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Research Article

Determination of Optimum Gamma Ray Irradiation Doses for Hulless Barley (*Hordeum vulgare* var. *nudum* L. Hook. f.) Genotypes

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Abstract: The limited germplasm resources of hulless barley restrict the breeding of hulless barley with improved traits. Mutation techniques are an effective tool for generating variation for plant breeding studies. This study aimed to evaluate the impact of gamma-ray at different doses on certain seedling properties of M_1 plants of two hulless barley genotypes, as well as determine the effective dose (ED_{50}). The seeds of two hulless two-row barley genotypes, cv. Yalin and hulless barley line YAA7050-14, were irradiated with 100, 150, 200, 250, and 300 gray Gamma-rays delivered by a Cobalt 60 source along with non-irradiated control samples. Gamma-ray irradiation affects the seedling properties of M_1 plants of both hulless barley genotypes significantly. The significant effect varied based on the doses, traits, and genotypes. While lower doses were found statistically identical to the control in the majority of qualities in the M_1 generation, 250-300 gray gamma ray doses caused statistically significant decreases in the majority of characteristics studied in both genotypes. The effective doses (ED_{50}) for hulless barley genotypes were determined by plotting growth reduction values of seedling lengths, then the polynomial regression equations were calculated for each genotype. It was determined that 50% growth reduction in shoot length was reached at 214.1 Gy and 253.4 Gy for cv. Yalin and line YAA7050-14, respectively.

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1. Introduction

Barley (*Hordeum vulgare* L.), one of the oldest cultivated crops, is the cereal with the highest cultivation area and production in the world, after wheat, corn, and rice. Today, around 157 million tons of barley grain are produced in 51 million hectares worldwide (FAOSTAT, 2022). Approximately 60% of barley is globally utilized for feed, 40% for malt, 5% for seed, and 3% for food (Ullrich, 2011). It can be assumed that the majority of the produced barley is covered (hulled) barley (Meints et al., 2021).

Hulless (naked) barley, which threshes freely from the hull, accounts for a small proportion of total barley production. Hulless barley is primarily grown for food end-uses because it is rich in nutritional constituents like beta-glucan, starch, and total dietary fiber compared to its hulled types (Meints et al., 2021). While hulless barley has been mainly cultivated in Japan, Korea, Nepal, Tibet of China, and Bhutan (Shaveta and Simarjit, 2019), regarding potential feed for non-ruminants and potential health benefits as a food, the utilization of hulless barley has been in increments in the developed countries (Dickin et al., 2012; Shaveta and Simarjit, 2019; Meints et al., 2021). On the other hand, the number of released hulless barley cultivars in the world is still few compared to its hulled type. One of the reasons for the limited number of developed hulless barley cultivars is limited germplasm resources. For instance, it is reported by Meints et al. (2021) that out of the 36.734 barley accessions entered into The Germplasm Resources Information Network (GRIN), 3.003 are classified as hulless.

A wide genetic variation of genotypes provides an effective and efficient breeding program to develop new varieties. The breeding of hulless barley with improved traits is restricted because the number of two-row hulless barley genotypes is limited to find out genotypes carrying desired traits. Crossing hulless barley with hulled type is an option to generate variation, but effectiveness could be less because hullessness is controlled by the recessive allele *nud* on chromosome 7HL (Franckowiack and Konishi, 1997; Duan et al., 2015). The other option to generate variation is mutation techniques. Mutation techniques provide tools for rapidly creating desired traits if genetic variability or a specific character is not available in a germplasm collection (Maluszynski et al., 2009). Mutations are sudden changes that occur in the genetic structure of plants. Mutant plants can be released directly as a new variety or serve as a parent in crossing programs (Ahloowalia et al., 2004). Mutation breeding is one of the most effective strategies for creating genetic diversity as well as identifying critical genetic variants for economically significant traits toward crop development (Chaudhary et al., 2019). Moreover, integrating mutagenesis techniques into newly developed molecular biology technologies such as molecular markers, high-throughput mutation screening techniques, and next-generation sequencing techniques has also become more powerful and effective in crop breeding (Suprasanna et al., 2015).

