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Development of a High Yielded Chlorsulfuron-Resistant Soybean (*Glycine max* L.) Variety through Mutation Breeding

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Abstract: This study was conducted to develop a novel herbicide resistance soybean using ethyl methanesulfonate (EMS) mutagen. In this study, 0.1% of EMS mutagen was applied to the soybean [*Glycine max* (L.) cv Arısoy] seeds. A single resistant mutant was selected in the M_2 population evaluated under field and greenhouse conditions. The AHAS gene regions of the herbicide-resistant mutant progeny were mapped, and the nucleotide changes were defined conferring herbicide resistance. The sequence analysis of the AHAS gene indicated that three nucleotide substitutions were detected such as 407 (C/T), 532 (C/T), and 1790 (C/T). According to the AHAS gene protein sequence of *Arabidopsis thaliana*, Ala155Val, Pro197Ser, and Thr616Met amino acid alterations were found in the progeny of the resistant mutant. Pro197Ser alteration was common in all the progeny, while the others were diverse. The wild-type and the mutant plants were compared for seed yield, number of pods per plant, stem height to the first pod, 1000-seed weight, and physiological maturity days for two subsequent years. No statistical difference was found between the mutant and wild types with respect to seed yield and its components. The agronomic data indicated that EMS provided target-site resistance to sulfonylureas (SU) with no tradeoff between yield components and resistance.

Keywords: AHAS gene; EMS mutagenesis; herbicide resistance; chlorsulfuron; *Glycine max*

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1. Introduction

Soybean (*Glycine max* L.) is one of the major oil crops, providing more than half of the world's oil crop production. It is an important food source in human and animal nutrition because of its high protein content, as well as essential amino and fatty acids. Fatty acids provide many health benefits such as preventing diabetes, heart disease, and arterial stiffness by decreasing cholesterol levels in the blood [1]. Further, they significantly decrease the risk of various cancers in humans with the soybean diet because of its highly valued phytochemicals such as lipids and phenolic acids [2]. Moreover, soybeans also contribute to soil fertility by nitrogen fixation with the help of symbiotic root bacteria [3].

Soybean cultivation is negatively affected by various abiotic and biotic stress factors involving less water, soil erosion, limited sunlight, viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds. Weeds can drastically reduce seed yield and quality in soybean production. Oerke [4] estimated that 37% of global soybean production decreased due to weed competition. On the other hand, Soltani et al. [5] stated that these losses in soybean reach up to 52%. Weed management can be achieved by many different methods such as preventative, cultural, mechanical, biological, and chemical methods. In developing countries, mechanical weed control is generally labor-intensive and costly. Although the chemical methods have different disadvantages, herbicide application is the most effective method globally. Herbicides have been a substantial component of weed management strategies since the 1940s [6].

Herbicide applications from the previous growing season can cause problems in different plant species and double-cropping systems. ALS-inhibiting herbicides are widely used for weeds control in wheat including mesosulfuron, chlorsulfuron combined with

metsulfuron, pyroxsulam, and propoxycarbazone [7,8]. Although SU herbicides are quite effective in controlling weeds in soybean fields, only a few SU groups are currently licensed for soybean weed management. Some studies have shown that chlorsulfuron applied in wheat causes a decrease in yield in second crop soybean after wheat [9]. This is mainly due to the sensitivity of soybean to these herbicides. The development of soybean varieties that are resistant to some of these herbicides may not reduce the yield of soybean planted after grains, and it may prevent the carryover injury to soybean that can result in yield losses.

The acetohydroxyacid synthase (AHAS) inhibiting herbicides are among the most preferred herbicidal groups for controlling weeds worldwide. Since the late 1980s, these herbicides have been regarded as practical tools in weed management due to their broad-spectrum weed control, persistent soil residual activity, low use rates, high margins of crop safety, and low toxicity to mammals [10]. Acetolactate synthase (ALS) (EC 4.1.3.18), also known as AHAS, is the responsible enzyme for the biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine) [11]. This enzyme is inhibited by group 2 herbicide families, including sulfonylureas (SU) triazolopyrimidines (TP), pyrimidinylthiobenzoates (PTB), sulfonlyaminocarbonyl-triazolinones (SCT), and imidazolinones (IMI) [12,13]. The AHAS-target herbicides suppress the synthesis of branched-chain amino acids, thereby leading to plant death resulting in amino acid inadequacy in herbicide-susceptible genotypes.

