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Development of Imazamox-Resistant Chickpea (Cicer arietinum L.) Genotypes via EMS-Induced Mutation

By

Aydogan A[1]*, Atasayar E[1], Kilinc H V[1], Ersin KAVLAK[1] Selin GUNDUZ[1]

vlak E[1], Gunduz S[1] [1]Central Research Institute of Field Crops, Ankara, Türkiye

ORCID: 0000-0002-9747-4004
ORCID: 0000-0001-8155-5152
ORCID: 0000-0002-7812-4041
ORCID: 0009-0005-1108-1146
ORCID: 0000-0003-4762-4876

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Abstract

Chickpea (Cicer arietinum L.) has crucial role in both human and animal diets owing to its high protein content. Furthermore, it presents considerable prospects for advancing sustainable agriculture and environmental conservation due to its low water requirements and carbon footprint. Türkiye is the gene centre of chickpeas and the fourth largest producer in the world. Weeds represent one of the major biotic constraints adversely impacting chickpea yield and quality. In certain cases, weed infestation can suppress chickpea productivity by over 60%. Current control methods are inadequate, especially against broad-leaved weed species. However, the release of varieties that are resistant to IMI group herbicides has increased the effectiveness of weed control. This study aims to develop chickpea genotypes that are resistant to IMI group herbicides by chemical mutation. This study, which was conducted from 2020 to 2024, involved the treatment of Azkan, Arda, Aksu, Aydoğan, and Göktürk cultivars of chickpea using EMS as the mutagen at doses of 0.10%, 0.20%, 0.40%, and 0.60%. Following the mutagenic treatment, 60 g/L imazamox (IMI group herbicide) was applied under field conditions. Herbicide resistance tests were conducted using the 1–5 scoring system. By using the pedigree selection method, 107 imidazolinone group herbicide-tolerant lines were successfully obtained at the M6 generation.

Keywords: *Chickpea, EMS, Mutation, IMI tolerant, weed*

Introduction

According to FAO (2024), chickpea (Cicer arietinum L.; 2n = 2x = 16) ranks second globally, with 18 million tonnes of production out of the 96 million tonnes of legumes produced worldwide, while Türkiye leads with 580,000 tonnes. It is consumed for both human and animal nutrition due to its high protein content and enriches the soil with organic matter through nitrogen fixation. Furthermore, chickpea cultivation is significantly beneficial to the environment and sustainable agriculture due to its low carbon and water footprints. However, various biotic and abiotic stressors — such as weeds, anthracnose disease, fusarium wilt, drought, high temperatures, and excessive soil salinity and lime content — limit chickpea productivity in Türkiye (Aydoğan, 2014).

Among these, weeds are one of the primary constraints restricting chickpea yield and production (Armin et al., 2016). Chickpea plants exhibit limited competitive ability and are particularly susceptible to weed competition due to their slow growth rate and restricted leaf area development during the early stages of growth (Barker, 2017; Chandrakar et al., 2018). Weeds are more efficient in utilizing soil water and nutrients, and their faster growth rates further suppress chickpea productivity by intercepting sunlight and reducing photosynthetic activity (Tudi et al., 2021). Additionally, weeds serve as hosts for various diseases and harmful organisms (Kumar et al., 2016).

Numerous studies have reported varying estimates regarding the reduction in chickpea yield due to weed interference. For instance, Mukherjee (2007) reported losses ranging from 40% to 90%, while Mohammadi et al. (2005) cited values of 66.4% and 48.3%. In contrast, Merga et al. (2019) documented a broader range of 17% to 105%.

Various control methods, such as cultural, mechanical, and chemical approaches, are applied to minimize the damage caused by weeds to chickpea yield. However, these methods do not eradicate weeds completely. Furthermore, while numerous post-emergence herbicides are licensed and effective against narrow-leaved weeds in chickpea, the absence of licensed and efficient herbicides for broad-leaved weeds exacerbates the weed problem. It has been reported that breeding herbicide-resistant chickpea varieties is necessary to address this issue in chickpea production (Galili et al., 2021).

To develop new herbicide-resistant chickpea varieties, acetohydroxyacid synthase (AHAS) enzymes must be rendered resistant to herbicides through mutation (Rizwan et al., 2015). Mutations induced by ethyl methanesulfonate (EMS) in the AHAS gene region alter the herbicide binding site, thereby preventing herbicide binding and subsequent inhibition of AHAS enzyme activity. This inhibition ordinarily disrupts essential amino acid biosynthesis, ultimately halting photosynthesis and leading to plant death (Tan et al., 2006; Han et al., 2012; McCourt et al., 2006). Thus, inhibition of the AHAS enzyme by herbicides offers a broad spectrum of weed control.

