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Research Article

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Evaluation of Possibilities to Improve Herbicide Tolerant Genotype in Cotton (*Gossypium hirsutum* L.) through Mutation Breeding

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Abstract The ethyl methane sulfonate (EMS) have been used for tolerance to the imidazolinone (IMI) class of herbicides in cotton. The probability of improving IMI tolerant cotton genotypes using both EMS and physical mutation was investigated in M_1 and M_2 generations. Imazamox was sprayed to plants at the early seedling stage and, tolerant cotton plants were observed for yield components and fiber quality after herbicide treatment. The EMS and increasing doses of Cobalt-60 negatively affected the germination and emergence rate in cotton. The genetic analysis using chi-square shown that a single and partially dominant gene was found the preponderance of tolerance to imidazolinone of cotton. The EMS and gamma rays with high-level imazamox decreased plant height, number of bolls, boll weight and fiber fineness. It was concluded that mutation via both EMS and gamma rays could be used in the breeding of IMI tolerant cotton.

Keywords Boll weight, fiber quality, imidazolinone, mutation, number of bolls

1. Introduction

Turkey is in a very favourable position for cotton growing due to its climate zone. For the 2020/21 season, Turkey ranks 9th in the world in terms of cotton cultivation area, 2nd in fiber cotton yield per unit area, 6th in cotton production and 5th in cotton imports [17].

Successful weed control is necessary to grow productive and high-quality cotton in the growing areas. It is known that weeds are one of the most important problems encountered in cotton growing and the increasing water deficiency increases the importance of controlling weeds. On the other hand, due to intensive labour and input costs, cotton is planted in high density and weed control with dilution/misting and manual hoeing is replaced by chemical control [1].

Imidazolinone (IMI) group herbicides are applied against important broadleaf and summer weeds such as common lambsquarters (*Chenopodium album*), rough cocklebur (*Xanthium strumarium*), jimsonweed (*Datura stramonium*) and black nightshade (*Solanum nigrum*). It has been determined that the use of this group of herbicides in cotton farming has many advantages. First of all, Imidazolinone herbicide groups have a low usage rate and residue amount (1-50 ppb), therefore it is the herbicide group with the least environmental risk [12]. Later, it was stated that the cotton was not damaged although imidazolinone herbicide was applied in the previous planting area during the rotation [16]. In addition, possible IMI tolerant cotton is an important factor in the development of no-till farming practices, resulting in soil and soil moisture management and low energy use [2].

IMI group herbicides (imazapyr, imazapic, imazethapyr, imazamox, imazametabenz and imazakine) are called acetolactate synthase (ALS) inhibitors. They control acetohydroxyacid enzyme synthesis (AHAS), which is a critical enzyme for the biosynthesis of amino acids [13]. Resistance to IMI group herbicides has been established in many cultivars and commercial seeds of this group of plants are named as Clearfield ®. It has been demonstrated that IMI resistance can be achieved in cotton as a result of mutation and that this group of herbicides can be used easily [3, 4]. On how resistance is formed in cultivated plants, it was emphasized that alkylating agents such as EMS (ethyl methane sulfonate) affect DNA bases, causing false base pairings or disrupting the structure of DNA [14]. When the structure of DNA is disrupted, repair mechanisms such as excision or misrepair come into play, and it has been determined that these repair mechanisms cause mutations while working. They reported that EMS reacts with guanine to form O6 – ethyl guanine. The resulting O6 – ethyl guanine base pairs with thymine and forms the CG → TA exchange.

The objectives of this research were to evaluate the possibilities of imidazolinone (IMI) tolerant genotypes in chemically (ethyl methane sulfonate) and gamma rays mutated population of cotton (*Gossypium hirsutum* L.) upland cotton and to conduct preliminary work on the inheritance of imidazolinone tolerance.