Previous reports revealed that there are two main resistance mechanisms related to resistance to AHAS inhibitors: target-site resistance (TSR) and non-target site resistance (NTSR) [14]. In general, TSR mainly occurs as a resistance mechanism by affecting the binding of herbicides due to a single amino acid change in the target enzyme. Target-site resistance (TSR) is due to the changes in the gene encoding the herbicide target protein that causes a reduction in the effectiveness of the direct effect of herbicide on its target [15]. However, NTSR mechanisms result from minimized herbicide uptake/translocation, enhanced metabolism, diminished rate of herbicide activation, and degraded herbicide [16,17]. Until recently, the detection of mutations in the target enzyme has emphasized importance in defining TSR mechanisms [18,19]. Other mutation points were also stated to provide resistance to group 2 herbicides, but the number of cases is very low compared to the other two (Pro197 and Trp574) mutation points [20].

Currently, 30 cases of resistance have been reported because of substitutions in the amino acid at eight positions in the AHAS gene region (Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654) [20,21]. Pro197 and Trp574 amino acid changes account for more than half of AHAS mutations [20]. While the amino acid mutation at Pro197 mainly provides resistance to SU group herbicides, the amino acid change of Trp574 offers resistance to both SU and IMI group herbicides [22].

Resistance to AHAS-inhibiting herbicides in many cultivated crops exemplified by corn (*Zea mays* L.), canola (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L.), and wheat (*Triticum aestivum* L.) has been developed by using various methods over the past few decades [23–27]. In most cases, resistance in plants was accomplished by amino acid changes in the AHAS gene.

In soybean, chromosomes (chr) 4, 6, 13, and 15 harbor the AHAS gene [28,29]. A study showed that the *Als*1 gene located on chr 4 shows resistance to SU herbicides due to an alteration at amino acids (Pro197) on this gene [28]. Another study indicated that mutations were identified in *Als*1 on chr 4 and *Als*2 on chr 6 (Pro197 and Trp574 in *A. thaliana*) genes [29]. Since these genes located on different chromosomes are unlinked, the combination of these two mutations provides increased tolerance to ALS-inhibiting herbicides.

EMS mutagenesis produces high point mutation, while very low rates of chromosome breaks can occur that cause aneuploidy, reduced fertility, dominant lethality [30]. These mutations achieve desired biotic and abiotic stress factors, herbicide resistance, yield, and quality, and they can also bring undesirable features together. Some researchers have noted that specific gene mutations conferring target site-based herbicide resistance have adverse pleiotropic effects on plant growth and vigor [31]. On the other hand, there have been some studies that have failed to detect any negative consequences of AHAS inhibitor resistance

on plant fitness [32,33]. AHAS inhibitor-resistant mutations have not been found to affect plant fitness due to lack of genetic background control [34]. This study will shed light on this uncertainty, since the genetic background was the same, and the differences were obviously caused by mutation.

The objectives of this work were to (i) develop chlorsulfuron-resistant (SU group) soybean mutants through EMS mutagenesis; (ii) confirm the resistance under field and greenhouse conditions; (iii) identify mutation points in the AHAS gene that confers SU resistance; (iv) determine agronomic potential of the mutant line.

2. Materials and Methods

2.1. Development of EMS-Mutated Populations

Ten thousand soybean seeds of Arısoy variety (*Glycine max* L.) were pre-soaked in tap water for two hours and then soaked in a 0.1% EMS (Sigma Aldrich, M0880-25, Taufkirchen, Germany) solution for nine hours. Seeds treated with the chemical mutagen were washed under tap water for three hours. All the treated seeds along with the wild type as a control (no treatment) were sown in the experimental field of Akdeniz University (36°53′ N, 38°30′ E and altitude 33 m) in Antalya, Turkey in 2018. Four thousand one hundred fifty M_1 plants were harvested and bulked to create three hundred thousand M_2 seed for the next planting.

2.2. Determination of Herbicide Effective Dose

A commercial herbicide named Hammer10 WP (Doğal Kimya Company, Antalya, Turkey), an herbicide classified as Group B2 according to its mechanism action, with 10% chlorsulfuron active ingredient was used. A range of application solutions (3, 7, 10, 13, 17, 20, 23, 27, and 30 g da^{-1}) were created using 10% a.i. chlorsulfuron. Three replications of each rate were applied to the Arısoy variety (*Glycine max* L.) using hand sprayer when the plants reached the V2–V3 stage. The suitable dose for the selection of herbicide resistance was determined as the lowest dose at which all plants died.

2.3. Detection of SU-Resistant Mutants

Three hundred thousand M_2 soybean seeds were planted in a 1200 m^2 experimental field with 70 cm row spacing in 2019. A 400-liter tractor sprayer with chlorsulfuron active ingredient was applied with a concentration of 10 g da^{-1} when the plants were at V2–V3 stages. Herbicide with the lowest dose of the herbicide to achieve full efficacy (10 g da^{-1}), a rate equivalent to 12 g (for 1.2 da), was used in this study. Observations were started to be taken 14 days after treatment. Following the herbicide application, symptomology included bruising on the main branch, chlorosis, necrosis, and stunting. Resistant plants, which are not affected by the herbicide application, were selected, while others were discarded.