In mutation breeding studies, there is a crucial ratio between plant deaths and the variation generated. In order to obtain the targeted variation, the mutagen to be applied must be at a dose that will provide a sufficient number of plants alive. Germination-viability rate (Kodym et al., 2012; Ahumada-Flores et al., 2020), growth decreasing in seedlings and the first leaf length (Kodym et al. 2012; Olgun et al., 2012), and chlorophyll mutations (Çiftçi and Şenay, 2005) are among the most accepted criteria to determine optimum irradiation dose. It is proposed that a dose that causes a 30% - 50% growth reduction could be accepted as the optimum dose. Currently, optimum irradiation doses for each type of mutagen have been determined in almost all cultivated plants, and for barley 150 - 400 gray (Gy) was reported (Suprasanna et al., 2015; FAO/IAEA, 2018). However, it is recommended that the optimum dose should be determined via a radiosensitivity test before large-scale experiment because it varies with the plant species, the cultivars, the type and status of the material, and the stage at which lethality is measured.

This study aimed to determine the optimum irradiation Gamma-ray doses for hulless two-row barley genotypes before conducting a large-scale mutagenesis experiment on hulless two-row barley to generate a variation. Effects of different Gamma-ray doses applied to seeds were investigated at M_1 plants by measuring the germination rate, survival rate, and shoot and root growth parameters. Optimum irradiation doses were determined by a dose-response curve.

2. Material and Methods

The elite seeds of the two-row hulless barley cv. Yalin and the two-row hulless barley line YAA7050-14 developed by the Central Research Institute for Field Crops were used. Gamma rays were obtained from the 381 Gray (Gy) hour^{-1} Cobalt 60 (^{60}Co) source in the Turkish Atomic Energy Agency (TAEK), Ankara Nuclear Research and Training Center (ANAEM). Five hundred seeds, uniform in size and containing approximately 12% moisture, were prepared for each irradiation dose and control group for each genotype. The seeds were irradiated with gamma rays obtained from the Cobalt 60 (^{60}Co) source at 100, 150, 200, 250, and 300 Gy doses. The seeds in the control group and irradiated at different doses were sown in a randomized complete block design with three replications separately in the greenhouse to grow M_1 plants. One day after the irradiation, the seeds were sown by hand in plastic pods (7.5 cm in diameter and 14 cm deep) containing 320 g of washed sand, with one seed per pod. Twenty-four seeds per replication of each treatment were planted. A nutrient solution containing 6% N, 5% K_2O, 4% P_2O_5,, 0.021% Fe (EDTA), 0.013% B, 0.011% Mn, 0.0058% Zn, 0.003% Cu and, 0.0011% Mo was given to the pods once a week to prevent seedlings from nutrient deficiency. The moisture of the pods was maintained with irrigation at two days intervals. During the four-week growing period, the temperature of the greenhouse was around 20 °C, and the seedlings were grown under daylight conditions.

Measurements on M_1 plants were conducted as described by Çiftçi and Şenay (2005), and FAO/IAEA (2018) as follows. The emergence rate (ER) was calculated as a percentage (%) by dividing the total number of plants that emerged four weeks after sowing by the number of seeds planted. The survival rate (SR) of the seedlings was determined as the ratio of the plants that survived at the end of the fourth week. The number of tillers (NOT), the number of leaves (NOL), the first leaf length (FLL), the seedling length (SL), the shoot fresh weight (SFW), and the shoot dry weight (SDW) were measured at four-week-old seedlings. The root length (RL), the root fresh weight (RFW), and the root dry weight (RDW) were determined after washing the roots of the same plant, of which seedling measurements were recorded. The root/shoot dry weight ratio (RSR) was obtained by dividing the root dry weight by the shoot dry weight. First leaf length, shoot and root length were recorded in centimeters (cm), root and shoot fresh weight, and root and shoot dry weight in miligrams (mg). The data obtained from M_1 plants were subjected to analysis of variance (ANOVA) according to the randomized complete blocks design, separately for the cv. Yalin and the line YAA7050-14. The significance level of the differences among the investigated parameters was determined according to the F test, and means were separated according to Duncan's multiple range test (Montgomery, 2013). In addition, regression analysis was performed to demonstrate the relationship between gamma-ray doses and the traits examined (Freund et al., 2006). The effective dose (ED_{50}) was determined according to the regression value, taking into account the value of seedling growth reduction (GR) and seedling survival rate (SR) by 50% compared to the value obtained with the control in both genotypes (Kodym et al., 2012). The shoot length values measured at the end of the fourth week were used to calculate the seedling growth reduction.

