*Article*

# Optimising Gamma Irradiation Seed Treatment of Sesame (*Sesamum indicum* L.) Varieties for Potential Future Application in Mutation Breeding

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## ABSTRACT

*Background*: Sesame (*Sesamum indicum* L.) is a nutritious and healthy seed oil crop with perspective for further improvement through plant breeding. Mutagenesis breeding generates new genetic variation with potentially favorable genetic changes for selection. Gamma irradiation has been applied to mutate sesame and other crops at seed stage. However, a wide range of doses have been applied, which can be ineffective or cause sterilization, and the different seed colors of sesame varieties influences sensitivity to irradiation.

*Methods*: Gamma irradiation equipment was calibrated for low gamma irradiation doses and different treatments were applied to three Turkish sesame varieties with the aim of optimizing treatment. The resulting phenotypic variation of the M0 generation were quantified in terms of seedling and field performance.

*Results*: Negative relationships were found between radiation dose and the measured traits; germination percentage, seedling shoot and root length, branch number, capsule number, capsule length, 100 seed weight, and yield, but not plant height or flowering time. Varieties generally maintained trait differences before and following radiation treatment, but for; germination, root length, branch and capsule number, and capsule length, varieties were affected differently by radiation dose.

*Conclusions*: These results highlight the value of optimizing gamma irradiation dose for sesame according to variety, seed color, and trait, while confirming that doses ranging from 250 to 350 Gy are effective to induce variation for most traits. The treated populations generated in this study will be progressed to later generations to confirm heritable mutations as part of a future plant breeding efforts.

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**KEYWORDS:** field trials; gamma irradiation; plant breeding; induced variation; seed color; yield

## INTRODUCTION

Sesame (*Sesamum indicum* L.) forms an important part of several regional diets and the nutritious seeds and seed oil (47 to 52 % oil and 22 to 34% protein by weight) of sesame mean that it could contribute to the current and expected global food crisis [1,2]. Further, seed meal of sesame left from oil extraction can be used as animal feed [3]. Consumption of sesame also brings health benefits due to its high levels of antioxidants, lignans, and essential fatty acids [4–7]. The top fraction of sesame oil, called serge, has been used to treat throat infection [8]. Sesame oil has been found to have protective gastrointestinal, cholesterol regulating and atherosclerosis preventing properties in animal studies [9–11]. Sesame varieties produce seed in several different colors, mostly white, red, and black, in addition to varieties that combine these colours [12,13]. Previous studies have indicated that white sesame seeds are suitable for confectionery production, while red sesame with greater levels of calcium can be used in food for the elderly who suffer from osteoporosis or bone weakness [14].

Desirable breeding targets in sesame include increased yield, improved nutrition, especially oil content, mineral content and color of seeds, environmental and disease tolerance [15–18]. Plant and capsule surface hairs or trichomes contribute to pest resistance [19]. Consistent plant height improves machine harvesting [20]. Flowering period in sesame is typically indeterminate over 97–110 days in Turkey and 120–150 days in USA, whereas determinate flowering over a shorter period would coordinate maturation better for efficient harvesting [18,21,22]. Shorter maturation that could allow two growing seasons in one year as another desirable trait. For example, in a few regions with suitable year-round temperature and precipitation, two growing seasons within one year are currently possible [23].

An important target for breeding in sesame is to reduce the seed capsule (also called pod) shattering trait. It is estimated that 99% of the sesame crop worldwide suffer from shattering [18,24]. Due to capsule opening upon dehiscence, when plants are cut for mechanical harvesting and turned, there is a loss of seeds ranging from 10% to 50%, depending on the varieties and equipment used [24]. For this reason, many countries still rely on manual harvesting methods. This leads to significant crop wastage, as well as the need for a large labor force, hindering the widespread cultivation of sesame more widely. If mechanical harvesting for sesame could be improved, it could make a valuable contribution to global food security. Research on the automated harvesting of sesame seeds has been conducted since the 1940s, aiming to achieve successful results [25]. However, so far, the success has been only partial. Mutant lines have been found that lead to fully closed capsules, but the seeds are difficult to extract from the hard casing and sesame oil subsequently becomes contaminated with capsule extracts. Alternatively, researchers have tried to utilize genetic mutations to make the capsules more elongated, allowing them to hold a larger quantity of seeds inside. However, during harvesting, the capsules open outward, resulting in significant losses of seeds that mature at different times over a period of up to two to three weeks. Sesame plants are cut at physiological maturity when 70%–90% of the capsules are mature and left to dry in the field for 7–10 days, before being fed into a seed thresher [18,26]. Cutting too early reduces yield as not all capsules are mature, while cutting too late reduces yield through seed dispersal [27]. The challenges for sesame include identifying the genes responsible for closed capsules and developing efficient methods for screening large populations of plants for closed capsule mutants [24,28].