To confirm the resistance, 10 seeds from the herbicide-resistant mutant named as AntSoy (sourced from the M_2 population under field conditions) and 10 wild-type Arısoy seeds were planted in 5 L pots and grown in the greenhouse. When the plants reached the stage of V2–V3, the lowest dose to achieve full efficacy (10 g da^{-1}) chlorsulfuron was applied in the greenhouse. The resistant progenies of AntSoy were also grown in a greenhouse to determine the resistance level at higher doses. For this purpose, 20 and 30 g da^{-1} (2× and 3×) herbicides were applied to the mutant progeny along with the wild type.

2.4. The AHAS Gene Analysis of the Mutant Progeny

Genomic DNAs from the SU resistant progeny and the wild-type Arısoy variety were extracted with the standard cetyltrimethyl-ammonium bromide (CTAB) method as previously described by Doyle and Doyle [35]. The quality and quantity of the extracted DNA from plant leaf tissue were determined by electrophoresis in 2% agarose gels with a DNA standard. The primer pairs used for the AHAS gene were previously designed by Ghio et al. [28]. The approximate size of the PCR fragments and the AHAS-specific primers are given in Table 1. PCR reaction templates were set up as follows: 2.5 µL of 10×

PCR buffer, 0.5 mM of the dNTPs mix, 2.5 mM of $MgCl_2$, 2 µL of each primer (10 pikamol), 1 U of Taq DNA polymerase (Thermo Scientific, Dreieich, Germany), 50 ng of genomic DNA template, and Milli-Q water to a final volume of 25 µL. In this study, PCR analysis was performed with a thermocycler (Bioneer, MyGenie™) under the following conditions: at 95 °C for 5 min then 40 cycles of 60 s each at 95 °C for template denaturation; 30 s at 55 or 60 °C for annealing (in according to primers); followed by 2 min at 72 °C for extension and a final extension at 72 °C for 5 min. Five µL of PCR products were analyzed with a 1 kb molecular weight marker (GeneRuler 1 kb DNA Ladder Plus, MBI Fermentas) by electrophoresis in agarose gels [1.5% (*w*/*v*) in 1× TBE pH 8.3 (Tris-acid boric-EDTA: 0.089 M Tris base, 0.089 mM boric acid, 0.002 M EDTA)] for 60 min to 80 V. The amplified PCR products were visualized using a gel imaging device (Genius brand), and then PCR products were cleaned and sequenced by Sanger Sequencing. DNA sequence information of the AHAS gene was analyzed with the Chromas 2.6.6 software (http://technelysium.com.au/wp/chromas/accessed on 15 April 2020). In addition, sequence information was aligned and transformed into protein sequences with the Mega-X program [36]. Protein sequence analysis was performed with the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo on 15 April 2020).

Table 1. Primers used to amplify the AHAS gene.

Primers	Sequence	Amplicon Size (bp)	Chromosome
GmAHAS1-F	ATGGCGGCCACCGCTTC	2041	4
GmAHAS1-R	ACAGGCCAAATCCTGCAACTAGGAC	2041	4
GmAHAS2-F	ATGGCGGCCACAGCTTCCAG	1936	6
GmAHAS2-R	TCAGTACCTCGTTCTACCGTCTCCCTCC	1936	6
GmAHAS3-F	TTTAGATTATTGTGGTATTGGAAGATG	2105	13
GmAHAS3-R	GAATATTTAGTACTAAAAGAAACCAACATC	2015	13
GmAHAS4-F	ACCTTTTGGTGCTATTTGAAAATG	2122	15
GmAHAS4-R	ACATATAATTAACAAAAATAACCAACATTG	2122	15

2.5. Comparison of Agronomic Characters between the Mutant and the Wild Type

The mutant and wild-type soybeans were grown in the experimental field of Akdeniz University during 2020 and 2021 growing seasons. All the experiments were designed as randomized complete block design (RCBD) with three replications, in which each plot consisted of four rows, with three meters in length and 70 cm apart. Agronomic characters such as seed yield, 1000-seed weight, plant height, stem height to the first pod, number of pods per plant, and number of days to physiological maturity were evaluated. For the yield data, plants from second and third rows (excluding peripheral one meter) were harvested, and the following formula was used to calculate yield/da;

$$\text{Yield (kg/da)} = \frac{1000\ \text{m}^2}{\text{harvested length of the row (2 m)} \times \text{row spacing (0.7)}} \times 1.4\ \text{m}^2\ \text{yield}$$

where the 1.4 m^2 yield is the amount of harvested soybean from two rows.