2. Materials and Methods

Non-delinted seeds of Gloria cultivar (*Gossypium hirsutum* L.) was used as material for different gamma rays (Gy) and ethyl methane sulfonate (EMS). Nine hundred seeds for each application were irradiated with gamma rays doses (100, 200 and 300 Gy) from cobalt 60 as physical mutagen at The Nuclear Research and Training Centre Saraykoy-Ankara Turkey. Also, 900 non-delinted seeds were treated with 0.5% v/v ethyl methane sulfonate. For EMS application, seeds were firstly imbibed in distilled water for 18 hours. In the next process, the material was treated with EMS for 4 hours. After the treatment with EMS, the seeds were washed in tap water for 4 hours, then dried on blotting paper for 1 hour and immediately planted in the field.

The field experiments were conducted in Nazilli Cotton Research Institute during the period from 2016 to 2017. The M_1 plants were planted to treated imazamox herbicide and to produce tolerant M_2 seeds in the field. In 2016, 900 seeds for each mutagen application and imazamox application (totally 9000 seeds) were planted in 15 rows plot of 12 m. Five rows non-treated with imazamox of EMS, 100 Gy, 200 Gy and 300 Gy were evaluated as control. The imazamox was sprayed to emerged and healthy plants after EMS and physical mutation at 1250 ml ha^{-1} (5 rows) and 2500 ml ha^{-1} (5 rows) at the stage of 5-6 true leaves. The levels of injury were observed at 14 days after imazamox herbicide and IMI tolerant and susceptible plants were counted. The number of bolls $plant^{-1}$, boll weight (g), plant height (cm), ginning out-turn (%), fiber fineness (mic.), fiber length (mm) and fiber strength (g tex^{-1}) were measured in the harvest period. All plants from M_1 generation was transferred to M_2 generation in 2017 and the all process for 2016 were repeated for 2017 as well.

The chi-square test was used to estimate plausible allelic ratio using the number of tolerant and susceptible plants after imazamox herbicide in the M_1 generation. The data obtained from M_1 and M_2 for measured characteristics were compared with t-statistics using Microsoft Excel. The mean value, variance and variance coefficient of M_1 and M_2 generation were estimated.

3. Results and Discussion

A total of 353 (39.2%), 343 (38.1%), 322 (35.7%) and 235 (26.1%) individual seeds for EMS, 100 Gy, 200 Gy and 300 Gy, respectively, germinated and remained viable until the period of 5-6 leaves (untabulated data). The increasing doses of EMS and Cobalt-60 negatively affected the germination and emergence rate in tomato [7]. In studies on cotton, as the amount of mutagen dose applied increased, the survival rate decreased, that is, the seeds were more damaged by the rays, and thus the physiological damage to the plants increased [5]. On the other hand, it was revealed that increasing gamma doses in cotton did not affect the germination and emergence rate [15]. In our study, it is clearly seen that the germination and emergence rate decreased below 40% and this value was 26.1% with increasing doses.

The results of chi-square analysis for 2500 ml imazamox application because of clearly distinguishing between tolerant and sensitive were given in Table 1. According to the results of the chi-square test, non-significant coefficients exhibited that IMI tolerance controlled a single, partially dominant gene [3, 18, 19]. Unlike similar studies, the confirmation of one dominant gene by physical mutation results other than EMS can be seen as the original finding of our study.

Table 1: Chi-square analysis of individual plant ratings at 14 days after application in M_1 populations tested against an expected 3:1 ratio.

Mutation	Observed		Expected		χ2
	Tolerant	Susceptible	Tolerant	Susceptible	
EMS	96	257	87	263	1.06 ns
100 Gy	94	249	85	255	1.09 ns
200 Gy	77	245	80	240	0.21 ns
300 Gy	65	170	59	176	0.81 ns