Up to 20 growing parameters have been reported to influence plant growth, seed harvest and quality [29]. The task of improving traits in sesame faces challenging obstacles due to the numerous potential mutational and epigenetic changes occurring in the genome, as well as the interactions of mutations with the physical and chemical conditions of the soil and the plant's environment. This process requires precise study and analysis to achieve meaningful results.

Mutations may be beneficial or harmful, but through field and laboratory study it is possible to select the desired traits, such as resistance to diseases, drought tolerance, or increased yield reference, and closed capsule, when the study continues for several generations. Mutations occur in slowly and can be accelerated either by radiation, genetic modification, or viral infection or strong reactive chemicals. Induced mutagenesis has been used as part of sesame breeding since the 1940s in the Americas [30] and the 1950s in Asia [31]. Mutation rate can be tracked by observing obvious traits such as leaf color variation reflecting chlorophyll mutations as well as the aforementioned traits of interest [32]. With the potential for genetic enhancements through mutations, ensuring consumer safety and assessing the effects of these alterations on food quality is vital.

Most mutagenesis methods have associated disadvantages. Ethyl methane sulfonate (EMS) has been used for sesame chemical mutation at treatments ranging from 0.5 to 2.0%, when the lowest treatment was found to have best mutation efficiency [20]. EMS is considered extremely toxic at low concentration, up to 100,000× greater than mercury for example [33]. Therefore, residual EMS is problematic during mutagenesis experiments, and it is desirable to minimize its use. The types of ionizing radiation capable of mutating plants are gamma rays, X-rays, neutrons, and elementary particles, but gamma radiation is the most widely used type of radiation, with applications in sterilization of medical equipment and food, and radiotherapy [34]. Radiation causes the plant's DNA to be broken, and errors to DNA repairs lead to induced mutations [35].

It is important to optimize the radiation treatment as excessive mutation can lead to chromosomal aberrations, impact DNA and RNA synthesis, and meiosis leading to sterility and mortality [32,36–38]. A wide range of gamma treatments have been reported for sesame mutagenesis ranging from 50 to 900 Gy, with the majority in the range between 200 to 400 Gy. With too small a treatment, the seed will recover and there will be no differences, but treatments greater than 450 Gy typically lead to sterilization [37,39,40]. Gamma radiation treatment above 1 kGy has been used to prevent fungal growth during post-harvest preservation and change the sesame oil properties [41]. Therefore, it is important to determine effective dosage. There might be relationship between radiation sensitivity and color classification of sesame seeds since colored seeds of other species have higher levels of protective antioxidant compounds [42]. White sesame requires a small radiation treatment to achieve mutations, while inducing mutations in red sesame requires a higher treatment of radiation to stimulate the genetic mutation process. However, the results have been inconsistent and varied in previous experiments, suggesting the need for further investigation and standardization.

These examples demonstrate some of the practical applications and challenges involved in studying the enhancement of traits in sesame plants and their treatment interactions. To create clear benefits for agriculture and food industries, detailed studies on this subject should be conducted, verifying the potential effects of these mutation treatments on crop quality and consumer safety. Additionally, providing specific information to the food industry will help them choose the most suitable type of sesame that meets their needs and requirements.

Here, experiments involving different gamma rays and their effects at different life stages on different sesame varieties were conducted. By comparing conditions used in these experiments, the aim was to identify the most promising approach for alteration of sesame traits and identify a suitable radiation treatment related to variety seed color. The presented results of this study are a first step towards confirming induced change in sesame varieties as part of breeding efforts to improve productivity.

## MATERIALS AND METHODS

### Material

We received samples from Haran University's Agricultural Center consisting of three different cultivars of local sesame seeds: 'Arslanbey', 'Boydak', and 'Hatipoğlu'. Cultivar 'Arslanbey' has mid-brown seed; 'Boydak' has pale brown seeds; and 'Hatipoğlu' has dark brown seeds (Figure 1a,b,c).