Five plants from the center two rows of each block at maturity (R8) were randomly selected to measure 1000-seed weight, plant height, stem height to the first pod, number of pods per plant, and physiological maturity days (past days from planting to harvesting time). Plant height was calculated from the ground level base of the stem to the tip of the top pod.

3. Results

3.1. Screening for the Resistance under Field Condition

At the V2–V3 stages of the M_2 plants, the herbicide was sprayed to the plants with a 10 g da^{-1} concentration, which was 33.3% higher than the manufacturer's recommendation.

It was first observed that the growing tips of the plants stopped within a few days after herbicide application. In the later stages, it was progressed as darkening on the plant stem, bruising, and necrosis spots in the leaf veins. Then, this blackening continued towards the side branches and leaves. In the last stage, the leaves and plants dried entirely and caused the death of the plant. Selection for the resistance was started 14 days after the application and was maintained regularly throughout the vegetation. The field observation showed that approximately 98% of germinated plants dried quickly after herbicide application. In 2% of the germinated plants, the growing tips of plants stopped within the first few days after herbicide application, but later the plants managed to grow side branches to resume growth. According to the observations taken under field conditions, only one mutant was selected with showing no herbicide symptoms and named as AntSoy. Thirty seeds were harvested from the AntSoy plant. Except for this resistant mutant, some plants showed minimal herbicide injury and were immediately recovered by suppressing herbicide damage. However, these recovered mutants were ignored as the main aim was TSR.

3.2. Confirmation Tests under Greenhouse Conditions

Of the ten seeds planted from the putative mutant and the control, eight AntSoy and all the Arısoy plants germinated and reached the V2–V3 stage. Of those, six were detected as herbicide resistant. Two plants were damaged from herbicide in the greenhouse. These six putative mutant plants were re-tested by the second herbicide application three weeks after the first herbicide application and continued to be free of herbicide injury symptoms. Leaf samples of six herbicide-resistant plants and two wild-type Arısoy plants were taken for molecular analyses. Although the herbicide dose was increased to 2X and 3X, no herbicide damage was observed on the resistant plants. On the other hand, the destructive effect of herbicide damage increased depending on the herbicide dose in the wild-type Arısoy variety (Figure 1).

Figure 1. (**a**) 15 days after herbicide applied susceptible (dried plants in front) and resistant plant (no symptoms); (**b**) 30 days after herbicide applied susceptible (more dried) and resistant plant (still no symptoms).

3.3. Identification of SNPs in the AHAS Gene

The gene controlling SU resistance was amplified with PCR for the mutant progeny. The nucleotide sequences of the soybean AHAS genes were successfully obtained, and the mutation points were detected using the Chromas program (Figure 2). The AHAS gene sequences were aligned with those of the resistant and susceptible controls (KC254821.1

and KC254825.1,) respectively, according to Ghio et al. [28]) by using the MEGA-X program (Figure 3). No difference was found between herbicide-resistant and sensitive in the sequence of the AHAS genes on chr 6, 13, and 15. However, a total of three SNPs were identified in the full-length coding sequence of the AHAS gene on chr 4, which were detected and resulted in distinct amino acid substitutions at different positions in herbicide-resistant plants. The changes at nucleotide points were 407 (C/T), 532 (C/T), and 1790 (C/T), respectively. The mutation points were numbered in parallel with Ghio et al. [28]. According to the AHAS gene protein sequence of the *Arabidopsis thaliana* model plant, three amino acid changes were detected due to these three SNPs, Ala155Val, Pro197Ser, and Thr616Met, respectively, and one was consistent with Ghio (Pro197Ser), but the other two were novel. (Figure 4).