Imidazolinone herbicides, which are effective against a wide range of both narrow- and broad-leaved weeds that significantly reduce crop yield, are considered ideal candidates for developing imidazolinone resistance. These herbicides are preferred due to their efficacy at low doses, low toxicity to mammals, and environmentally friendly use (Tan et al., 2005). Imidazolinone-tolerant products, referred to as Clearfield® products, do not contain exogenous DNA and are therefore compliant with conventional breeding standards rather than transgenic methods.

Through mutation breeding, various herbicide-resistant lines and varieties inhibiting the AHAS enzyme have been successfully developed worldwide. Such herbicide resistance has been introduced in several species, including sugar beet (Wright and Penner, 1998), cereals (Newhouse et al., 1992), cotton (Rajasekaran et al., 1996), soybean (Sebastian et al., 1989), lentil (Aydoğan et al., 2016), tobacco (Chaleff and Ray, 1984), rice (Croughan, 1998), and chickpea (Thompson and Taran, 2014; Galili et al., 2021; Mugerwa, 2022).

The objective of this study is to develop herbicide-resistant chickpea lines using EMS-induced chemical mutagenesis and to enhance chickpea yield and profitability by establishing an effective weed management strategy to address this major constraint in chickpea production.

Materials and Methods

In the study, Azkan, Arda, Aksu, Aydoğan, and Göktürk commercial chickpea varieties used as the experimental materials. As a mutagen, the varieties treated with Ethyl Methanesulfonate (EMS). EMS (Ethyl Methanesulfonate) has a molecular weight of 124.154 g/mol and a density of 1.15 g/cm³.

In terms of IMI group herbicide, 40 g/l Imazamoxolan active ingredient herbicide used. The recommended dose of Imazamox: (2-[4,5-dihydro-4-methyl-4-(1-methyl)-5-oxo-1H-imidazol- 2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt) is 40 g a.i. ha^{-1}.

The materials and doses used in the mutation are given in Table 1. Initially, all of the material was softened by leaving it in running water for 8 hours. Then, 100 pieces of each of the project materials were treated with EMS for 8 hours at the following doses. Then, each material was rinsed in running water for 8 hours. The material was dried on blotting paper and IMI-chickpea seeds were obtained.

Table 1: Chickpea varieties exposed to EMS mutation in 2020 and doses administered.

Dose/Variety	Azkan	Arda	Aksu	Aydoğan	Göktürk
% 0,10 (0,0010)	100 seeds	100 seeds	100 seeds	100 seeds	100 seeds
% 0,20 (0,0020)	100 seeds	100 seeds	100 seeds	100 seeds	100 seeds
% 0,40 (0,0040)	100 seeds	100 seeds	100 seeds	100 seeds	100 seeds
% 0,60 (0,0060)	100 seeds	100 seeds	100 seeds	100 seeds	100 seeds

After the IMI material has been obtained; the applied method, selection and material grades are given in Table 2 according to years.

Table 2: Methods applied to IMI chickpea genotypes by year

Year	Material Stage	Planted materials	Selection	Methods	IMI herbicide applied
2019	MI	20 populations	-	Bulk method	-

*Corresponding Author: **Aydogan A**

2020	M2	20 populations	-	Bulk method	-
2021	M3	20 populations	150 ml	Bulk method	150 ml
2022	M4	20 populations	1396	Single plant selection method	150ml
2023	M5	1396 single plants	421	Single plant selection method	150ml
2024	M6	421 single plants	107	Single plant selection method	150ml

In the experiments:

1. Anthracnose observation was carried out based on 1-9 scale of (Ready and Singh, 1984).
2. Plant height (cm), 50% flowering and maturation days data were recorded.
3. Herbiside application; 150 ml/da of a 40 g/l Imazamox was applied when the chickpeas reached the 6-12th node.
4. Herbicide resistance observation was taken and scored 21 days after the herbicide application. It was done according to Gaur et al. (2013) scoring criteria as follows:

1. High tolerance, no yellowing and drying in the plant,
2. Good tolerance, very little yellowing and burning in the leaves,
3. Moderate tolerance, moderate burning, shrinking and yellowing in the leaves,
4. Sensitive, severe and very much yellowing, curling, shrinking and burning in the leaves, 5= Very sensitive, most of the plants death, and the remaining leaves burn and dry.