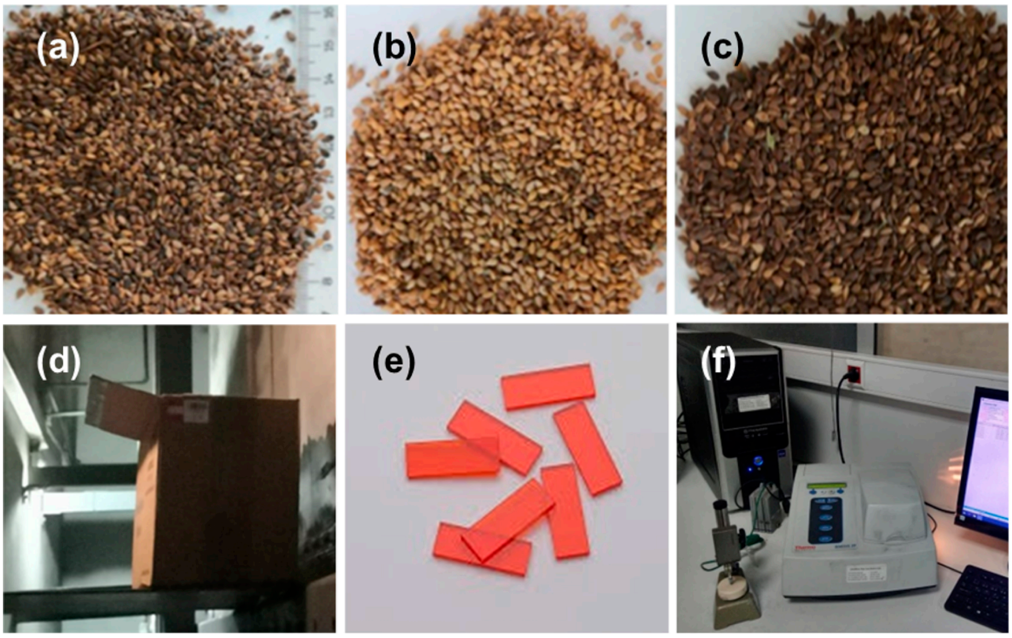


**Figure 1.** Images of irradiation experiments. Seed samples of sesame cultivars: (**a**) 'Arslanbey' (**b**) 'Boydak' 'Arslanbey' and (**c**) 'Hatipoğlu'. (**d**) Location of radiation area behind the main equipment. (**e**) Radiation dosimeters. (**f**) Dosimeter light absorbance measurement.

**Irradiation Experiments**

The irradiation device used was JS 9600 model 4th category and automatic tote box carrier type serial number IR-185 at Gamma-Pak Irradiation Facility (Tekirdağ, Çerkezköy Organized Industry Zone, Turkey). The radiation device uses double encapsulated Co-60 radioactive pencils as source. The total capacity of the source panel is 101 PBq (3,000,000 Ci). The energy level of the gamma rays emitted by Co-60 isotopes is not enough to make the seeds of sesame radioactive.

Normally, radiation doses in irradiation facilities from 3 to 25 kGy are used when irradiating food, medical, cosmetic, and industrial products for sterilization [34]. Lower doses up to 400 Gy are sufficient to induce mutation of sesame without causing greater than 50% seed mortality and other negative phenotypic effects such as slow flowering [35]. For this reason, only doses of 100, 200 and 300 Gy were considered in this study. These lower doses could be achieved in the space behind the main radiation chamber. Radiation doses were measured using three replicated Amber 3042 dosimeters (Harwell, Didcot, Oxfordshire, UK) (Figure 1d,e,f). Upon irradiation, these dosimeters darken, increasing their light absorbance, which is measured to calculate the dose exposure. Further, Gamma-Pak station conducts regular calibration using reference dosimeters from Aerial (Illkirch, France), an accredited laboratory. The time required to achieve doses of 150 Gy, 250 Gy, and 350 Gy was calculated, and additional dosimeters were used for confirmation. Due to the small doses used, there is a 20% margin of error in the energy field. A control sample of 150 g of seed from each variety was left untreated as a control against which to compare the effects of radiation. The remaining seeds of each of the varieties were exposed in samples of 50 g each using three gamma radiation dose treatments at each dose of approximately 150, 250, and 350 Gy (nine samples per variety, Table 1). After irradiation, the samples were bulked by variety and treatment dose to form nine M0 samples of 150 g each. These samples were split into two parts, one part was preserved for future study and the second part was for classification and morphological characteristics of the M0 treated sesame seeds.

