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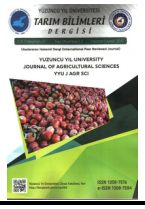

Research Article

Tolerance to Imazamox Herbicide Found after Screening of Advanced Generation Lentil Mutant Genotypes

Zian HAMID AHMAD[1], Abdulkarim LAKMES[*2], Havva AKKURAK[3], Nefise EREN UNSAL[4], Abdullah KAHRAMAN[5]

[1,2,4,5]Harran University, Department of Field Crops, Sanliurfa, Turkey
[3]Harran University, Department of Plant Protection, Sanliurfa, Turkey
[1]https://orcid.org/0000-0003-4699-7654 [2]https://orcid.org/0000-0002-8167-7085 [3]https://orcid.org/0000-0003-1196-5230
[4]https://orcid.org/0000-0003-0140-9820 [5]https://orcid.org/0000-0002-8829-3797
Corresponding author e-mail: abdulkarimlakmes@harran.edu.tr

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Lentil,
Ethyl methane sulfonate (EMS),
Imazamox tolerance.

Abstract: In Turkey, one of the essential grain legumes is lentil. It is usually perceived as a weak competitor with weeds. The research objective was to determine the tolerance of selected 145 mutagenized lentil genotypes at M5 generation to imazamox herbicide including 139 M5 lentil genotypes derived from Ethyl Methane Sulfonate (EMS) mutagenized seeds of cultivar Firat-87 and 6 control lentil cultivars were screened for imazamox herbicide tolerance. Experiments were carried out in the greenhouse and field. Herbicide was applied at 150% of the recommended dose of (100 ml/ha, or 40 g a.i/ha) imazamox when the plants were between 5 - 6 node stage. The response of the genotypes to the herbicide was evaluated by measuring the plant height as a sign of the growth and also by visual scoring of foliar damage with a 1 to 5 scale at 45 and 60 days after a spraying in the field experiment and at 30 and 60 days after a spraying in the greenhouse experiment. The genotypes were categorized based on their reactions to herbicides as highly tolerant, tolerant, moderately tolerant, sensitive, and highly sensitive. The results showed significant differences among the genotypes for tolerance to the herbicide. At 60 days after spray, most of the genotypes showed some of the recoveries in both experiments. Five genotypes (IMI-124, IMI-128, IMI-130, IMI-138, and IMI-139), displayed high herbicide tolerance in both experiments. The tolerant genotypes can be exploited in future breeding programs for improving herbicide tolerant lentil varieties.

İleri Generasyon Mercimek Mutant Genotiplerinin İmazamoks Herbisitine Toleranslarının Belirlenmesi

Makale Bilgileri

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Anahtar Kelimeler
Mercimek,
Etil metan sulfonat (EMS),
İmazamoks tolerans.

Öz: Yabancı otlara karşı rekabet gücü zayıf olan mercimek, Türkiye'de üretilen önemli yemeklik dane baklagillerdendir. Araştırma, Etil Metan Sülfonat (EMS) ile mutajenize edilen Fırat 87 çeşidinden M5 generasyonunda seçilen 139 mercimek mutant genotipleri ve 6 kontrol çeşitin imazamoks herbisite toleransını belirlemek amacıyla yürütülmüştür. Denemeler hem sera hem arazi şartlarında yapılmıştır. İmazamoks etken maddeli herbisit, bitkiler 5 - 6 boğumlu olduğu dönemde önerilen dozun (100 ml/ha veya 40 g a.i/ha) 1.5 katı (150 ml/ha) olarak uygulandı. Genotiplerin herbisite tepkisi, büyümenin bir işareti olarak bitki boyunun ölçülmesiyle ve ayrıca, bitki aksamında oluşan herbisit zararının görsel skorlanması (1= dayanıklı 5= % 100 ölü) yapılarak değerlendirildi. Ölçüm ve skorlamalar tarla denemelerinde ilaçlama yapıldıktan 45 ve 60 gün sonra, sera

denemelerinde ise 30 ve 60 gün sonra yapılmıştır. Genotiplerin herbisite karşı reaksiyonları; yüksek toleranslı, toleranslı, orta derecede toleranslı, hassas ve oldukça hassas olarak gruplandırıldı. Elde edilen sonuçlara göre, herbisite tolerans bakımından genotipler arasında önemli farklılıklar gözlenmiş olup ilaçlamadan 60 gün sonra yapılan değerlendirmelerde, hem tarla hem de sera denemelerinde genotiplerin çoğunda bir miktar iyileşmeler gözlenmiştir. Beş genotip (IMI-124, IMI-128, IMI-130, IMI-138 ve IMI-139), tarla ve sera denemelerinde herbisit toleransı en yüksek genotipler oalrak belirlenmiştir. Bu genotipler, imazamoks herbisite toleranslı mercimek çeşiti geliştirmek için ıslah programlarında kullanılabilir.