All experimental data were performed using JMP 11 software (SAS Institute Inc., Cary, NC, USA).

Results

Table 3 presents the results of a dose study that was carried out on the material that was submitted to mutation application to determine the dose that would be used in the studies in 2020.

In the trial, when the material reached the 8th stage, 60, 80, 100, and 120 millilitres of IMI herbicide (40 g/l Imazamox active component SL) were applied 23 days after planting. 17 days after spraying, an observation of herbicide resistance was made after it was observed that the material was died. Table 3 makes clear that all doses beyond 60 ml were fatal to populations.

Table 3: Variety, dose observations

Varieti es	IMI Dos es (ml)	HRS	Height before chemica l applicat ion (cm)	IMI Dos es (ml)	HR S	Height before chemica l applicat ion (cm)	IMI Dos es (ml)	HR S	Height before chemica l applicat ion (cm)	IMI Dos es (ml)	HR S	Height before chemica l applicat ion (cm)
Azkan	60	4,0	15	80	5	18	100	5	12	120	5	13
Arda		4,0	10		5	12		5	12		5	13
Aksu		4,0	12		5	9		5	16		5	18
Aydoğ an		4,0	13		5	13		5	13		5	15
Göktü rk		4,0	14		5	13		5	14		5	15

HRS: Herbicide resistance score (1: Resistant; 9: Sensetive)

In 2020, M1 populations were planted to obtain higher genetic expansion. M2 seeds were obtained without any treatment or selection of the material. It was observed that the number of days to 50% flowering was 84-88 days and the plant height was between 40-50 cm

*Corresponding Author: **Aydogan A**

in 20 M2 populations (Table 4). Bulk method was applied to each population separately.

Table 4: Observations taken in M2 populations

Varieties	EMS Doses	DF	PH)	AB	EMS Doses	DF	PH	AB	E EMS Doses	DF	PH	AB	EMS Doses	DF	PH	AB.
Azkan		88	45	2		88	50	2		88	46	2		87	45	2
Arda	0,10 %	87	45	2	0,20 %	87	45	2		87	50	2		87	40	2
Aksu		84	45	3		85	40	2	0,40%	84	45	2	0,60%	84	46	2
Aydoğan		89	47	2		87	45	2		88	45	2		88	45	2
Göktürk		88	50	2		88	42	2		89	40	2		87	42	2

AB: Ascochyta blight (1: Resistance; 9: Susceptible) ; PH:Plant height (cm); DF: Days to %50 flowering; DM: Days to maturity

In 2021, it was planted in rows according to the amount of seeds as M3 population. When the plant height was 5-8 cm and the number of nodes was 6-7, IMI group herbicide (40 g/l Imazamox active ingredient SL) was applied as 150 ml. Observations of plant height before and after chemical usage, as well as herbicide resistance, were made 27 days following chemical use. The variation analysis for herbicide resistance is given in Table 5.

Table 5: Variation analysis for herbicide resistance in M3 population by variety and EMS dose used

	(SD)	(DF)	(MS)	(F ratio)(P)	(CV%)	(LSD) 0,05
Doses	0,5624	**3**	0,1875	1,9141 ^{ns} 0,1812		0,4312
Population	0,8728	**4**	0,2182	2,2279 ^{ns} 0,1270		0,4822
Error	1,1753	**12**	0,0979		21,01	
Total	2,6105	**19**				

ns: no significance

In the variation analysis of the 20 M3 populations and doses for herbicide resistance, the differences in both dose and populations in terms of herbicide resistance were not found to be statistically significant.

