) mutants 1-184A, 1-166A, and 1-126A were uniformly tolerant (Table 4) to continuous chlorsulfuron exposure (able to form only unifoliolate leaves). These mutants were shown to carry recessive, non-ALS based tolerance at the *Hs1*, *Hs2*, and *Hs3* loci, respectively. If the SU-resistance mutation in W20 segregated independently of these three loci, one would expect the $F_2$ progeny from a dominant resistant × recessive tolerant cross to segregate 12 resistant: 1 tolerant: 3 sensitive (assuming that resistance was epistatic to tolerance). If resistance was allelic with any of the *hs* genes, one would expect no sensitive $F_2$ segregants from such a cross. Linkage would reduce the frequency of sensitive $F_2$ segregants to less than 3/16. At least 3/16 sensitive $F_2$ segregants were observed in all three crosses (Table 4) and two of the three crosses displayed a close fit to the theoretical 12 resistant : 1 tolerant: 3 sensitive ratio. The high proportion of sensitive $F_2$ segregants from the cross W20 × 1-184A caused a poor fit to the theoretical 12:1:3 ratio. This aberrant segregation was difficult to interpret as anything more than random deviation due to small sample size. Although $F_2$ segregation data from all three crosses fit a 3 resistant+tolerant: 1 sensitive ratio (when tolerant and resistant segregants were pooled), monogenic inheritance was not a logical conclusion. Qualitative transgressive segregation (nonparental sensitive segregants) indicated that more than one locus was involved in each of the resistant × tolerant crosses. The sensitive segregants clearly indicated that the resistance mutation in W20 is not allelic with any of the previously described *hs* genes. Because of the limited numbers of $F_2$ progeny screened, it was premature to conclude that the new locus defined by the W20 mutation is unlinked with *Hs1*, *Hs2*, or *Hs3*.

**Table 4. Chlorsulfuron reaction of parents, F1, F2, and F3 progenies form crosses between chlorsulfuron resistant mutant (W20) and chlorsulfuron sensitive (Williams 82) or chlorsulfuron tolerant mutants (1-184A, 1-166A, and 1-126A).**

| Line or Population | Resistant | Tolerant | Segregating $F_3$ Families | Sensitive | Chi-square value (ratio tested)† |
|---|---|---|---|---|---|
| | no. of plants or families | | | | |
| Williams 82 | 0 | | | 53 | |
| W20 | 29 | | | 0 | |
| 1-184A | | 14 | | 0 | |
| 1-166A | | 15 | | 0 | |
| 1-126A | | 14 | | 0 | |
| W20 × Williams 82 | | | | | |
| $F_1$ plants | 5 | | | 0 | |
| $F_2$ plants | 22 | | | 10 | 0.67 (3:1) |
| $F_3$ families | 10 | | 12 | 6 | 1.71 (1:2:1) |
| W20 × 1-184A | | | | | |
| $F_2$ plants | 19 | 4 | | 12 | 8.01*(12:1:3)<br>1.61 (3:1) |
| W20 × 1-166A | | | | | |
| $F_2$ plants | 18 | 2 | | 7 | 1.05 (12:1:3)<br>0.01 (3:1) |
| W20 × 1-126A | | | | | |
| $F_2$ plants | 24 | 0 | | 6 | 2.00 (12:1:3)<br>0.40 (3:1) |

* Significantly different than theoretical ratio at $\alpha = 0.05$.

† 3:1 (resistant + tolerant : sensitive) theoretical $F_2$ ratio assuming segregation of a single dominant gene. 1:2:1 (resistant : segregating : sensitive) theoretical $F_3$ family ratio for segregation of a single dominant gene. 12:1:3 (resistant : tolerant : sensitive) theoretical $F_2$ ratio assuming independent assortment of one dominant gene for resistance and one recessive gene for tolerance.

The ALS activity from leaves of W20 plants was less sensitive to chlorsulfuron inhibition than was ALS activity from leaves of Williams 82 (Fig. 2). At the highest chlorsulfuron concentration tested, 200 $\mu g$ $L^{-1}$, the percentage of ALS activity remaining in W20 extracts was threefold greater than the percentage of activity remaining in Williams 82 extracts.