1. Introduction

Lentil (*Lens culinaris* Medik.) is a member of the Fabaceae family. It is grown as a winter crop in most parts of the world. Lentil can grow well in depleted soil, and a lack of rain and freezing conditions do not necessarily affect their growth. It has nutritional and health importance for humans because it contains a high percentage of vegetable protein (up to 30%) and is a good source of vitamins and other important nutrients, such as 0.5% phosphorus content. Furthermore, lentil brings good economic returns (Sarker, 2006). It is cultivated in most parts of the world and the countries with the greatest production are Canada, India, Australia and Turkey. Crushed crust from the processing of lentils is also used to feed cattle and poultry. Therefore, its cultivation brings secondary benefits such as animal feed and, via nitrogen fixation, increases the fertility of the land in which they are grown.

Lentil cultivation suffers from considerable annual variations in yield, and a clear decline has emerged over the last five years in Turkey due particularly to management problems, and susceptibility to various biotic and abiotic stresses (Figure. 1). The yield of current cultivars of lentil ranges between 1450 and 1950 kg/ha, but the yield can be considerably depressed due to poor weed management (Aktar et al., 2013). In Turkey, the lentil area harvested was 292 455 ha, production was 430 000 tonnes and yield was 1 470 Kg/ha, in 2017 (FAO, 2018).

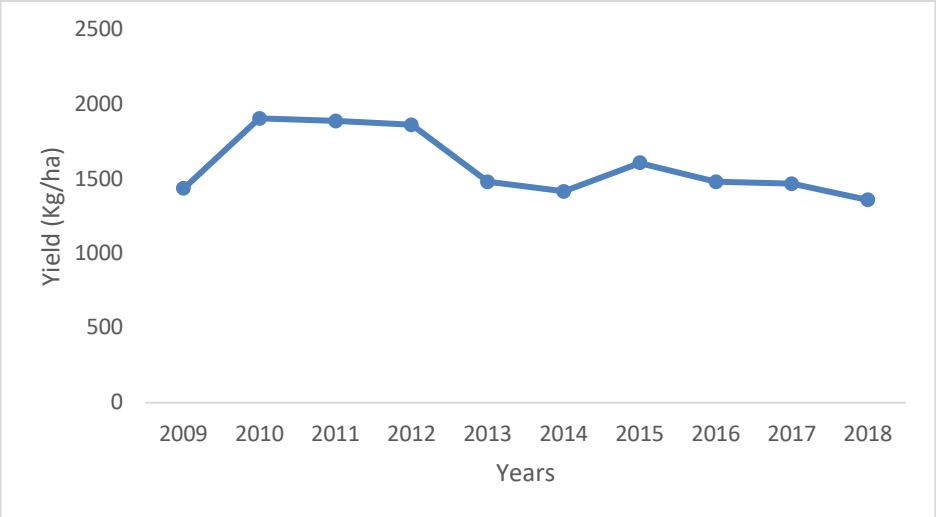

Figure 1.Variations in lentil yield in Turkey (FAO, 2018).

Weeds have a major impact on lentil production. Yield losses in lentil range between 20% and 80% because lentil is a poor weed competitor (Al Thahabi et al., 1994; Saxena and Wassimi 1980; Boerboom and Young, 1995; Brand et al., 2007). Many broadleaf species and annual grasses are in competition with lentils for water, nutrients, and sunlight, which affects lentil production and the quality of grain while also allowing diseases and other pests to thrive (Rizwan, 2015). Losses due to weed competition in the lentil yield are dependent on the level of weed infestation, and the types of weed species which are prevalent (Al-Thahabi et al., 1994; Yenish et al., 2009; Saxena and Wassimi 1980; Hattori, 1995).

In lentils, weeds are commonly controlled manually. However, hand weeding is impractical in the extensive production areas because is an expensive process and labour intensive (Baumgartner and Al-Khatib, 1999; Iler and Pauls,1993), and if delayed, the operation does not prevent the adverse effect of the weeds on crop yield. It is therefore necessary to use effective herbicides to decrease unwanted competition (Ashigh et al., 2009). As such, lentil genotypes with improved herbicide tolerance can offer more suitability for the use of broad-spectrum post-emergence herbicides which are required by farmers. In light of this information, lentil varieties with improved herbicide tolerance which can offer greater flexibility for the use of broad-spectrum post-emergence herbicides are required by farmers.

The IMI class of herbicides, including imazethapyr, imazamox and imazapic, provides a broad spectrum of weed control activity, adjustability in the timing of application, low usage rates, and low mammalian toxicity. Other advantages of using IMI herbicides include low environmental damage, control of broadleaf weeds and a low herbicide dose per hectare (Weed Science Society of America 2007). Furthermore, IMI-tolerant genotypes have been identified in many species, which has enabled the development of several tolerant crops (Ashigh et al., 2009). At present, IMI herbicides are used on non-pulse crops such as barley, spring wheat, sunflower, oats, oilseed mustard, canola and alfalfa and pulse crops including lentil, field pea, soybean and dry bean (Saskatchewan Ministry of Agriculture 2013).

There is the possibility of finding highly tolerant genotypes by the screening of a large number of genotypes to exploit natural differences or screening of genotypes created through induced mutations (Toker et al., 2012). Other studies have reported the creation of herbicide-tolerant mutants by selection from spontaneous mutation (Bernasconi, 1995; Tan, 2005). Herbicide tolerant mutant plants have been discovered in many crops like lentil (Sharma, 2017), wheat (Newhouse, 1992) and maize (Anderson, 1989; Newhouse, 1992). In particular, many studies have found that in many crops, the mutations were efficient in the creation of genotypes tolerant to herbicide (Tan et al., 2005; Rizwan et al., 2017; Ndungu, 2009; Malkawi, 2003; Sharma et al., 2018; Chant 2004; Bernasconi et al., 1995; Beckie at al., 2006).