**Table 1**. Irradiation treatments.

| Step | Dose (Gy) | Exposure time (s) | Irradiation rate (Gy/s) |
|---|---|---|---|
| Mean for calibration | 2090 | 3600 | 0.58 |
| Treatment 1 | 153.86 | 265 | 0.58 |
| Treatment 2 | 252.56 | 435 | 0.58 |
| Treatment 3 | 351.20 | 605 | 0.58 |

**Seed Tests**

Because radiation causes cellular and genetic damage, seedlings are at high risk for reduced growth and mortality at all radiation levels. For each cultivar and treatment, four replicates of 100 seeds were sown on damp filter paper in separate plastic containers with lids to maintain high humidity and left for 4 days in a laboratory with natural light at ambient temperature conditions to encourage germination (Figure 2 top left panels). The percentage of germination was recorded. Seedling vigor was assessed by measuring the length of seedling shoots and roots with a ruler. Summary statistics were calculated using least fit squares analysis of variety, treatment, and block, and their interactions, was performed with JMP software (JMP Statistical Discovery LLC, Cary, NC, USA).

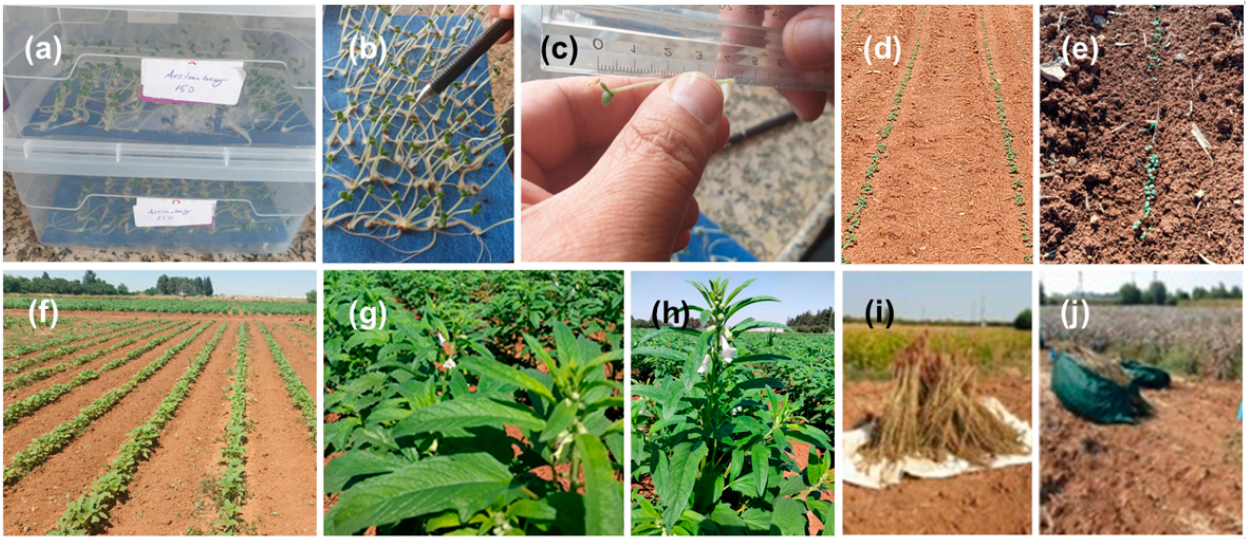


**Figure 2**. Images of seed germination and field experiment at different stages. (**a**) Seed germination experiment (**b**) Seed germination count (**c**) Seedling measures (**d**) Field growing experiment (**e**) Seedling emergence (**f**) Young plants (**g**) Mature plants (**h**) Flowering plant (**i**) Plants harvested to dry (**j**) Plants collected.

### Field Tests

The research was carried out in 2023 at the GAP Agricultural Research Institute Directorate, Talat Demirören Research Station (N36.901261, E38.917615), located in the semi-arid climate zone. Prior to sowing, the land was fertilized with 60 kg/ha Gübretaş 20-20-0 N-P-K compound fertilizer (Gübretaş, Istanbul, Türkiye). The sesame seeds from each treated and untreated group were sown with three replications according to split-plot trial design in random blocks on 16th June 2023. Varieties and radiation treatments were randomly distributed in the sub-plots. In each plot, there were four rows of 6 m length with row distance of 70 cm and intra row distance of 10 cm. The plot area was 16.8 $m^2$ (6 × 0.7 × 4) and the harvest area was 14 $m^2$. A distance of 1 m was left between the plots and 3 m between the main blocks.

