Yield and Fiber Quality Characteristics of Mutation-Based IMI Tolerant Cotton (*Gossypium hirsutum* L.) Lines

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Abstract: The economical and effective weed control in cotton can succeed by breeding IMI-tolerant genotypes. For this purpose, we treated seeds with gamma rays at different doses and sprayed the plants with imazamox in M_1 - M_5 generations. Thirty-three M_4 lines in 2020 and seventeen M_5 lines in 2021 with two comparative varieties were arranged in a Randomized Complete Block Design with three replications. M_4 lines generally had superior fiber properties than standard varieties. We transferred 17 M_4 lines, superior in terms of yield, ginning out-turn and fiber quality, to the M_5 generation. All M_5 lines exhibited finer fibers than comparative varieties. Seed cotton yield and ginning out-turn of eight M_5 lines were superior to the comparative varieties. Five IMI-tolerant genotypes with high yield and favourable fiber quality were transferred to further generations.

Keywords: Fiber quality, gamma rays, IMI-tolerant, mutation, variation

Mutasyon Yoluyla Elde Edilmiş IMI Toleranslı Pamuk (Gossypium hirsutum L.) Hatlarının Verim ve Lif Kalite Özellikleri

 $\ddot{o}z$: IMI-toleranslı pamuk ıslahı ile pamukta etkin ve ekonomik bir şekilde yabancı ot mücadelesi gerçekleştirilebilir. Bu amaçla pamuk tohumları farklı dozlarda gama ışınları ile muamele edildi ve M₁-M₅ generasyonlarında bitkilere imazamox uygulandı. 2020 yılında 33 M₄ ve 2021 yılında 17 M₅ hattı 2 kontrol çeşit ile birlikte 3 tekerrürlü olarak Tesadüf Blokları Deneme Desenine göre ekildi. M₄ hatlarının kalite özellikleri çoğunlukla kontrol çeşitlerden daha üstün bulundu. Verim, çırçır randımanı ve lif kalite özellikleri yönünden 17 M₄ hattı M₅ generasyonuna aktarıldı. Tüm M₅ hatlarının lif incelikleri kontrol çeşitlerden daha düşük bulundu. Benzer şekilde 8 M₅ hattının kütlü pamuk verimi ve çırçır randımanı performansı kontrol çeşitlerden daha yüksek olarak belirlendi. IMI-toleranslı ve verim ve lif kalite özellikleri üstün 5 hattın sonraki generasyonlara aktarılması gerektiği sonucuna varıldı.

Anahtar kelimeler: Gama ışınları, IMI-toleranslı, lif kalite özellikleri, mutasyon, varyasyon

INTRODUCTION

Although imidazolinone (IMI) herbicides have controlled weeds in many IMI-tolerant crops, they have not been found to be used in cotton farming (Tcach et al., 2022). Imidazolinone (IMI) is a group of herbicides that inhibit acetolactate synthesis (ALS). This enzyme (also called acetohydroxyacid synthase; AHAS) is involved in the first step of valine, leucine and isoleucine synthesis. Low doses of IMI group herbicides are effective on grass and broadleaf weeds and are defined as environmentally friendly herbicides. IMI group herbicides such as imazapyr, imazapic and imazamox controlled the important broadleaf and summer weeds such as common lambsquarters (Chenopodium album), rough cocklebur (Xanthium strumarium), jimsonweed (Datura stramonium) and black nightshade (Solanum nigrum). Therefore, improving IMItolerant crops has been recognized as an important strategy for effective weed control (Tan et al., 2005).

The use of IMI-tolerant cotton varieties in cotton farming will positively affect the success of weed control (Bechere et al., 2009). At the same time, cotton can be included in the crop rotation with IMI-tolerant crops (York et al., 2000). Maize (*Zea mays* L.), canola (*Brassica napus* L.), sunflower

(Helianthus annuus L.), soybean (Glycine max L.), and wheat (Triticum aestivum L.) are important crops that have recently developed resistance to AHAS inhibitory herbicides using various methods (Ustun and Uzun, 2023). Previous studies show that it is possible to confer herbicide resistance to cultivated plants by mutation of ALS genes as a result of amino acid substitution (Sala et al., 2012; Li et al., 2015; Hu et al., 2017; Zhang et al., 2019; Li et al., 2020; Chen et al., 2021). Although IMI-tolerant cotton cultivars are not yet registered, some mutant lines that can be used in obtaining IMI-tolerant commercial cotton cultivars were obtained (Bechere et al., 2009; Cutts, 2013; Tcach et al., 2022). However, it was emphasized that plant growth and vigour were adversely affected by mutations due to negative pleiotropic effects (Vila-Aiub et al., 2009).