The average plant height treated with EMS and Cobalt-60 was 63.17 cm. The lowest plant heights were obtained in both the chemical mutagen treatment and the 2500 ml ha^{-1} imazamox-treated plots of the lower doses of physical treatment (44, 42, 36 and 36 cm, respectively). This difference resulted in high variance (465.64) and coefficient of variation (35.68%) values. It is noteworthy that the highest plant height decrease was obtained from the parcels that were treated with mutagen at a high dose of 300 Gy and imazamox at a high dose of 2500 ml ha^{-1}. Since imazamox was not applied to the parcels with M_2 mutagen applications, the average of this year's average was 106.67 cm, the variance was 66.89 and the coefficient of variation was 8%. The difference between the mean of M_1 data and the mean of M_2 was found to be statistically significant (t probability = 0.001). As a result, imazamox application created a strong pressure on plant heights as expected, whereas, in 2017, it can be said that this decrease in plant height disappeared in plants that survived 2016. Despite all this, less plant height was detected in the plants transferred to the next year from the plots with high dose mutagen applications and 2500 ml da^{-1} imazamox applied in both chemical and physical mutagen applications. This situation can be clearly observed from the images of the M_2 population. It was stated that mutagens cause positive or negative effects on plant height in cotton as in other plants, or in other words, they create variations in the genetic structure [6, 8]. In this study, it is clearly seen that the plant height decreases as the mutagen doses increase.

It is seen that the number of bolls in M_1 varies between 8 and 54 (Table 2). The overall mean of boll number for the applications was found to be 23.67 and the variance was 247.55 and the coefficient of variation was 69.43%. In M_2, the change interval was narrowed and the number of bolls was changed from 25 to 44. The coefficient of variation decreased to 17.26% in 2017. When the mean of M_1 and its offspring M_2 were compared, the difference was found to be statistically significant. It can be said that the fact that the spacing of the plants on the row is not uniform in M_2, especially in M_1, and the difference in the number of plants that survived in 2016 is high, caused the large variability in the number of bolls. In addition, mutagen applications also showed an effect on increasing the number of bolls. Especially in mutagen applications, the higher number of bolls in M_2 of subjects treated with 2500 ml da^{-1} imazamox apart from the 300 Gy dose proves this. It was revealed that different doses of chemical (EMS) and physical (Cobalt-60) mutagens positively affect the number of bolls in the plant and the number of bolls in the plant increases in mutant plants [9, 10]. Similarly, in this study, it was observed that the number of bolls in the plant was 35 on average, especially in M_2.

Table 2: The yield components of performances of EMS and physical mutation after imazamox in M_1 and M_2 generation in 2016 and 2017

Tre.	Imaz.	Plant Height (cm)		Number of bolls / plant		Boll Weight (g)		Ginning (%)	Out-turn
		M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
EMS	-	99	115	54	28	4.6	5.8	41.65	39.51
EMS	1250	77	104	33	30	4.6	5.6	40.68	36.08
EMS	2500	44	100	21	38	4.2	5.6	39.87	40.33
100 Gy	-	93	120	46	40	4.5	5.3	41.62	40.65
100 Gy	1250	66	101	41	40	4.6	4.7	40.31	40.35

100 Gy	2500	42	100	21	44	3.5	4.7	38.97	41.00
200 Gy	-	82	123	24	37	4.3	5.6	40.74	40.21
200 Gy	1250	54	100	12	35	4.6	5.1	39.53	40.24
200 Gy	2500	36	100	4	39	3.2	4.9	31.53	41.32
300 Gy	-	80	110	12	35	4.1	5.0	40.67	39.19
300 Gy	1250	49	108	8	27	3.8	4.7	38.48	38.72
300 Gy	2500	36	99	8	25	3.5	4.9	31.97	38.50
Average		63.17	106.67	23.67	34.83	4.13	5.16	38.84	39.68
Variance		465.64	66.89	247.55	33.13	0.32	0.15	10.87	1.87
CV (%)		35.68	8.00	69.43	17.26	12.04	7.86	8.86	3.59
t possibility		0.001		0.021		0.002		0.233	

The boll weight varies between 3.2 – 4.6 g and 4.7 – 5.8 g in M_1 and M_2 populations, respectively. Boll weight averages were 4.13 and 5.16 g. There was a high variance element in M_1 and it was statistically different from the mean value of M_2. It is noteworthy that as both EMS and physical doses increase, the boll weight decreases. Similarly, when imazamox doses were added to all mutagen applications, it was determined that the boll weight decreased. It was emphasized that in mutant lines with different genetic content, especially in plants treated with EMS, boll weight increased compared to both control and 100 and 200 Gy doses [10]. In parallel, in our study, it was found that the number of boll increased and there were differences between applications in terms of boll weights.