Plants were monitored regularly during development (Figure 2). First irrigations following emergence were made by sprinkler, and subsequent irrigations were made by the furrow method (four irrigations in total). A top dressing of 100 kg/ha Gübretaş urea (Gübretaş, Istanbul, Türkiye) was added to plants before flowering. Seed ripening uniformity was assessed visually, until the stage of manual plant harvest (physiological maturity with approximately 70% mature capsules) at 120 days. Harvested plants were left to mature and dry in the field for seven days before threshing to separate the seeds. The following parameters were measured for each plot: plant height, branch number, flowering time, capsule numbers, first capsule length, 100 seed weight, and yield. Summary statistics were calculated using least fit squares analysis of variety, treatment, and block, and their interactions, was performed with JMP software.

## RESULTS

### Seed Germination

Mean seed germination measures and their least significant differences are summarized for each trait in Table 2 and Figure 3, while the data measures can be found in Supplementary Table S1. There were significant differences for germination between varieties (F = 346.4836, $p < 0.0001$), treatments (F = 453.1545, $p < 0.0001$), and variety × treatment interaction (F = 32.2312, $p < 0.0001$). Each variety showed reduced germination with increasing radiation dose, but they showed different sensitivities. There were significant differences for stem length only between treatments (F = 191.9787, $p < 0.0001$). Higher radiation doses caused shorter stem lengths. There were significant differences for root length between varieties (F = 68.1821, $p < 0.0001$), treatments (F = 329.2148, $p < 0.0001$), and variety × treatment interaction (F = 6.6676, $p = 0.0002$). Each variety showed shorter root lengths with increasing radiation dose, but they showed different sensitivities.

**Table 2.** Summary of least fit squares analysis of field traits in sesame varieties after different radiation treatments.

| Trait | Block F, *p* DF = 3, 27 | Variety F, *p* DF = 2, 27 | Treatment F, *p* DF = 3, 27 | Variety × Treatment F, *p* DF = 6, 27 |
|---|---|---|---|---|
| Germination (%) | 0.07 | 346.48***** | 453.15***** | 32.23***** |
| Stem length (cm) | 0.39 | 0.21 | 191.98***** | 1.04 |
| Root length (cm) | 3.08 | 68.18***** | 329.21***** | 6.67**** |

Note: F is F ratio statistic, DF is partial and full degrees of freedom. Significance of F values are indicated with asterisks. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$, **** means $p < 0.0001$, ***** means $p < 0.00001$.