We conducted this study to test IMI tolerant lines of M_4 and M_5 generations for yield and quality traits. In addition, we also planned to test the selection success in terms of the examined traits.

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MATERIAL AND METHODS

Seeds of standard cotton cultivar Gloria (Gossypium hirsutum L.) were irradiated with 100, 200 and 300 Gy gamma rays. Results of M1 and M2 generation have been reported by Altintas and Unay (2021). At the M₃, M₄ and M₅ generations, mutant plants were grown in Nazilli Cotton Research Institute, and at a dose of 1250 ml/ha, imazamox was sprayed to plants at the stage of 5-6 true leaves emerged. During the M₄ generation, 33 mutant lines were selected, and 17 of their mutant lines, superior in yield, ginning out-turn and fiber quality were transferred to the M₅ generation. In this study, 33 M₄ and 17 M₅ mutant lines and two comparative cultivars, ST-468 and Gloria, were used as genetic material according to the method applied by Key et al. (1998) and Jankowicz-Cieslak et al. (2017). Mutant lines were planted in a Randomized Complete Block Design with three replications. Each plot consisted of 4 rows of 12 m in length. The distances between rows and plant to plant were 0.7 m and 0.20 m, respectively. Standard cultural management for regional cotton growing was fulfilled. Twenty plants were randomly sampled in the harvest period. Seed cotton yield per plant (SCY; g), ginning out-turn (GOT; %), fiber fineness (FF; mic.), fiber length (FL; mm) and fiber strength (FS; g tex⁻¹) were measured. The fiber quality traits were determined by Uster[®] High Volume Instrument (HVI) 1000 (USTER Technologies, Inc., Knoxville, TN, USA). Data for each trait were analysed using two-way ANOVA according to Randomized Complete Block Design using the 'agricolae'

package (de Mendiburu and de Mendiburu, 2019) in R studio. Tukey's HSD (Honestly Significant Differences) test with a probability level of P = 0.05 was used to compare genotypes (Tukey, 1949).

RESULTS AND DISCUSSION

Stunted shoots and reddening of leaf veins are the most common symptoms after imazamox treatment (Bechere et al., 2009), and original images of these symptoms are presented in Figure 1. These symptoms were not observed in M₄ and M₅ plants in our study. We compared mutant lines with standard check varieties, Gloria and St-468. In the M₄ generation, genotypic differences were significant for all observed traits. The seed cotton yield per plant of all mutant lines except GR-200 V and GR-200 VIII was significantly lower than that of standard varieties. Mutant lines GR-100 VI, GR-100 III, GR-200 X and GR-200 XI had higher GOT than standard varieties. Regarding fiber length, 15 of the 33 mutant lines performed better than the standard varieties. Although all mutant lines had fine fiber compared with standard varieties, fiber fineness values of four lines were below 3.0 micronaire. Fourteen lines exhibited superior fiber strength than standard varieties. Unlike the results in our study, Cutts (2013) reported that the differences among M₆ and M7 lines (treated with imazamox) and comparative varieties were non-significant for yield, ginning out-turn, fiber strength, fiber length and micronaire.



Figure 1. Original images (A) plant adversely affected by imazamox; (B) rows adversely affected by imazamox; (C) nonaffected plant and (D) non-affected rows

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Table 1. Average seed cotton yield (SCY), ginning out-turn (GOT), fiber length (FL), fiber fineness (FF) and fiber strength (FS) in M4 generation