Seed mutagenesis followed by herbicide selective pressure has been utilized widely to develop crop resistance to herbicides (Mulwa and Mwanza, 2006). This technique obtains a wide array of advantages for farmers of enhanced economical weed control and improves economic returns. Enhanced economical weed control are advantage to farmers from the technique of seed mutagenesis. Plant -tolerance to herbicides has been developed by the widespread utilization of seed mutagenesis followed by herbicide selective pressure. The selected- mutations can be evaluated for herbicide resistance. Because most herbicide-resistant mutants have been created via chemical mutagenesis, this process was understood to be a significant source of producing genetic variability.

Among chemical mutagens, ethyl methanesulfonate (EMS) is the most popular method for obtained an effective method (Rizwan et al., 2017; Tan et al., 2005). EMS generally causes little nucleotide changes or small point mutations within the genome, as compared to other chemical and physical mutagens that cause huge changes such as the disappearance of the large part of the genome that causes significant changes and can also destroy the characteristics of the cultivar (Weil and Monde, 2007).

This study aims to explore the potential of EMS induced mutation to generate lentil genotypes that are tolerant of the imidazolinone herbicide that could be included in plant breeding programs.

2. Material and Methods

2.1. Material

The present study was conducted at Harran University, Faculty of Agriculture Experiment Station field and greenhouse facilities in the 2017-2018 growing season. A total of 145 genotypes including 139 genotypes of EMS mutagenized seeds of cultivar Firat-87 and 6 lentil cultivars used as imidazolinone sensitive controls (Firat-87, Cagil and 4 Canadian cultivars with the unknown name) were screened against imazamox herbicide tolerance in the experiment. The protocol for ethyl methanesulfonate (EMS) mutation has been described by Bukun and Kahraman, 2013.

EMS mutated Firat 87 genotypes were selected at M2 generations based on healthy appearance after two doses of imazamox spray in the field and were advanced to M6 generation using single seed descent (SSD) to obtain homozygosity and also to generate enough seed for the experiment.

A commercial herbicide of imazamox was used in the experiment. The herbicide is registered for use on Clearfield Sunflower cultivars with a recommended dose of 1250 ml/ha (50 g a.i/ha) in Turkey. Currently, there is no imidazolinone herbicide registered for use in lentil in Turkey but imidazolinone herbicides have been used on Clearfield lentil cultivars grown in Canada with a recommended application dose of 40 g a.i/ha. Based on this information, it was decided to apply with an extra 50% of the recommended dose of imazamox (1500 ml/ha, e.g 60 g a.i/ha) to determine the tolerant genotypes. Any genotype that survived at this dose of herbicide would be considered tolerant to the herbicide. A shoulder-mounted hand operated knapsack sprayer was used to spray agents the herbicide by100 L/ha of water during cooler hours of the day when there was slow or no wind.

Two experiments were carried out both in the greenhouse and in the field in a randomized complete block design (RCBD) with three replications. Since the aim of the experiment was to determine genetic resistance to the herbicide, seed yield was not evaluated, and the experiments were not carried out at the usual planting time of lentil. About 30 seeds from each genotype were planted in 1 m rows with 20 cm of row spacing in the field experiment. For the greenhouse experiment, 10 seeds from each genotype were planted in a 50 cm row length with a 20 cm of row spacing.

The imazamox herbicide was applied when the plants had grown between 5 - 6 nodes in size. A 1 to 5 scale was used to evaluate the damage caused by the herbicide as proposed in chickpea [(Gaur et al., 2013), (Table 1)]. The damage was scored in the whole row. The damage response of lentil genotypes against imazamox herbicide in the field experiment was observed after 45 and 60 days after spraying (DAS), or after 30 and 60 DAS for the greenhouse experiment.

Table 1. The scale used for categorizing plants for their reaction against herbicides (Gaur et al., 2013)

Damages	**Reaction**
Highly tolerant	Very good genotype growth with no chlorosis/burning/narrowing of leaves
Tolerant	Good genotype growth with a lıtle chlorosis /narrowing/burning of leaves
Moderately	moderate genotype growth with medium chlorosis/narrowing/burning of leaves,
Sensitive	Weak growth genotype growth with severe chlorosis /narrowing/burning of leaves
Highly sensitive	Very Weak growth genotype with complete chlorosis/narrowing/burning leading to mortality of most plants

In addition to the susceptibility score, plant height was measured immediately before spray (BS). Resistance to the herbicide was observed by measuring plant height as a sign of post-treatment growth after 15 and 45 DAS for the field experiment, or after 30 and 60 DAS for the greenhouse experiment of the spray. Stalling or stunting of plant growth was considered as susceptibility to the herbicide, while regular normal growth of any genotype was considered as resistance to the herbicide.