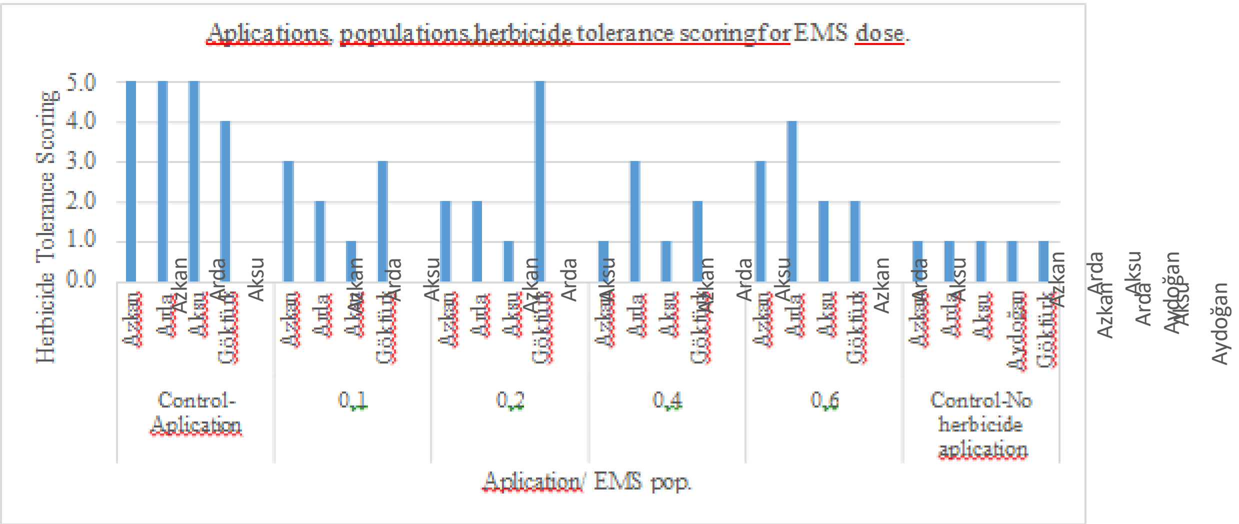
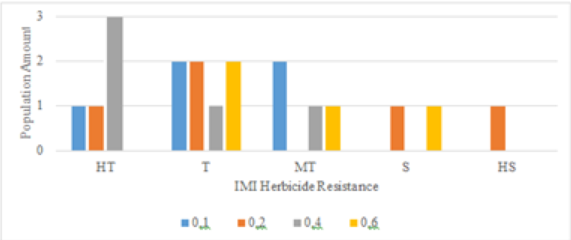


Figure 1. Herbicide resistance scores of M3 populations and controls

In the M3 population, control-herbiside application, the population and the control-untreated material were resistant to herbicide without control application (score: 1), and sensitive to control-herbiside application (score: 5). Twenty populations with different levels of EMS application responded differently to herbicide application (scores varied between 1-5) (Figure 1). The distribution of herbicide resistance of M3 populations according to EMS doses is given in Figure 2.

For the IMI herbicide, the highest herbicide tolerance was observed in 3 populations treated with a 0.4% EMS dose, whereas no herbicide tolerance was detected at a 0.6% EMS dose. The most sensitive populations to IMI group herbicides were observed at 0.2% and 0.6% EMS doses. An insignificant relationship was found between herbicide resistance score and EMS application dose (r: 0.0529ns) and variety (r: 0.1596ns).



HT: High tolerance; (Score:1) T: Tolerance (Score:2); MT: Medium tolerance (Score:3); S: Sensetive (Score: 4; HS: High Sensitivity (Score:5)

Figure 2. Distribution of herbicide resistance of M3 populations by EMS doses

After herbicide application, the maximum plant height was 19.3 cm in the control varieties without EMS application and without herbicide use, while the shortest plants at 2 cm in the sensitive varieties with EMS application (Figure 3).

An inverse and statistically significant (r: -0.8978**) relationship was found between the herbicide resistance score and plant height extension after herbicide application in M3 material.

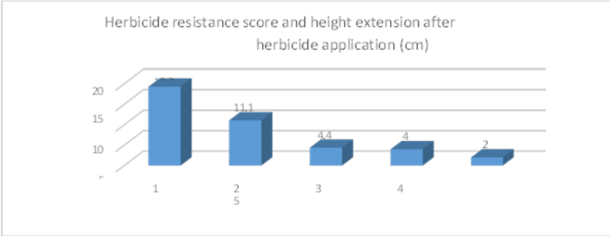


Figure 3. Changes in plant height after herbicide application

The tolerant materials from M3 were bulked to obtain the M4 population. In 2022, 150 ml of IMI herbicide was applied to the material planted as M4 when the plant reached the 6th node.