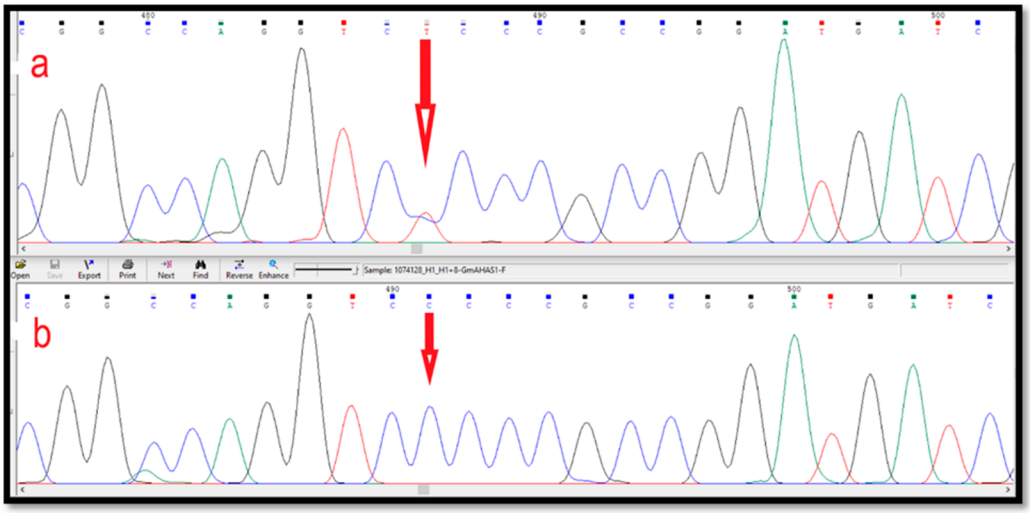

Figure 2. Mutation detected at point 532 that causes Pro197Ser alteration (**a**) The resistant mutant, AntSoy (TCC); (**b**) Wild-type, Arısoy (CCC).

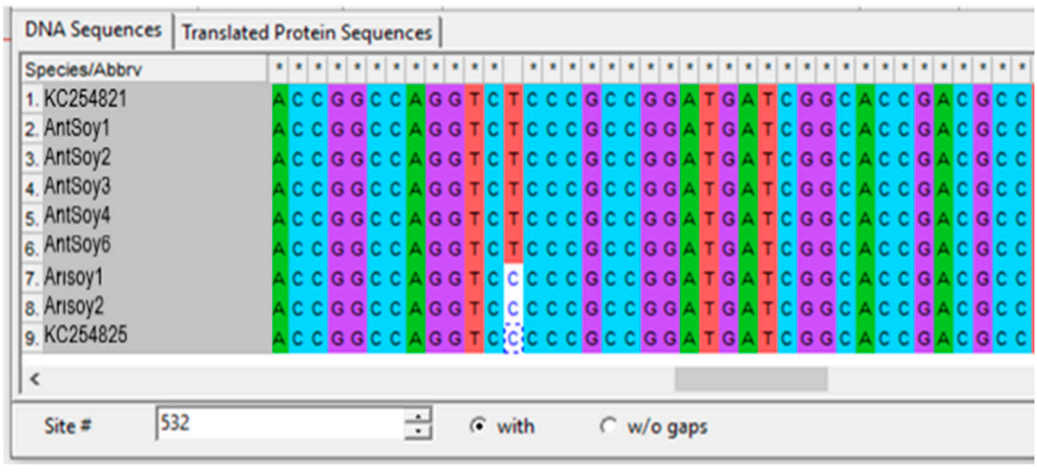

Figure 3. Alignment image made with Mega-X program. KC254821 (1) and KC254825 (9) resistant and sensitive plant reference sequence, respectively. 2, 3, 4, 5 and 6 were the progeny of AntSoy. 7 and 8 were herbicide sensitive Arısoy variety (the wild type).

3.4. Comparison of Agronomic Characters between the Mutant and the Wild Type

Six quantitative traits of the wild type and the mutant were compared under field conditions (Table 2). The yield of the wild type was calculated as 340.87 and 353.97 kg da^{-1}, whereas the mutant yield was recorded as 341.93 and 351.21 kg da^{-1} in 2020 and 2021 growing seasons, respectively. The slight seed yield differences between these two geno-

types were statistically insignificant (Table 3). The 1000-seed weight of the wild type was measured 109.1 and 110.43 g in 2020 and 2021 years, respectively, and the two-years average of this trait was calculated as 109.76 g. The same character of the mutant was determined as 96.7 and 99.4 g in 2020 and 2021 years, respectively, and the two-years average of the trait was found 98.07 g. Although 1000-seed weight is one of the yield components, it did not affect the total yield and this difference between two genotypes was statistically insignificant. The mean number of pods per plant ranged from 47.33 to 54.6 in this study. In the average of two years, the number of pods per plant was found to be 50.1 in the wild type and 51.5 in the mutant. The first pod height was recorded in the wild type with a value of 19.07 and 21.4 cm, while the mutant had the first pod height 20.73 and 22.06 cm in 2020 and 2021 growing seasons, respectively. The highest mean of plant height was found in the wild type with a value of 99.33 cm in 2022, while the shortest mean plant height was the wild type with 92.4 value in 2020. In the average of plant height measurements in two years, the mutant gave slightly higher values than the wild type. Physiological maturity days were measured as 120.67 in the first year and 117.0 in the second year in the wild type. In the mutant type, the same feature was measured as 121.33 and 118.66, respectively. When the two-year averages of these two types were compared, the mutant type was found to be approximately 1.5 days longer which was statistically insignificant. According to the analysis of variance, there were no significant differences between the wild type and the mutant for yield components such as seed yield, 1000-seed weight, number of pods per plant, the first pod height, plant height, and physiological maturity.