The GOT values in M_1 varied between 31.97% and 41.65%, the variance is 10.87 and the coefficient of variation is 8.86% because of high differences among applications. Among these values, the highest GOT was observed only in plants with EMS (41.65%) application. This high GOT was followed by 100Gy (41.62%). The lowest GOT was 200 Gy + 2500 ml ha^{-1} imazamox (31.53%). When evaluating the GOT in M_2, it is clearly understood that the values are closer to each other since the variance is 1.87 and the coefficient of variation is 3.59%. The highest value was recorded in 200Gy + 2500 ml ha^{-1} imazamox (41.32%) and the lowest value belongs to EMS + 1250 ml ha^{-1} imazamox (36.08%). It was determined that the difference between the means of both generations was not significant (t probability = 0.233). When the data of M_1, in which imazamox was added to mutagen applications in terms of GOT, are examined, it is noteworthy that only mutagen-treated plants are higher, whereas GOT decreases with imazamox application. On the other hand, the GOT of 41.32% in the progeny of plants treated with 200 Gy + 2500 ml/da imazamox in M_2 indicates that there is a variation that can be considered positive in terms of GOT. In a similar study, it was stated that the differences in GOT were not significant in the M_4 generation between different mutant lines treated with EMS [3]. Moreover, it was stated that the GOT increased from 36.5% to 46.5% in mutant lines in the M_4 generation but without imazamox application [9].

As for the fiber fineness, among the examined fiber samples, 300 Gy + 1250 ml ha^{-1} imazamox (2.73 mic.), 300 Gy + 2500 ml ha^{-1} imazamox (2.74 mic.) and EMS + 2500 ml ha^{-1} imazamox (2.37 mic.) cottons gave results well below the normal values (Table 3). Especially when high mutagen doses were combined with 2500 ml ha^{-1} imazamox application, immature fibers were encountered. When the physical mutagen application was compared among themselves, 200 Gy (5.34 mic.) gave the coarsest fiber. This difference in M_1 can also be understood from the coefficient of variation, which was found to be 31.31%. The evaluations made in terms of fiber fineness in M_2 were within the standard values. Among these results, which are close to each other, the lowest value still belongs to 300 Gy + 1250 ml ha^{-1} imazamox (4.01 mic.). The highest fiber fineness value was found at 100 Gy (5.26 mic.). Results were evaluated with the t-test and the difference between M_1 and M_2 populations was not found to be significant (t probability = 0.09). It was cited stated that 85 mutant lines in the M_3 generation continued their characteristics in the M_2 generation, while 31 mutant lines were superior in terms of yield and ginning efficiency, while 13 mutant lines were superior in terms of yield ginning and fiber quality characteristics [5]. Similarly, fiber coarseness of mutant lines was detected significantly increasing [9], whereas non-significantly differences in plants treated with imazamox on mutant lines were determined [3]. It was concluded that there is a variation that can be used in our study, but most importantly, the values within commercial limits (3.9 - 4.9 mic.) are also promising in mutagen + imazamox applied plants.