2.1. Statistical Analysis

Statistical analysis of the data was performed with Microsoft Excel and Genstat v12 and involved analysis of variance (ANOVA) to test for differences in plant height between genotypes at each measured timepoint in the field and greenhouse experiments. Histograms were constructed to show the frequency distribution of plant height and plant damage reaction scales. The dynamic development of plant height in each response category was assessed by comparing mean plant height across timepoints and using ANOVA to test the significance of differences between each category and the interaction between category and time point. The relationship between plant height and tolerance scores was assessed with least squares difference (LSD) tests of plant height in each category.

3. Results

3.1. Plant damage reaction scale

Based on the visual scoring at 45 DAS and classification, the herbicide tolerance score of the genotypes ranged from 1 to 5 in the field experiment. Out of 139 genotypes tested, one genotype (IMI-128) was scored as highly tolerant, 4 genotypes as tolerant (IMI-124, IMI-130, IMI-138 and IMI-139), 9 genotypes as moderately tolerant, 7 genotypes as sensitive, and the rest of the genotypes (124) were

highly sensitive (Table. 2) shows the results of variance analysis. The response of the genotypes for imazamox at 60 DAS indicated some differences in the herbicide tolerance scores among the genotypes with some recovery observed in plants possibly due to late rains in the field. Based on the second visual scoring at 60 DAS (Figure 2), it was observed that 3 genotypes (IMI-124, IMI-128, IMI-130) were highly tolerant, 7 genotypes (IMI- 125, IMI -132, IMI- 135, IMI- 136, IMI- 137, IMI- 138, IMI- 139), were tolerant, 8 genotypes were moderately tolerant, 19 sensitive, and the rest of the genotypes (108) were highly sensitive (Table 3).

The herbicide tolerance score of the genotypes in the greenhouse at 30 DAS ranged from 1.3 to 5.0. No genotype was scored as highly tolerant, 4 genotypes (IMI-128, IMI- 129, IMI- 130 and IMI-138) were tolerant, 11 genotypes were moderately tolerant, 4 genotypes were sensitive and the rest of the 126 genotypes were highly sensitive (Table 3). Similar to the field results, the second visual scoring at 60 DAS recorded that some genotypes showed recovery. According to the second visual scoring, it was observed that 2 genotypes (IMI-129, and IMI-130) were highly tolerant, 5 genotypes (IMI- 123, IMI- 124, IMI- 128, IMI- 138, IMI- 139) were tolerant, 10 genotypes were moderate tolerant, one genotype was sensitive, and the rest of the genotypes (127) were highly sensitive. (Table 3 and Figure 3).

For the control genotypes, little tolerance was observed against imazamox herbicide in field and greenhouse experiments. All these genotypes were highly sensitive and sensitive, except a Canadian genotype (CL-Lentil-4) that was moderately tolerant at first and second observation in the field and greenhouse experiments.

The ANOVA test (Table. 2) showed significant differences between field and greenhouse experiments. Most of the differences between field and greenhouse experiments were among sensitive and highly sensitive genotypes (Figure 4).

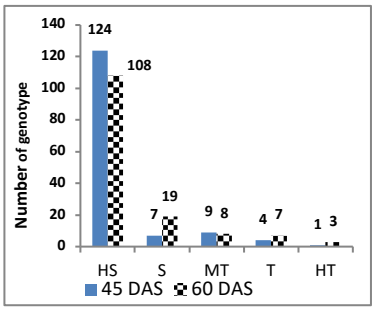

Figure 2. Frequency distribution of lentil genotypes for imazamox herbicide on a 1-5 scale in the field experiment.

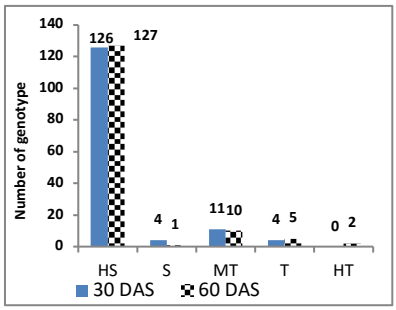

Figure 3. Frequency distribution of lentil genotypes for imazamox herbicide on a 1-5 scale in the greenhouse experiment.

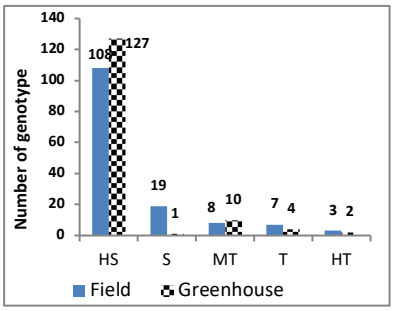

Figure 4. Frequency distribution of lentil genotypes for imazamox herbicide on 1-5 scale in field and greenhouse experiments, 60 DAS.

HS: Highly sensitive, S: sensitive, M: Moderately tolerant, T: Tolerant, HT: Highly tolerant.

Table. 2. Analysis of variance for scales according to location, genotype and replication in the field and greenhouse experiments

Source	DF	Sum of Squares	F Ratio	Prob > F
Location	1	139.58	395.01	<0.001*
Replication	2	3.87	5.48	0.043*
Genotype	144	1011.99	19.89	<0. 001*

Asterisks indicate significant ANOVA results.