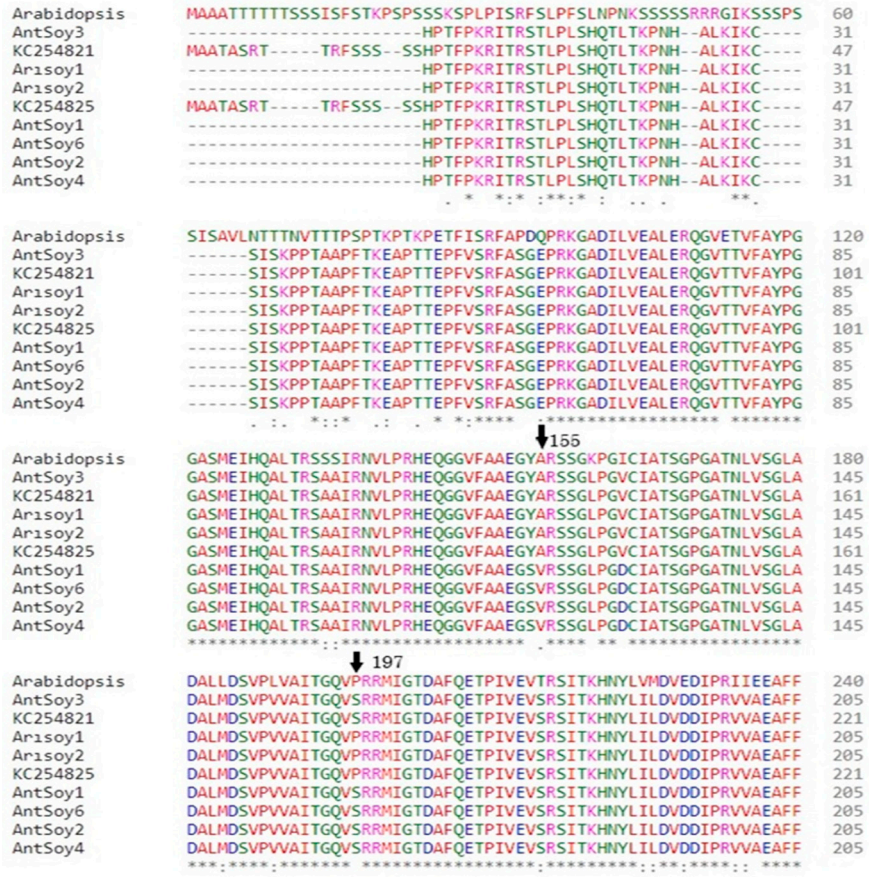

Figure 4. *Cont.*

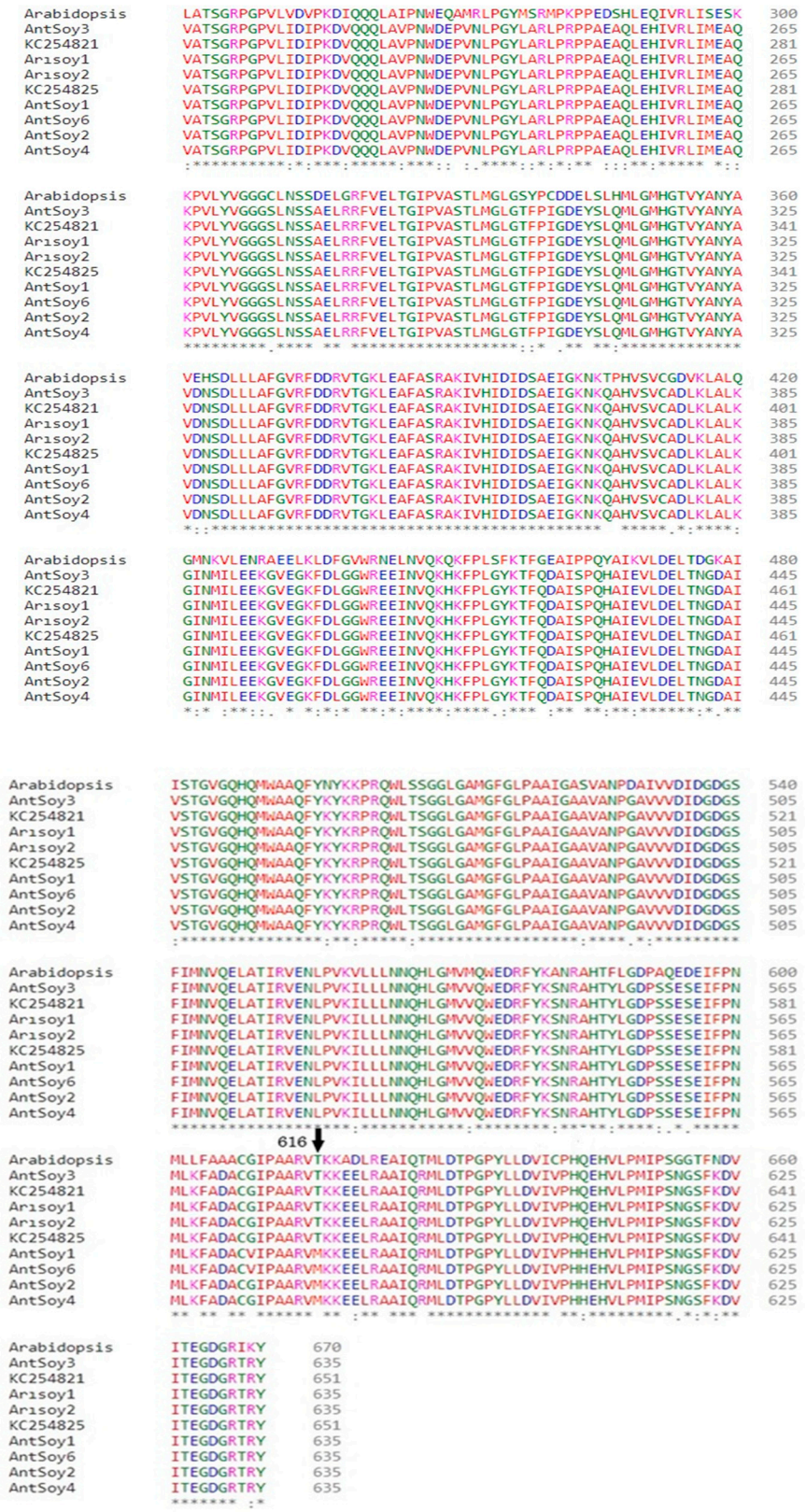

Figure 4. Protein sequence analysis amplified with the GmAHAS1 primer and made with the Clustal Omega program; *Arabidopsis thaliana* reference plant; KC254821, herbicide resistance soybean plant; KC254825 herbicide susceptible soybean plant; Progeny of herbicide resistant soybean mutant AntSoy 1, 2, 3, 4, 6; herbicide sensitive wild-type Arısoy1, 2 variety (arrows indicate with amino acid change points).

Table 2. The agronomic comparison between the wild type and the mutant for the growing years of 2020 and 2021.

Characters/Years	Wild-Type			AntSoy		
	2020	**2021**	**Average**	**2021**	**2022**	**Average**
Yield (kg da^{-1})	340.87	353.97	347.43	341.93	351.21	346.57
1000 seeds weight (g)	109.1	110.43	109.76	96.7	99.4	98.07
Number of pods per plant	47.33	53.0	50.16	48.47	54.6	51.53
First pod height (cm)	19.07	21.4	20.24	20.73	22.06	21.40
Plant height (cm)	92.4	98.73	95.56	97.07	99.33	98.2
Physiological maturity (day)	120.67	117	118.56	121.33	118.66	120.0

Table 3. The analysis of variance of agronomic characters.

Genotypes	Sum of Squares	Mean Square	F Ratio	Prob > F	
Yield	2.20	2.20	<0.001	0.99	NS
1000 seeds weight	410.67	410.67	3.20	0.10	NS
Number of pods per plant	5.60	5.60	0.06	0.81	NS
First pod height	4.08	4.08	1.66	0.24	NS
Plant height	3.85	3.85	0.13	0.73	NS
Physiological maturity	6.16	6.16	1.40	0.26	NS

NS: not significant.

4. Discussion