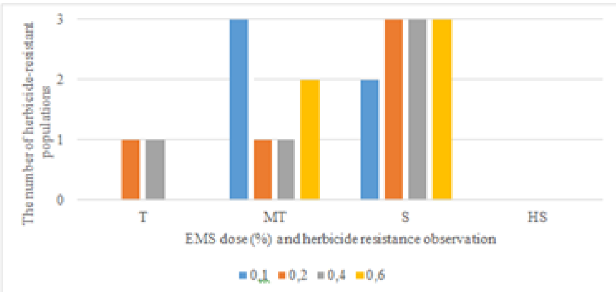
Resistance observation was taken 21 days after herbicide application. Variation analysis was performed for EMS dose and populations in the experiment (Table 6). While there was no statistical significance in the differences between EMS doses and herbicide resistance in the analysis, this difference was found to be significant at the 0.05% level in varieties and populations.

Table 6: EMS dose of M4 population and variation analysis table for variety

Model	Sum of Squares (SS)	Degrees of Freedom (DF)	Mean Squares (MS)	F Ratio	Probability (P)	%CV	LSD (0.05)
EMS Dose	0,15	3	0,05	0,146^{ns}	0,930		0,805
Population	4,7	4	1,175	3,439*	0,043*		0,901
Error	4,1	12	0,342			16,943	
Total	8,95	19					

*: Significant at 0.05% level

The resistance scores obtained against each EMS dose in the M4 population are given in Figure 4. The highest tolerance was found in EMS 0.2 and 0.4% doses with 1 population each, while the most sensitive population was found in EMS doses of 0.2-0.4 and 0.6% with 3 populations each.



HT: High tolerance; (Score:1) T: Tolerance (Score:2); MT: Medium tolerance (Score:3); S: Sensetive (Score: 4; HS: High Sensitivity (Score:5)

Figure 4. Herbicide resistance score for each dose in the M4 population

A total of 1396 single plants were selected from the herbicide tolerant M4 population. In 2023, these 1,396 M4 plants were planted as M5 on April 3, 2024. 150 ml of IMI herbicide was applied when the plants reached the 6th node 14 days after planting. Due to excessive rainfall, there were not any observations taken and only 421 materials that were selected as herbicide tolerant.

In 2024, 421 materials were planted as M6 on 02.04.2024. When the plants reached the 6th and 7th nodes, 150 ml of IMI herbicide was used 22 days after planting on 24.04.2024. Herbicide resistance observation was also taken 21 days after the herbicide use. Herbicide resistance observation analysis for herbicide dose and population is given in Table 7.

Table 7: Analysis of variance for herbicide resistance observations in M6 lines based on variety and EMS doses.

Model	**Sum of Squares (SS)**	**Degrees of Freedom (DF)**	**Mean Squares (MS)**	**F Ratio**	**Probability (P)**	**%CV**	**LSD (0.05)**
EMS Dose	20,718	3	6,906	3,823	0,039*		1,852
Population	64,529	4	16,132	8,930	0,001**		2,071
Error	21,678	12	1,807			41,471	
Total	106,926	19					

*: statistically significant at the level of 5%, **: significant at the level of 1%

The difference between the herbicide resistance observation and EMS dose was found to be statistically significant at the 5% level. The differences between the resistance scores of the populations were determined to be significant at the 1% level. EMS dose level and LSD grouping for varieties are given in Table 8.

Table 8: Grouping of EMS doses and varieties for M6 lines

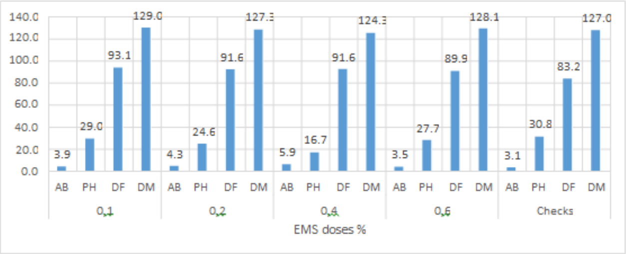
Variety	Doses %
Azkan^{ab}	0,1^{a}
Arda^{a}	0,2^{ab}
Aksu^{bc}	0,4^{b}
Aydoğan^{c}	0,6$_{a}$
Göktürk^{c}	

Variety LSD (5%) : 2,071 ; Doses LSD(5%) : 1,852

While the most sensitive populations to IMI herbicide among the varieties were in the Arda variety, the most resistant material was found in the Göktürk populations. In terms of EMS doses, the most sensitive material was obtained at 0.1% and 0.6% doses, and the most resistant genotypes were obtained at 0.1% and 0.2% doses.