3.2. Plant height

Visual symptoms started appearing on plants from about 15 DAS, where the imazamox herbicide killed the growing tips of the branches and affected the vegetative growth of the highly sensitive and sensitive genotypes. A high level of injuries on various plant parts was observed in highly sensitive genotypes that led to the death of many plants. According to the analysis of variance for plant

height, there were highly significant differences between the height of treated genotypes in the field and greenhouse experiments ($p<0.001$) at each timepoint (Tables 4 & 5).

Plant height in the field experiment at BS ranged from 2.33 to 5.70 cm (IMI-75and IMI-20, Figure 5). Plant height in the field at 15 DAS ranged from 0.00 (plants were dead) to 7.67 cm (IMI-68, IMI-127 Figure 6), while plant height in the field at 45 DAS ranged from 2.67 to 26.33 cm (IMI-86 and IMI-128, Figure 7), with only a few genotypes growing taller than 20 cm.

Plant height in the greenhouse experiment at BS was 7.33 to 12.00 cm (IMI-65 and IMI-141) (Figure 8), while at 30 DAS plant height ranged between 0.00 (plants were dead) for many highly sensitive and sensitive genotypes to 17.00 cm (IMI-123, and IMI-130, Figure. 9), and at 60 DAS, the range was 0.00 cm to 27.67 cm (CL-Lentil-4, Figure 10). Only a few tolerant and highly tolerant genotypes had plant heights greater than 15 and 20 cm at 30 and 60 DAS, respectively (Figure. 9 & 10).

The results indicated that all control genotypes showed low tolerance responses for plant height against imazamox herbicide, except CL-Lentil-4 that showed a high tolerance response for plant height against imazamox herbicide in both field and greenhouse experiments (Figures 11 & 12, Table 3).

Table7 indicates no significant differences for plant height between the field and the glasshouse, confirming that relative plant height per genotype is consistent across environments.

The correlation for plant height between each consecutive timepoint showed a significant increase between BS and 15 DAS, also a highly significant increase was between 15 DAS and 45 DAS in the field experiment, while in greenhouse no significant change between BS and 30 DAS was seen because strongly growing plants could still be herbicide sensitive, but a highly significant increase was seen between 30 DAS and 60 DAS because the tolerance continues across these timepoints (Table.6).

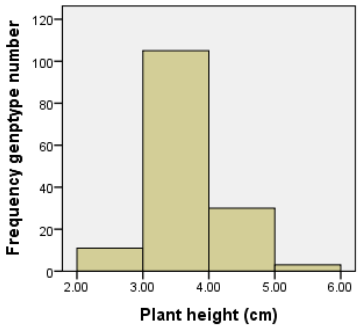

Figure. 5. Frequency distribution for plant height in field experiment before spray.

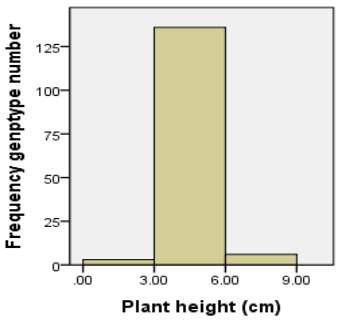

Figure.6.Frequency distribution for plant height in field experiment 15 DAS.

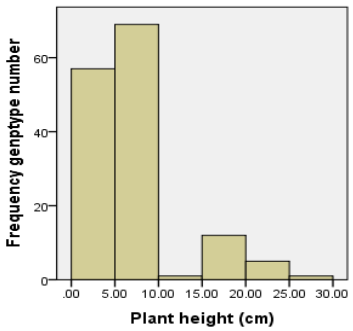

Figure 7. Frequency distribution for plant height in field experiment, 45 DAS.

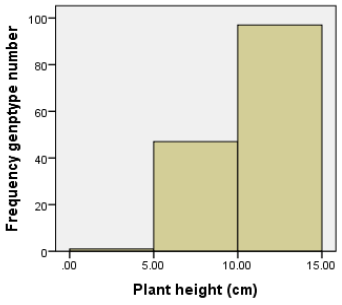

Figure 8. Frequency distribution for plant height in greenhouse experiment before spray.

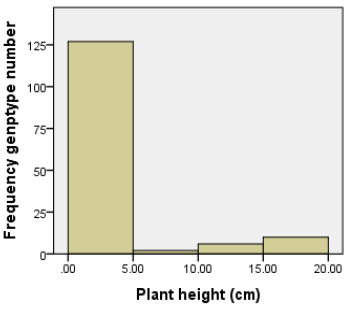

Figure 9. Frequency distribution for plant height in greenhouse experiment 30 DAS.

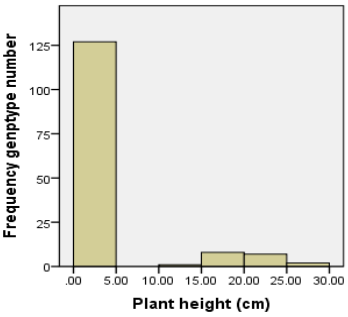

Figure 10. Frequency distribution o for plant height in greenhouse experiment 60 DAS.