| Genotypes | SCY (g plant ⁻¹) | GOT (%) | FL | FF | FS (g tex⁻¹) |
|---------------|---------------------------------|------------|-----------|----------|-----------------|
| | | | (mm) | (mic) | |
| GR-100 I | 29.60 r | 41.89 f | 28.84 g-i | 4.30 c | 28.53 j |
| GR-100 II | 45.00 j | 40.44 ij | 27.44 lm | 3.86 g-j | 31.78 b-d |
| GR-100 III | 31.30 q | 43.12 b | 28.75 g-l | 4.13 c-e | 28.82 ij |
| GR-100 IV | 50.80 gh | 40.35 i-k | 26.27 o | 4.06 d-f | 23.62 lm |
| GR-100 V | 19.60 v | 40.62 i | 31.20 b | 3.23 rs | 30.92 d-f |
| GR-100 VI | 47.40 i | 43.66 a | 29.02 fg | 3.82 h-k | 30.79 e-g |
| GR-100 A I | 40.00 m | 40.49 i | 28.17 i-k | 3.50 m-o | 29.61 hi |
| GR-100 A II | 33.60 o | 36.30 o | 30.93 bc | 3.46 n-p | 34.98 a |
| GR-100 A III | 50.40 h | 42.06 ef | 26.47 no | 3.45 n-p | 28.53 j |
| GR-200 I | 99.20 a | 39.01 m | 30.23 c-d | 3.71 j-l | 34.98 a |
| GR-200 II | 37.00 n | 39.99 j-l | 32.03 a | 3.78 i-k | 34.18 a |
| GR-200 III | 57.30 e | 41.88 d | 28.42 g-i | 3.80 h-k | 29.55 hi |
| GR-200 IV | 45.80 j | 42.35 de | 30.40 cd | 3.59 l-n | 31.64 b-e |
| GR-200 V | 76.70 b | 40.64 i | 28.23 h-k | 3.49 m-o | 32.53 b |
| GR-200 VI | 55.80 f | 41.39 gh | 29.68 ef | 3.51 mn | 34.94 a |
| GR-200 VII | 32.20 pg | 40.68 i | 29.95 de | 2.87 t | 29.64 hi |
| GR-200 VIII | 77.10 b | 41.82 fg | 28.67 g-i | 4.16 cd | 31.10 c-f |
| GR-200 IX | 51.43 g | 39.99 j-l | 29.81 de | 3.56 l-n | 31.28 c-f |
| GR-200 X | 17.05 w | 42.89 bc | 28.41 g-j | 3.90 f-i | 30.66 fg |
| GR-200 XI | 63.20 c | 42.87 bc | 28.49 g-i | 3.29 p-s | 30.89 d-g |
| GR-200 XII | 30.30 r | 39.60 | 27.73 j-l | 3.43 n-q | 29.97 gh |
| GR-200 XIII | 32.30 p | 37.76 n | 27.56 k-m | 2.58 u | 26.45 k |
| GR-200 XIV | 41.50 l | 36.62 o | 29.08 fg | 3.19 rs | 30.69 fg |
| GR-200 XV | 41.60 l | 41.34 h | 26.87 m-o | 3.97 e-h | 23.67 I |
| GR-200 XVI | 28.30 s | 42.40 h | 27.12 l-n | 3.33 o-r | 25.86 k |
| GR-200 XVII | 45.00 j | 39.55 l | 27.34 lm | 3.66 k-m | 22.66 n |
| GR-300 I | 43.50 k | 40.73 i | 28.56 g-l | 3.12 s | 32.00 bc |
| GR-300 II | 22.50 tu | 40.67 i | 29.10 fg | 3.99 d-g | 31.20 c-f |
| GR-300 III | 22.87 t | 42.01 ef | 28.87 gh | 2.90 t | 23.50 l-n |
| GR-300 IV | 16.87 w | 42.65 cd | 28.20 h-k | 3.13 s | 22.70 mn |
| GR-300 V | 14.10 x | 40.70 i | 27.25 lm | 3.26 q-s | 25.60 k |
| GR-300 VI | 14.27 x | 41.99 ef | 28.18 h-k | 2.83 t | 23.30 l-n |
| GR-300 VII | 21.88 u | 39.90 kl | 29.03 fg | 3.12 s | 25.90 k |
| Gloria | 61.30 d | 41.37 gh | 30.15 de | 4.88 b | 30.70 e-g |
| ST-468 | 61.53 d | 42.71 b-d | 28.63 g-i | 5.10 a | 30.37 f-h |
| Genotype | ** | ** | ** | ** | ** |
| Mutant's Mean | 40.46 | 40.72 | 28.67 | 3.51 | 29.17 |
| HSD | 0.97 | 0.47 | 0.69 | 0.17 | 0.95 |

**: $p \le 0.01$. Means within a column for each trait followed by the same letter are not significantly different at the 0.05 probability level by Tukey's HSD test.