Plants can develop target-site resistance and/or non-target-site resistance mechanisms against AHAS-inhibiting herbicides. Target-site resistance is a mechanism that occurs by preventing the herbicide binding to the target enzyme due to a mutation in the AHAS gene in the herbicide binding site [15,37]. Many scientists have studied the AHAS gene extensively in many plants. Plants may have a variable number of AHAS gene copies depending on the level of ploidy, and these genes with multiple copies could be more complex than those with one gene [25]. In the previous study, four AHAS genes were found on different chromosomes in soybean, and separate primers were designed for each [28]. This study supports the previous research by replicating four other AHAS genes using the primers. While Ghio et al. (2013) found mutations only in the *Als1* gene on chromosome 4, Walter et al. (2014) detected mutations in the *Als1* and *Als2* genes on chr 4 and 6, respectively. Despite the possibility of mutation occurring in the *Als1* and *Als2* genes on chr 4 and 6, only mutations in the AHAS (*Als1*) gene on chromosome 4 was detected in that one was consistent with Ghio (Pro197Ser), but the other two were novel. In three studies on AHAS-inhibiting herbicide resistance in soybean, no mutation was seen in the AHAS genes on chr 13 and 15. It raises the question whether transformation does not occur in these genes due to conserved genes or lack of functionality of these genes in the plant.