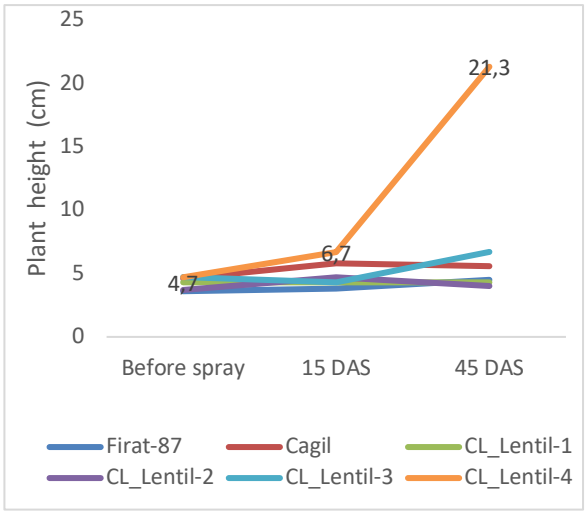

Figure 11. Response of plant height for control genotypes against imazamox herbicide in the field experiment.

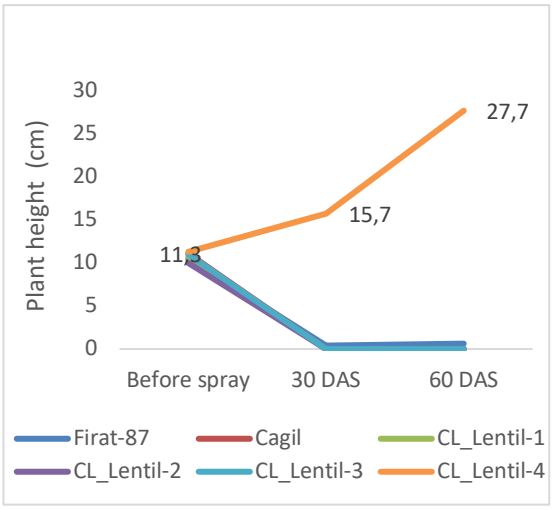

Figure 12. Response of plant height for control genotypes against imazamox herbicide in the greenhouse experiment.

Table 3. Damage scale and plant height responses of tolerant lentil genotypes and controls for imazamox herbicide in field and greenhouse experiments at different timepoints

Genotype		**1-5 (scale)**			**Plant height (cm)**						
		Field		Greenhouse	Field				Greenhouse		
		45 DAS	60 DAS	30 DAS	60 DAS	BS	15 DAS	45 DAS	BS	30 DAS	60 DAS
Firat-87	Control	HS	HS	HS	HS	3.60	3.80	4.50	10.50	0.40	0.60
Cagil	Control	HS	HS	HS	HS	4.60	5.80	5.60	11.10	0.00	0.00
CL_Lentil-1	Control	HS	HS	HS	HS	4.30	4.30	4.30	10.70	0.00	0.00
CL_Lentil-2	Control	HS	HS	HS	HS	3.70	4.70	4.00	10.00	0.00	0.00
CL_Lentil-3	Control	HS	HS	HS	HS	4.70	4.30	6.70	11.00	0.00	0.00
CL_Lentil-4	Control	MT	MT	MT	MT	4.70	6.70	21.30	11.30	15.70	27.70
IMI-123	M5	MT	MT	MT	T	3.30	5.30	15.70	11.70	17.00	21.70
IMI-124	M5	T	HT	MT	T	3.70	5.70	19.00	10.70	16.70	22.30
IMI-125	M5	MT	T	MT	MT	3.30	5.30	18.30	11.30	8.30	18.00
IMI-128	M5	HT	HT	T	T	3.70	6.70	26.30	10.30	16.30	21.30
IMI-129	M5	MT	MT	T	HT	3.00	5.00	20.70	9.70	16.70	25.00
IMI-130	M5	T	HT	T	HT	4.30	7.70	23.00	9.70	17.00	24.00
IMI-132	M5	MT	T	MT	MT	2.33	4.33	17.33	9.00	12.67	16.67
IMI-135	M5	MT	T	MT	MT	3.00	5.00	18.30	10.30	13.70	18.30
IMI-136	M5	MT	T	MT	MT	3.00	5.00	19.30	9.30	15.00	19.30
IMI-137	M5	MT	T	MT	MT	3.70	5.30	19.70	11.30	15.30	20.00
IMI-138	M5	T	T	T	T	3.30	6.00	23.00	10.70	15.30	22.30
IMI-139	M5	T	T	MT	T	3.30	5.70	21.70	11.00	16.00	21.70

BS: Before spray, DAS: Days after spray, HS: Highly sensitive, S: sensitive, M: Moderately tolerant, T: Tolerant, HT: Highly tolerant.