We evaluated all the traits of the mutant lines collectively. We choose the seed cotton yield per plant of 30 g \leq , the ginning out-turn of 36% ≤, fiber length of 28 mm ≤, fiber fineness between 3.0 and 4.6 mic. and fiber strength of 28 g tex⁻¹≤ as criteria. As a result, 17 mutant lines were selected and transferred to the M₅ generation. In the M₅ generation, significant differences were observed among mutant lines and standard varieties for all studied traits (Table 2). Earlier studies reported the genetic variations caused by gamma radiation (Raffat, 1995; Muthusamy et al., 2005; Muthusamy and Jayabalan, 2011; Yilmaz et al., 2018). In contrast to the M₃ and M₄ generations, non-significant variations were obtained for yield in the M5 generation (Herring et al., 2004). Eight mutant lines for seed cotton yield and ginning out-turn, 13 mutant lines for fiber length and 7 mutant lines for fiber strength were highly performed compared to standard varieties, while all mutant lines had fine fiber. GR-200 III, GR-

200 V, GR-200 I and GR-200 XII, especially GR-100 A II, drew attention as mutant lines with high ginning efficiency and superior fiber properties, as productive as or more than the standard varieties. Our study's most successful mutant lines were obtained from a moderate level of gamma radiation (200 GR), which was in agreement with Muthusamy and Jayabalan (2011). In addition, the doses of effective gamma radiations were different in the studied traits and cotton varieties (Khan et al., 2017). The distribution of M₄ and M₅ populations based on studied traits was presented in Figures 2 and 3. Curve analysis indicated that the frequency distribution of both populations was a normal curve for all traits except the seed cotton yield of M₄ populations. The formed curve was lognormal and skewed to the left side with the highest frequency at a class of 22.0 - 44.0 seed cotton yield per plant for M₄ populations.

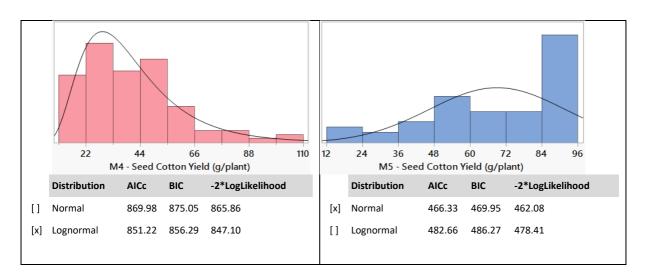
Yield and Fiber Quality Characteristics of Mutation-Based IMI Tolerant Cotton (Gossypium hirsutum L.) Lines

Table 2. Average seed cotton yield (SCY), ginning out-turn (GOT), fiber length (FL), fiber fineness (FF) and fiber strength (FS) in M₅ generation

| Genotypes | SCY | GOT | FL | FF | FS |
|--------------|-------------|----------|-----------|----------|------------------------|
| | (g plant⁻¹) | (%) | (mm) | (mic) | (g tex ⁻¹) |
| GR-100 VI | 21.10 m | 39.34 | 29.75 ef | 5.12 ab | 34.38 ab |
| GR-100 A I | 55.20 i | 46.28 cd | 26.74 i | 2.97 j | 31.70 ef |
| GR-100 A II | 89.90 cd | 43.36 g | 30.91 ab | 4.16 fg | 34.43 ab |
| GR-100 A III | 90.20 cd | 44.46 ef | 29.08 fg | 5.24 ab | 32.25 de |
| GR-200 I | 85.70 e | 46.32 cd | 30.88 ab | 4.61 c-e | 32.51 cd |
| GR-200 II | 40.00 k | 46.25 cd | 27.48 h | 4.48 d-f | 29.48 i |
| GR-200 III | 73.00 g | 46.85 bc | 31.18 a | 4.83 d-b | 33.94 b |
| GR-200 IV | 35.70 | 47.90 a | 29.84 de | 4.12 fg | 30.36 h |
| GR-200 V | 91.50 bc | 42.51 gh | 30.85 a-c | 3.99 gh | 31.13 fg |
| GR-200 VI | 88.70 d | 42.16 hi | 29.90 de | 4.42 d-f | 30.31 h |
| GR-200 VIII | 94.90 a | 42.15 hi | 28.87 g | 4.70 vd | 31.09 fg |
| GR-200 IX | 71.10 g | 45.43 de | 30.48 b-d | 4.10 fg | 31.65 ef |
| GR-200 X | 51.30 j | 46.00 cd | 30.87 a-c | 3.64 hi | 34.81 a |
| GR-200 XII | 78.90 f | 42.21 hi | 30.20 с-е | 4.26 e-g | 33.13 c |
| GR-200 IVX | 50.10 j | 47.70 ab | 30.25 b-e | 3.91 gh | 30.69 gh |
| GR-300 I | 64.50 h | 41.40 i | 30.19 с-е | 3.33 ij | 33.07 c |
| GR-300 II | 92.90 ab | 44.38 f | 27.60 h | 5.02 a-c | 34.96 a |
| Gloria | 73.06 ef | 42.49 gh | 28.91 g | 5.34 a | 32.79 cd |
| ST-468 | 76.68 de | 44.61 ef | 27.43 h | 5.40 a | 30.21 h |
| Genotype | ** | ** | ** | ** | ** |
| Mutants Mean | 68.97 | 44.39 | 29.71 | 4.29 | 32.35 |
| HSD | 2.51 | 1.01 | 0.69 | 0.42 | 0.70 |

**: $p \le 0.01$. Means within a column for each trait followed by the same letter are not significantly different at the 0.05 probability level by Tukey's HSD test.