3. Results

To determine the effective irradiation doses on hulless barley seeds to generate a variation for hulless barley breeding studies, five gamma-ray doses were evaluated on two hulless barley genotypes. The data obtained from each genotype were individually subjected to analysis of variance (ANOVA). The differences among the irradiation doses were found to be statistically significant in terms of all measured traits (Table 1).

Table 1. ANOVA table of data obtained from M_1 plants

	Yalin				YAA7050-14			
	Replication	Doses	Error	CV	Replication	Doses	Error	
DF	2	5	10	(%)	2	5	10	
	Mean square				Mean square			CV (%)
ER	117.4	163.4*	31.3	5.9	191.5	279.7*	52.9	7.9
SR	66.1	3130.8**	20.2	5.9	52.6	2289.1**	18.6	5.5
NOT	0.01	0.5**	0.01	7.1	0.002	0.05**	0.004	5.5
NOL	0.03	2.9**	0.08	6.5	0.19	0.92**	0.11	8.1
FLL	0.004	8.3**	0.06	5.9	0.25	11.3**	0.12	5.9
SL	0.96	212.6**	0.4	4.3	5.60	258.0**	2.3	7.1
RL	6.97	182.3**	2.2	6.4	1.79	114.1**	2.2	5.8
SFW	0.003	0.3**	0.005	11.4	0.005	0.7**	0.012	12.0
RFW	0.002	0.16**	0.002	11.8	0.01	0.4**	0.009	13.8
SDW	131.08	10865.5**	153.6	12.6	76.33	17124.4**	175.0	10.3
RDW	69.19	1803.1**	32.9	11.6	92.60	2449.2**	42.7	9.6
RSR	0.01	0.04*	0.01	14.4	0.05	0.37**	0.02	23.0

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ER Emergence rate; SR Seedling survival rate; NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio: CV Coefficient of variation (%); DF Degrees of freedom.

The emergence rate of the genotypes showed a different response to irradiation doses. The emergence rate in the cv. Yalin was significantly reduced over 200 Gy, while in the line, the YAA7050-

14 emergence rate was significantly reduced over 150 Gy (Figure 1.a). On the other hand, survival rates of both genotypes were significantly reduced at 200 Gy and overdoses, and at 300 Gy, only 12.3% and 25.5% of seedlings could be survived for four weeks, for cv. Yalin and YAA7050-14, respectively (Figure 1.b).