Table 4. Analysis of variance for plant height according to genotype and replication in the field experiment

Source of variation		BS			15 DAS			45 DAS		
	DF	MS	F	F pr.	MS	F	F pr.	MS	F	F pr.
Genotypes	144	0.89	1.34	<0.001**	2.45	2.23	<0.001*	75.94	16.59	<0. 001**
Replications	2	11.57	18.21	<0.001**	44.17	40.21	<0.001*	293.24	64.07	<0. 001**

Table 5. Analysis of variance for plant height according to genotype and replication in the greenhouse experiment

Source of variation	DF	BS MS	F	F pr.	30 DAS MS	F	F pr.	60 DAS MS	F	F pr.
Genotypes	144	2.83	2.40	<0.001**	71.40	43.44	<0.001**	140.02	38.80	<0.001**
Replications	2	25.27	21.52	<0.001**	2.30	1.823	0.16	7.63	2.13	0.12

Table 6. The correlation for plant height between each consecutive time point in the field and greenhouse experiments

Field experiment				Greenhouse experiment			
Correlations	**BS1**	**15 DAS**	**45 DAS**	**Correlations**	**BS2**	**30 DAS**	**60 DAS**
Bs1	1	0.146*	0.17*	**Bs2**	1	0.11	0.08
15 DAS		1	**0.57****	**30 DAS**		1	**0.98****
45 DAS			1	**60 DAS**			1

*Correlation is significant at the 0.05 level ** Correlation is significant at the 0.01 level.

Table 7. Analysis of variance for plant height according to the location on, genotype, and replication in the field and greenhouse experiment

Source	DF	Sum of Squares	F Ratio	Prob > F
Location	1	4.72	0.25	0.62
Replication	2	436.46	11.48	<0.001**
Genotype	144	21307.72	7.79	<0.001**

3.3. The dynamic development of plant height in each response category against imazamox herbicide

Analysis of variance for the interaction between category and timepoint showed highly significant differences ($p<0.001$) in plant height for response categories, timepoint, and category × timepoint interaction in the greenhouse and the field experiments (Table. 8). These are due to differences in the response of these categories against imazamox herbicide in field and greenhouse experiments. Also, Table. 9 indicates significant differences between response categories for plant height in the field and greenhouse experiments, except between tolerant and moderately tolerant in field and between highly tolerant and tolerant in the greenhouse.

Figure 13 shows the dynamic development of average plant height by time for each imazamox damage response category in the field experiment. Firstly, before spray, the average plant height for all categories was about 4 cm. Secondly, at 15 DAS, some differences between categories were observed, that the average plant height for highly tolerant and tolerant categories was mostly 6.67 cm, moderately tolerant category was 5.30 cm, while the sensitive and highly sensitive categories were about 4.00 cm. Thirdly, at 45 DAS, large differences between categories were observed, where the average of plant height for; highly tolerant, tolerant, moderately tolerant, sensitive, and highly sensitive categories were respectively; 24.11, 22.33, 18.70, 8.50, and 5.12 cm.

Also, Figure 14 indicates large differences in average plant height of genotypes in each response category against imazamox herbicide in the greenhouse experiment at 30 and 60 DAS. The average plant height before spray for all categories was about 10 cm. While at 30 DAS, the average of plant height in each category; highly tolerant, tolerant, moderately tolerant, sensitive, and highly sensitive, was respectively; 16.83, 15.38, 13.17, 5.33, and zero cm. Finally, at 60 DAS, the average plant height for each respective category was; 24.50, 21.10, 18.70, 12.67, and zero cm.

Table 8. Analysis of variance for the interaction between category and timepoint in the field and greenhouse experiment

Source	DF	Field experiment			Greenhouse experiment		
		Sum of Squares	F Ratio	Prob >F	Sum of Squares	F Ratio	Prob > F
Category	4	1357.04	274.48	<0.001**	6606.41	1748.90	<0. 001**
Timepoint	2	2617.18	1058.71	<0.001**	279.02	147.73	<0. 001**
Category× Timepoint	8	1874.90	189.61	<0.001**	3471.4	459.49	<0. 001**

Table 9. Significant differences between categories for plant height, in-field and greenhouse experiment

	Category	Field experiment		Greenhouse experiment	
		LSD group	Mean plant height (cm) /category	LSD group	Mean plant height (cm) /category
1	Highly tolerant	A	11.09	A	17.00
2	Tolerant	B	9.35	A	16.33
3	Moderately tolerant	B	8.79	B	14.37
4	Sensitive	C	4.95	C	9.33
5	Highly sensitive	D	4.11	D	3.35

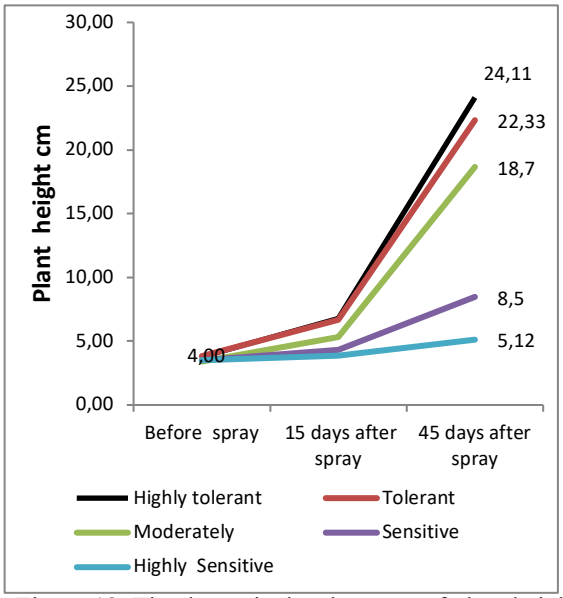

Figure 13. The dynamic development of plant height in each category against imazamox herbicide in the field experiment.