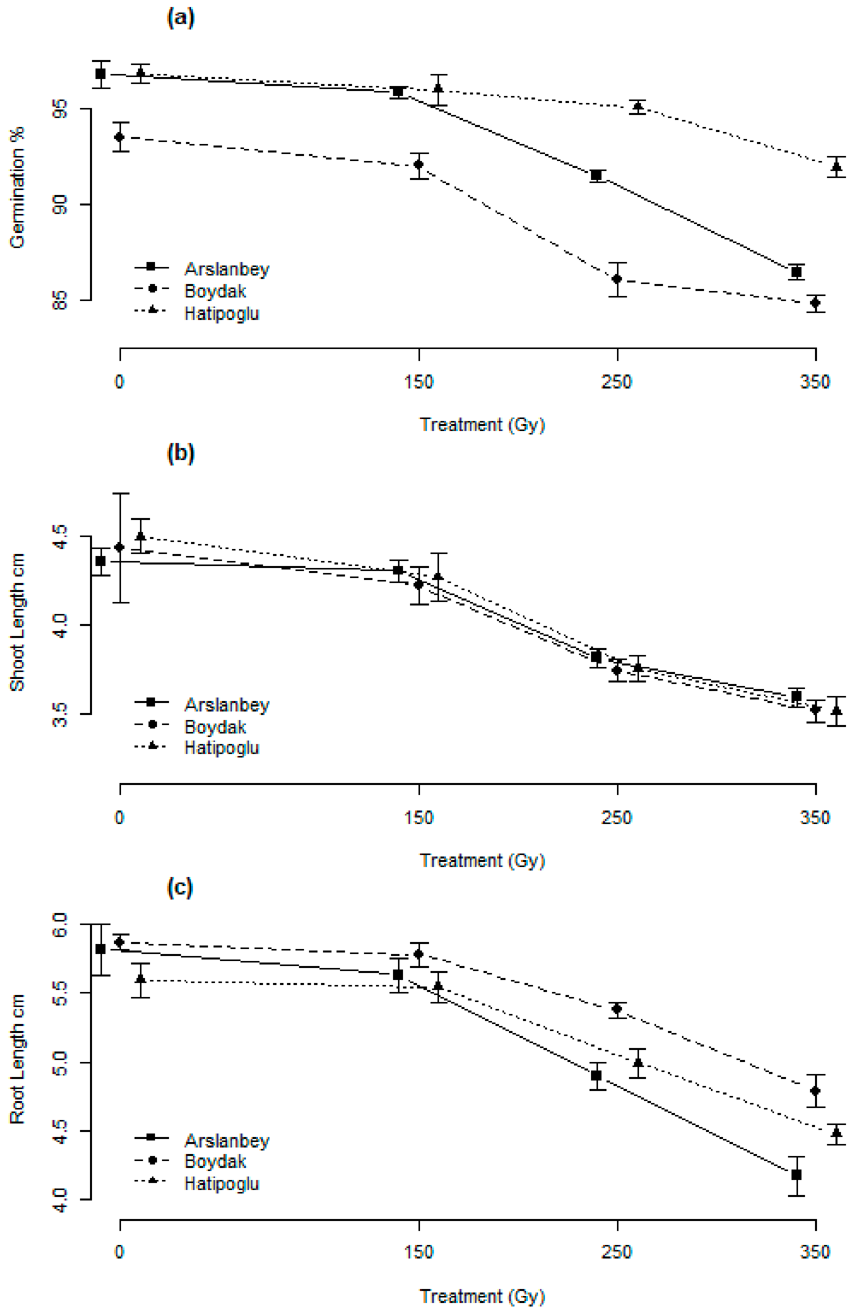


**Figure 3.** Connected scatterplots of seedling measures for each variety and radiation treatment. Legend: **(a)** Germination **(b)** Shoot length **(c)** Root length. Points are mean values and bars are standard deviation values across replications.

### Plant Field Performance

Field trait results are summarized in Table 3 and Figure 4, while the data measures can be found in Supplementary Table S2. Significant differences for plant height were observed between varieties (F = 5.8155, $p$ = 0.0394) but not for treatment or variety × treatment interaction. Significant differences for branch number were observed between varieties (F = 327.6195, $p$ < 0.0001), treatments (F = 9.9970, $p$ = 0.0001), and variety × treatment interaction (F = 5.9377, $p$ = 0.0005). Flowering time showed fixed differences between varieties ('Arslanbey' = 34 days, 'Boydak' = 36 days, 'Hatipoğlu' = 38 days) but no effects of treatment or variety × treatment interaction. Significant differences for capsule number were observed between varieties (F = 391.9338, $p$ < 0.0001), treatments (F = 73.62029, $p$ < 0.0001) and variety × treatment interaction (F = 6.4270, $p$ = 0.0003). Although the interactions between variety and treatment were significant, in each case, capsule number decreased from the control with increasing radiation and variety 'Hatipoğlu' was most affected by higher radiation doses. Significant differences for capsule length were observed between varieties (F = 61.9274, $p$ < 0.0001), treatments (F = 14.3212, $p$ < 0.0001) and variety × treatment interaction (F = 6.6953, $p$ = 0.0002). Significant differences for 100 seed weight were observed between varieties (F = 136.8513, $p$ < 0.0001), treatments (F = 43.3744, $p$ < 0.0001) but not variety × treatment interaction. This means that each variety was affected in the same way by increasing radiation doses, with greater doses decreasing seed weight. This reflects the greater deleterious consequences of greater radiation treatments. Significant differences for yield in kg/ha were observed between varieties (F = 821.2442, $p$ < 0.0001), treatments (F = 350.0578, $p$ < 0.0001) but not variety × treatment interaction. Similar to 100 seed weight, this means that each variety was affected in the same way by increasing radiation doses, with greater doses decreasing yield.

**Table 3.** Summary of least fit squares analysis of field traits in sesame varieties after different radiation treatments.

| **Trait** | **Block F, *p*** **DF = 3, 27** | **Variety F, *p*** **DF = 2, 27** | **Treatment F, *p*** **DF = 3, 27** | **Variety × Treatment F, *p*** **DF = 6, 27** |
|---|---|---|---|---|
| Plant height (cm) | 0.75 | 5.82* | 1.18 | 1.84 |
| Branch number | 0.50 | 327.62***** | 10.00**** | 5.94**** |
| Capsule number | 1.59 | 391.93***** | 73.60***** | 6.43**** |
| Capsule length (mm) | 0.29 | 69.93***** | 14.32***** | 6.70**** |
| 100 seed weight (g) | 3.86 | 136.85***** | 43.37***** | 0.18 |
| Yield (kg/ha) | 16.31** | 821.24***** | 350.06***** | 2.37 |

Note: F is F ratio statistic, DF is partial and full degrees of freedom. Significance of F values are indicated with asterisks. * means $p$ < 0.05, ** means $p$ < 0.01, *** means $p$ < 0.001, **** means $p$ < 0.0001, ***** means $p$ < 0.00001.