When the fiber length values were evaluated in our study, it was determined that the worst values in M_1 ranged between 200 Gy + 2500 ml ha^{-1} imazamox (28.00 mm) and 200 Gy (31.50 mm). Also, the coefficient of variation and variance was very low, such as 3.47% and 1.01. Although values improved in M_2 compared to M_1 results, 100 Gy (28.99 mm) remained at lower results. The highest value in M_2 was seen at 300 Gy + 1250 ml ha^{-1} imazamox (31.61 mm). When the applications are evaluated within themselves, the EMS application (non-treated herbicide) gave higher results than the EMS + 1250 ml ha^{-1} imazamox (30.37 mm) and EMS + 2500 ml ha^{-1} imazamox (30.9 mm) applications. When both years are compared statistically, it is seen that the difference between the means is not significant (t probability = 0.152). It was concluded that the applications did not cause significant differences in fiber length values. The use of mutation-inducing factors in appropriate doses and times caused the fiber length to be affected positively or negatively in cotton, or in other words, it created variations in the genetic structure, but this result could not be reached in terms of fiber length in our study [6, 8]. However, in a similar study [3], it was emphasized that the difference in fiber length was not significant in mutant lines.

Table 3: The quality characteristics of performances of EMS and physical mutation after imazamox in M_1 and M_2 generation in 2016 and 2017

Tre.	**Imaz.**	**Fiber Fineness (mic.)**		**Fiber Length (mm)**		**Fiber Strength (g tex^{-1})**	
		M_1	**M_2**	**M_1**	**M_2**	**M_1**	**M_2**
EMS	-	5.36	4.67	30.68	31.06	35.2	36.3
EMS	1250	4.66	4.03	30.38	30.37	40.2	32.
EMS	2500	2.37	4.08	31.41	30.90	31.2	35.4
100 Gy	-	5.49	5.26	30.94	28.99	38.1	34.9
100 Gy	1250	4.19	4.47	31.33	30.55	40.2	35.5
100 Gy	2500	3.31	4.07	29.63	31.53	34.9	37.8
200 Gy	-	5.34	4.41	31.50	30.83	37.2	37.8
200 Gy	1250	3.73	4.69	31.40	31.32	35.8	35.4
200 Gy	2500	2.53	4.78	28.00	30.12	30.0	36.9
300 Gy	-	5.53	4.75	29.97	30.70	37.9	38.8
300 Gy	1250	2.73	4.01	29.83	31.61	40.2	37.1
300 Gy	2500	2.74	4.42	29.45	31.45	31.2	38.9
Average		4.00	4.47	30.38	30.79	36.01	36.45
Variance		1.43	0.13	1.01	0.49	12.19	3.01
CV (%)		31.31	8.56	3.47	2.39	10.10	5.00
t possibility		0.09		0.15		0.372	

The highest fiber strength recorded in EMS + 1250 ml ha^{-1} imazamox (40.2 g tex^{-1}), 100Gy + 1250 ml ha^{-1} imazamox (40.2 g tex^{-1}) and 300Gy + 1250 ml ha^{-1} imazamox (40.2 g tex^{-1}) in M_1. On the other hand, the lowest values were observed in 200Gy + 2500 ml ha^{-1} imazamox (30.0 g tex^{-1}) and 300Gy + 2500 ml ha^{-1} imazamox (31.2 g tex^{-1}) applications. 12.19 variance and 10.10% coefficient of variation show significant differences in mutagen + imazamox applications in M_1. When the M_2 results are examined, it is seen that fiber strength varied between 32.6 g tex^{-1} and 38.9 g tex^{-1}, average was 36.45g tex^{-1}. The variance value was 3.01 and the coefficient of variation was 5.00%, which indicates that the differences have become stationary in the applications. It is understood from the t statistics that the mean difference in M_1 and $M_2$was not significant (t probability = 0.372). The fiber strength results in our study are in agreement with the results found by [3], who stated that there was no difference in fiber quality in the M_4 generation of plants treated with mutagen + imazamox.

4. Conclusions

The result of the chi-square test exhibited that IMI tolerance conferred by a single and partially dominant gene. Moreover, possible tolerance genes were present at the same locus or closely linked. This study was revealed that both chemical and physical mutation sources could be used in the breeding of IMI tolerant cotton genotype development together with imazamox applications. Especially, it was concluded that the selection of IMI tolerant single plants with high yielding and fiber quality from mutated seeds could be an indicator of success in breeding.

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