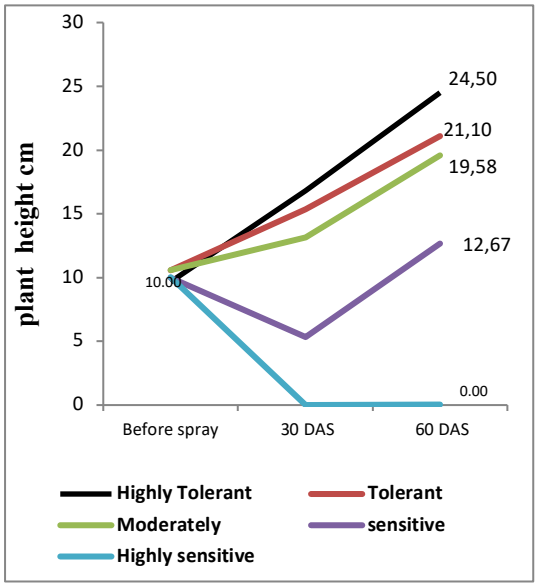

Figure 14. The dynamic development of plant height in each category against imazamox herbicide in the greenhouse experiment.

4. Discussion and Conclusion

Lentils are a weak competitor of weeds and their sensitivity to herbicides is a major hurdle for large scale production. It is crucial to control the sensitivity of lentil to herbicides because the selection of herbicides targeting only weeds is difficult to achieve. Due to the limited responsiveness of leguminous crops to transformation, scientists have instead tried seed mutagenesis to develop imidazolinone resistant crops, with many mutagens being used with seeds in different crops, including physical (gamma irradiation) and chemical (ethyl methanesulfonate, N-nitroso-N-methylurea, ethylnitrosourea and sodium azide) treatments. For example, Malkawi et al., (2003) treated lentil cultivars with gamma radiation to develop tolerance against chlorsulfuron herbicide. In the present study, chemical mutagen EMS (ethyl methane sulfonate) was used to create variability in the genotypes.

The resulting lentil genotypes were evaluated for enhanced tolerance against imidazolinone herbicide. The screening and selection of imidazolinone herbicide resistant mutants is a necessary first step for further plant breeding efforts.

In the present study, the EMS mutagenized lentil genotypes showed a range of different responses against imazamox herbicide measured on a 1-5 scale and as plant height in both field and greenhouse experiments. Differences in induced mutations of these genotypes is a likely reason for the observed variability intolerance as evidenced by the consistent results across replicates, experiments and timepoints. In earlier studies, the classification of tested lentil genotypes into different categories revealed considerable genetic variations in tolerance to imazethapyr herbicide, such as in lentil (Sharma et al., 2018; Rizwan et al., 2017), chickpea (Gaur et al., 2013; Chaturvedi et al., 2014) and ryegrass (Preston and Powles 2002). Our findings are consistent with that of Taran et al., (2010) who showed the existence of a significant range of natural differences for resistance to the IMI class of herbicides (imazethapyr and imazamox) in chickpea (Taran et al., 2010) and field pea (Hanson and Thill 2001). A possible explanation for the different levels of resistance in different varieties might be the different levels of resistance in different genotypes which can be attributed to differential metabolic degradation rates (Sharma et al., 2018).

This study indicated that EMS is an efficient tool to develop herbicide-resistant genotypes in lentil. In particular, the second visual scoring revealed that some genotypes showed symptoms of recovery in the field experiment and greenhouse experiment. A similar study performed by Sharma et al. (2018) screening 180 lentil genotypes for tolerance against imazethapyr herbicide by visual scoring to determine the resistance at 14 and 45 DAS indicated that some lentil genotypes showed recovery in the second visual scoring. This result corroborates our findings that some mutant genotypes can show recovery after herbicide spray.

Lentil genotypes showed a range of different responses against imazamox herbicide in both field experiments and greenhouse experiments. The field and greenhouse experiments had contrasting environments (i.e the greenhouse was protected from the rain while the field was not). The change in the category of a few genotypes between field and greenhouse experiments appears to be due to environmental conditions such as late rains in the field experiment, which might have reduced the efficacy of the herbicide. The limited difference in genotypes' relative imazomox tolerance were observed between the field and greenhouse experiments, indicating that these differences were under genetic control. The significant genetic differences determined in these lentil genotypes for imazamox herbicide resistance will encourage further study efforts towards the development of herbicide-tolerant varieties.

Five genotypes (IMI-124, IMI-128, IMI-130, IMI-138, and IMI-139), were observed to have a high tolerance response for imazamox herbicide in the field and greenhouse experiments. These genotypes can be used in future breeding programs for creating herbicide-resistant lentil varieties. Based on the second visual scoring, some studied genotypes showed some recovery in both locations. The herbicide-tolerant genotypes that have been examined in this study would be helpful in genetic and physiological studies aimed at determining the molecular mechanisms of imazamox herbicide tolerance. .3.2000Greater knowledge about the mechanisms of imazamox herbicide tolerance could facilitate future progress in the development of herbicide tolerant lentil cultivars.

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