The average seed cotton yield in the M_4 generation increased by 28.64 g in the M_5 generation after selection, and a class of 84.0 - 96.0 g seed cotton yield constituted the highest frequency. The fact that the range in the M_5 generation is higher than the M_4 generation shows that the segregation continues in the M_5 generation for all traits except fiber length, and the induced variability in the mutant population could not be fixed by selection. (Figure 3). On the contrary, it was reported that the later mutant generations remained uniform compared with earlier mutant generations in soybean (Inis, 1972), wheat (Sarkar (1986; Borojevic, 1991) and mungbean (Khan and Goyal, 2009). M_5 generation mean performance to M_4 was 3.67% for ginning out-turn, 1.04 mm for fiber length and 3.18 g tex⁻¹ for fiber strength, whereas fiber fineness of M_5 populations coarsened to 0.78 micronaire. We commented that the fibers, which were at quite fine class in the M_4 generation, coarsed in the M_5 generation as an indicator of maturity.



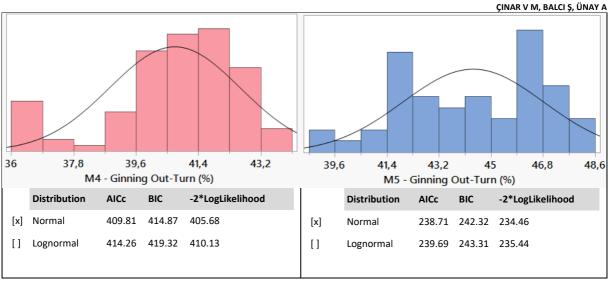
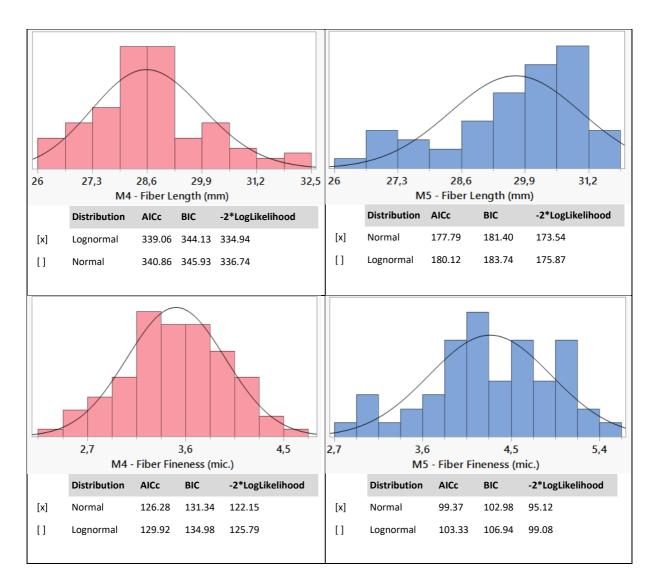
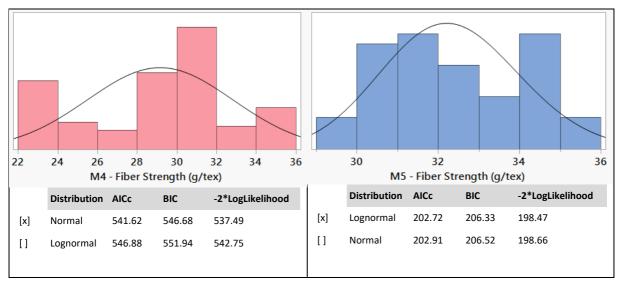


Figure 2. Density graphs of seed cotton yield and ginning out-turn in M_4 and M_5 generations



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CONCLUSION

We have shown that IMI-tolerant genotypes with superior agronomic characteristics can be developed in cotton by mutation and have identified five promising genotypes that we can transfer to the next generations. This result clearly proved that there is no negative linkage between IMI tolerant and agronomic traits.

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