Chickpea blight, height, flowering and maturation days of M6 materials according to EMS doses are given in Figure 5. Ascochyta blight score varied between 3.1-5.9. The lowest score was seen in control averages.

Plant height varied between 1.7-30.8 cm and the longest height was 30.8 cm in control averages. Average 50% flowering days ranged between 83.2-93.1 days. The longest 50% flowering days were reached with 93.1 days at 1% dose. Average maturation days ranged between 124.3-129 days.



AB: Ascochyta blight (1: Resistance; 9: Susceptible) ; PH:Plant height (cm); DF: Days to %50 flowering; DM: Days to maturity

Figure 5. Some observation recorded for M6 material based on different EMS doses.

Conclusion

Chickpeas have very low competitiveness against weeds, that leads to significant yield losses in production. There is no fully effective chemical or cultural method for weed control for chickpeas. In particular most chemical control methods are ineffective against broadleaf weeds. One of the most effective strategies to combat weeds is to develop IMI-tolerant chickpea varieties by developing herbicide resistance in EMS-mutated genotypes.

The gene region which is responsible for amino acid synthesis in plants, fungi and bacteria (AHAS) is prevented from doing so by chemical mutation. The chemicals commonly used are ethyl methane sulfonate (EMS), ethyl nitrosourea, N-nitroso-N methyl urea, and sodium aside are utilised. Among them EMS has proven to be the most influential way to achieve herbicide resistance in plant breeding (Naveed, et al., 2024). In this study similar strategy has been adopted that is based on testing various doses of to develop herbicide resistance.

While Galili et al. (2021) used 144 g of a.i ha-1 with imazamox, in this study, imazamox 60 g ha-1 was applied to all chickpea populations obtained by mutation. In such studies, 3 times the dose of herbicide applied is recommended

(Prakash et al., 2017). There were not any selection was subjected to until sufficient variation obtained in the M2 seed population, and when the plant reached M2 (when the seed would be M3), Imazamox was used to select for the herbicide.

Herbicide applied on EMS mutated material starting from M3. After the application, Galili et al. (2021) after 21 days, in our study, after determining that the standards were damaged (21-28 days), plant damage (1-5 class), plant height, 50% number of flowering days, ripening time observations were taken (Taran et al., 2010; Prakash et al., 2017; Gaur et al., 2013). In our study, the observations taken after the use of herbicides, plant height increased according to resistance levels and sensitivities, and 50% flowering and maturity days could be determined. In sensitive genotypes, these observations could not be taken or there was not any improvement in this regard because the development stopped.

In our study, no statistically significant difference was found in the herbicide resistance scores between varieties and doses in the M3 population. However, a statistically significant difference was observed in the response of the varieties to the herbicide in the M4 population, with a significance level of 5%.Thomson (2013) did not find Chi-square statistics for herbicide resistance important in his study on 2 EMS-opened chickpea populations and attributed herbicide resistance to both dominant and semi-dominance.

As a result of the study, it was determined that 107 materials were herbicide tolerant. In a study conducted in India, it was reported that there was a mortality rate of 80-100% in 40 materials developed with EMS and a moderate herbicide tolerant in 24 materials (Teggi, 2017). Tar'an et al. (2010) developed 4 IMI-resistant lines (ICC2242, ICC2580, ICC3325 and ICCX860047–9) from the EMS population by classical breeding.

Based on these findings, future chickpea breeding studies will be integrated with these lines found tolerant to herbicide stress. Moreover, molecular validation of AHAS gene mutations will be appropriate to confirm the resistance mechanism and accelerate marker-assisted selection. The implementation of these breeding strategies will serve to increase chickpea cultivation with improved competitiveness against broadleaf weeds. Thus, it will enable increased productivity in sustainable agricultural ecosystems.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Researchers, who want to access data for further analysis should use relevant data and documents for any questions or requests regarding the dataset, please contact the corresponding author.

Keypoints

- It is very important to solve the problem that limits the yield and quality of chickpeas, as it is one of the legume crops that is widely cultivated worldwide.
- Weeds are one of the main problems that cause yield losses of up to 60% in chickpea production and need to be solved urgently.
- Within the scope of this comprehensive study, Ethyl Methane Sulfonate (EMS) was used at various doses to induce mutations.
- (IMI group herbicide) was applied in field trials to select resistant lines and to evaluate performance and stability between years.
- The research enables the development of chickpea varieties resistant to IMI herbicide.

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*Corresponding Author: **Aydogan A**