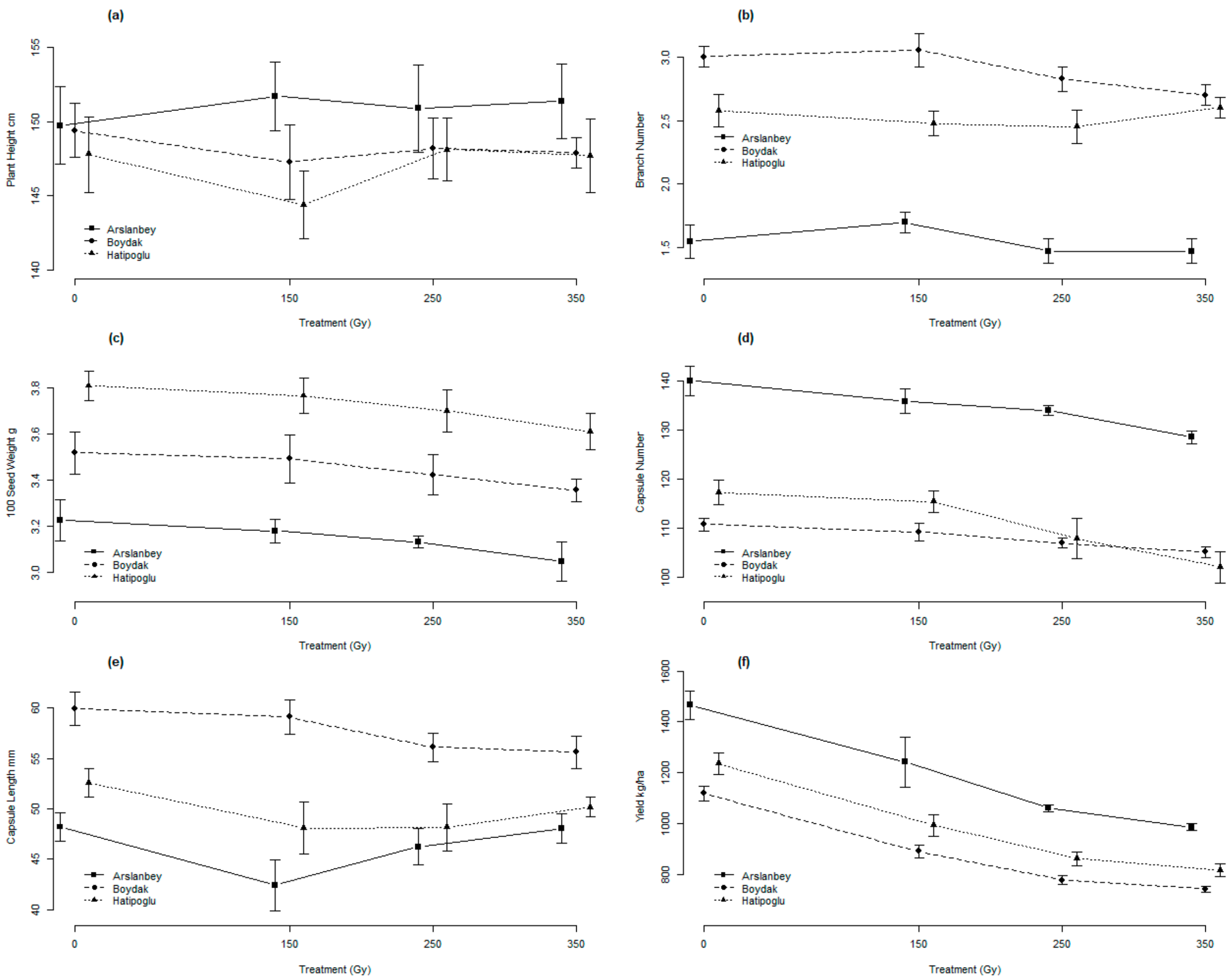


**Figure 4.** Connected scatterplots of field measures for each variety and radiation treatment. Legend: (**a**) Plant height (**b**) Branch number (**c**) 100 seed weight (**d**) Capsule number (**e**) Capsule length (**f**) Yield. Points are mean values and bars are standard deviation values across replications.