Until now, eight different mutation points in the AHAS genes have been associated with mechanisms of resistance to AHAS inhibitors. The Pro197 mutation point is the most detected one, providing SU group herbicides resistance [22]. This mutation (Pro197Ser) has been identified as the basis for AHAS-inhibition resistance in several species, such as soybean [28,29], rapeseed [38], and several weed species [20]. The study by Ghio et al. (2013) revealed that the sulfonylurea-resistant line has a serine residue (TCC) at position 197 (refer to *A. thaliana*). In contrast, the herbicide-susceptible Williams 82 has a proline residue (CCC) at this position. In the current study, all the progeny derived from the AntSoy mutant have serine amino acid (TCC) at position 197 in the AHAS gene. However, the wild-type Arısoy variety has proline at this point. In addition to Pro197 mutation, AntSoy progeny showed two more novel mutations in the AHAS gene. Since Pro197Ser mutation is the common, it is challenging to identify whether these two other mutations have an increasing or decreasing effect on herbicide resistance. It may be possible to give

precise information by detecting and testing each mutation in individual progeny. Despite the application of 2X and 3X herbicide doses, no difference was found among the progeny.

SU herbicides such as mesosulfuron, chlorsulfuron, and metsulfuron are used worldwide to manage broad-spectrum weed control in cereals [7,8]. As a result of the intensive use of chlorsulfuron in wheat fields, it may cause residue problems in the soil. Some reports show that soybean yield is reduced due to planting soybean after wheat in these fields [9], likely due to the carryover injury from the residual herbicides in the soil. AntSoy has the potential to overcome these negative impacts, thereby preventing any yield losses that might be observed in a soybean/wheat rotational or second crop after wheat program.

Commercial target-site herbicide resistance varieties have been developed for some plant species against AHAS inhibitors. In some studies, it has been reported that resistant lines obtained because genetic mutations do not differ from susceptible lines in terms of growth, development, and yield. In general, mutations that usually involve a single base change are essential in improving the AHAS herbicide resistance trait of the plants. Since chemical mutagens mainly cause single nucleotide changes, it is unlikely that significant changes will occur in the plant. These point mutations can affect protein synthesis, but the chance of causing major adverse effects in the plant could be rare. No considerable differences were observed phenotypically between the herbicide-resistant mutants obtained due to the EMS applied and Arısoy variety in terms of plant growth and development characteristics.

Plant yield is one of the most important characteristics for breeders and farmers and plays an important role in the selection of genotypes. There are various agronomic traits of soybean that affect plant yield, such as pods per plant, first pod height, and 1000-seed weight. A lot of genotypes with herbicide resistance or some abiotic and biotic stress factor resistance obtained through mutagenesis are not preferred due to low yield and quality. It has been noted by some researchers that specific gene mutations conferring target site-based herbicide resistance have adverse pleiotropic effects on plant growth and viability [31]. Nonetheless, some researchers did not identify this pleiotropic effect [32,33]. The problem with these studies is that the genetic background is usually not the same between the initial wild type and the mutant type. The question arises whether the agronomic and phenotypic differences between them are due to mutation only or there were differences before the mutation. Since the background is the same before the mutation, it is very clear that the differences are only caused by mutations in this study. The mutations only in the AHAS gene were identified in this research. In addition, it is possible that there may be mutations in different genes or genomes. Despite all this, the differences in terms of characteristics such as yield, 1000-seed weight, plant height, first pod height, number of pods per plant, and number of days to physiological maturity remained at very small levels and were statistically insignificant. In this study, pleiotropic effect study was carried out since the starting material has almost the same genetic similarity, the resulting difference occurred mainly due to mutation. The effect of the mutation was revealed more clearly by looking at the difference between the resistant mutant, AntSoy, and Arısoy variety. Results of the present study clearly showed that there is no pleotropic effect between this Antsoy genotype developed by EMS mutagenesis and wild type. This study clearly removes this ambiguity in the literature.

The AntSoy mutant represents a combination of previously identified and novel mutations of the AHAS gene on chr 4 in *Glycine max* L. This novel mutant can provide a source of non-GM resistance to sulfonylurea herbicides, which can benefit both breeders and researchers. From a commercial standpoint, it can provide value to farmers by not only providing protection from sulfonylurea carryover in a rotational program but also allowing a new mode of action of chemistry to be applied in season to control a different spectrum of weeds. Particularly in soybean-growing regions that do not permit the planting of GM soybean, AntSoy has the potential to provide a unique value to the farmers.

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