The percentage of ALS activity remaining at 200 $\mu g$ $L^{-1}$ chlorsulfuron (Fig. 3) was highly correlated ($R^2$ = 0.91) with the number of resistance alleles present in $F_2$ plants from the W20 × Williams 82 cross. Leaf extracts from homozygous resistant plants had consistently higher levels of ALS activity in the presence of chlorsulfuron than did extracts from homozygous sensitive plants. In the presence of chlorsulfuron, ALS activity from heterozygous plants was consistently intermediate between the extremes observed for the two types of homozygotes. The cosegregation of ALS and whole plant resistance provided strong evidence that the whole plant SU resistance displayed by W20 is conferred by a SU-resistant form of the ALS enzyme. This study and previous studies (Chaleff and Mauvais, 1984; Yadav et al., 1986) confirm ALS as a primary site of action of SU herbicides. Although the whole plant hydroponics test indicated that resistance was dominant, enzyme assays provide clear evidence for a dosage effect (i.e., semidominance) of the resistance allele. Apparently, the hydroponic test was not stringent enough to differentiate heterozygous from homozygous resistant individuals.

The *Als1* (acetolactate synthase locus 1) is proposed as the gene symbol for the semidominant SU-resistant allele in W20. The symbol, *als1*, is proposed for the corresponding SU-sensitive wild type allele.

## DISCUSSION

The present study documents *Als1* as the first semidominant soybean mutation conferring resistance to SU herbicides. Seed mutagenesis has provided a viable alternative to soybean cell culture and transformation systems for introducing such a mutation into the soybean genome. Although more extensive field evaluations are necessary, preliminary phenotypic characterizations indicate that the *Als1* allele provides an agronomically useful level of resistance to a variety of postemergence and preemergence SU treatments. Field testing is also required to determine if *Als1* has any pleiotropic effects on agronomic performance. If commercialized, the development of SU-resistant soybean cultivars will greatly expand the utility of SU herbicides and provide the farmer with more options for weed control. SU resistance should also increase the safety margin for application of SU herbicides that are currently registered for use on soybean.

Because *Als1* is selectable in the heterozygous condition, it will also serve as a useful marker in genetic studies, breeding, and seed production. For example, when crossing a SU-resistant male with a sensitive female, the resulting $F_1$ hybrids will express the resistance trait. The undesirable selfed progeny of the sensitive plants can be removed from the hybrid population with a SU treatment that is selectively lethal to sensitive plants. The same treatment could be used to rogue sensitive plants from resistant populations that have been contaminated through careless seed handling operations. Currently, the use of other dominant markers (such as purple hypocotyls/flowers and tawny pubescence) requires careful visual inspection of each plant for expression of the marker and hand roguing of undesirable types. With semidominant herbicide resistance, large seed production fields can be chemically rogued by merely spraying the entire field with a selective herbicide treatment. Such chemical roguing would be particularly useful for commercial-scale purification of inbred lines or production of $F_2$ seed that is free of the selfed female parent.

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## REFERENCES

Beyer, E.M., M.J. Duffy, J.V. Hay, and D.D. Schlueter. 1988. Sulfonylurea herbicides. p. 117–189. *In* P.C. Kearney and D.D. Kaufman (ed.) Herbicides: Chemistry, degradation, and mode of action. Vol. 3. Marcel Dekker, Inc., NY.

Chaleff, R.S., and C.J. Mauvais. 1984. Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science (Washington, DC) 224:1443–1445.

Chaleff, R.S., C.J. Mauvais, and T.B. Ray. 1984. Herbicide-resistant mutants from tobacco cell cultures. Science (Washington, DC) 223:1148–1151.

Sebastian, S.A., and R.S. Chaleff. 1987. Soybean mutants with increased tolerance for sulfonylurea herbicides. Crop Sci. 27:948–952.

Yadav, N., R.E. McDevitt, S. Benard, and S.C. Falco. 1986. Single amino acid substitutions in the enzyme acetolactate synthase confer resistance to the herbicide sulfometuron methyl. Proc. Natl. Acad. Sci. 83:1–5.