## DISCUSSION

The results confirm our literature review [30,33] that gamma radiation doses in the range 150 to 350 Gy are appropriate to cause morphological changes in the M0 generation of sesame without causing sterilization and excessive mortality [39,40]. It is important to perform preliminary experiments to calibrate radiation equipment and carefully quantify radiation dose to properly control this step for desired phenotypic variation outcomes. While it is tempting to equate these changes with the mutation load since gamma irradiation treatments typically lead to heritable changes, it is important to confirm that genetic change has occurred by testing subsequent generations. Epigenetic changes affecting gene expression might also have been induced by irradiation that also affects DNA methylation, chromatin, and RNA regulation [43–45].

Plant performance decreased with increasing radiation dose, showing that induced phenotypic variation can be controlled with this parameter. The smallest changes were observed for the lowest radiation dose of 150

Gy, suggesting limited molecular damage at low radiation dose. This low dose could prove most useful for breeding programs if the changes could be confirmed to be heritable genetic mutations as lines with specific beneficial changes could be identified among later generation offspring without a large associated mutation load.

Overall yield is influenced by multiple traits including increased branch number, numbers of capsules, capsule length, and seed weight [15,18]. The radiation treatment doses generated significant reductions the size and quantity of most measured traits with increasing treatment, with the exception of plant height, which was unchanged. The smaller trait values could indicate the presence of more induced deleterious changes in these individuals that decrease plant performance and fitness. However, individual lines might still show desirable trait characteristics that can be selected for breeding. The selfing nature of sesame means that identification of desirable traits in individual lines would require growing the treated populations for more generations to confirm that the changes are caused by heritable mutations and to express these mutations in homozygous state. Artificial selection for desirable trait variation by the breeder could then take place from M3 to M8 to obtain new varieties.

The varieties tested in this study showed significant differences for all measured traits, apart from seedling shoot length. However, there was no obvious trend in terms of original variety seed color. For example, germination was least affected in 'Hatipoğlu' with the darkest brown seeds, but root length was least affected in 'Boydak' with the palest brown seeds. The variety differences mean that pre-existing variety differences should be considered when planning irradiation experiments for breeding targets. It might be easier to generate desirable trait outcomes from varieties that are already similar to the desired trait range. Inclusion of multiple varieties with different radiation sensitivities at the start of the breeding program could also improve the chances of detecting desirable trait changes, should resources allow.

No radiation induced differences were observed for flowering time in this experiment, which is important for maturation time of capsules and harvestability [21]. This means that the radiation treatments were unsuccessful in this case of providing genetic variation in flowering time. It might be that individual differences in flowering time were masked at the level of block measures made in this study. In addition, it will be necessary to test later generations to confirm the heritability of changes and to detect the effects of any recessive mutations as they become expressed in homozygous state.

Some traits at both life stages; germination rate, root length, branch number, and capsule number, showed significant variety × treatment interactions meaning that the sensitivity to strength of gamma radiation dose varied between varieties. The different seed color of the varieties could be contributing to the treatment sensitivity, emphasizing the importance of optimizing the radiation treatment according to variety characteristics.

## CONCLUSIONS

This study represents a promising first stage in a breeding program for sesame improvement in Turkey. The results show that relatively low gamma radiation doses from 150 to 350 Gy are all suitable to introduce variation into sesame varieties. There are interactions between radiation dose and sesame varieties but that these can be difficult to predict based on variety characteristics such as seed color. There is now a need to advance to later generations to identify individual line with desirable trait characteristics for selection. Although we have discussed the critical importance of seed harvesting [24], it was not possible to address it directly during the period of this study. This trait will be examined as part of future research as we develop later irradiated sesame generations. Controlled crossing experiments might be needed to combine desirable trait combinations to develop superior varieties across several yield related traits. For example, crossing studies to determine if lines could be produced both with more branches and more capsules without trade-off. Future genetic studies can compare the DNA sequence of desirable lines with untreated varieties to identify the potential causal mutations and the affected genes. These studies would contribute to understanding the genetic basis of traits of interest in sesame.

## SUPPLEMENTARY MATERIALS

The following supplementary materials are available online at https://doi.org/10.20900/cbgg20240004. Supplementary Table S1: Seed germination and performance data; Supplementary Table S2: Plant performance and yield data.

## DATA AVAILABILITY

All data generated from the study are available in the supplementary files.

## AUTHOR CONTRIBUTIONS

AJ, ACB, SS, and EA designed the study. SS, AJ, SA, and HH performed the experiments. AJ, ACB, SS, and EA analyzed the data. AJ, ACB, SS, and EA wrote the paper with input from all authors.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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