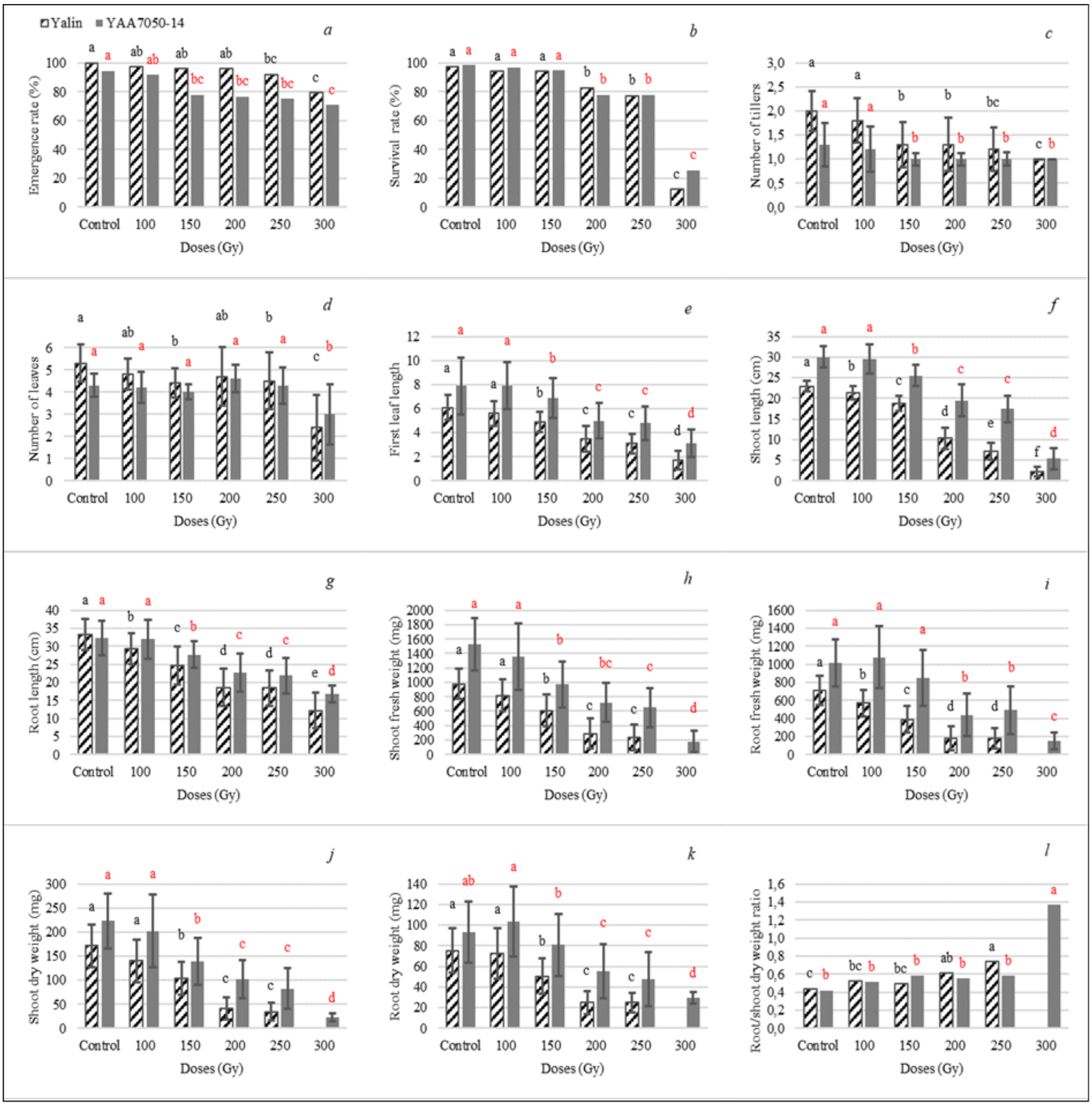

Figure 1. Effect of gamma irradiation on germination, shoot and root characters of hulless barley genotypes. *a*: Emergence rate (%); *b*: Seedling survival rate (%); *c*: Number of tillers; *d*: Number of leaves; *e*: First leaf length (cm); *f*: Shoot length (cm); *g*: Root length (cm); *h*: Shoot fresh weight (mg); *i*: Root fresh weight (mg); *j*: Shoot dry weight (mg); *k*: Root dry weight (mg); *l*: Root/shoot dry weight ratio, The same letters in bars are not significantly different at P 0.05 (black letters for cv Yalin, red letters for barley line YAA7050-14).

A significant decrease was found in the number of tillers of cv. Yalin and line YAA7050-14 at high doses compared to those in control, and even several plants were not tillering at these doses (Figure 1.c). Among the traits, the number of leaves was almost identical when the gamma-ray doses reached 250 Gy compared to those in control, and a significant reduction was only observed in both genotypes at 300 Gy doses, while the other traits investigated significantly affected the treatments at 150 Gy and overdoses (Figure 1.d). Growth reduction in seedling is a crucial indicator to determine optimal irradiation doses (Maluszynski et al., 2009; Forster and Shu, 2012; FAO/IAEA, 2018). Therefore, several parameters related to seedling growth were measured, including first leaf length, shoot length, shoot fresh weight, and shoot dry weight. Growth parameters measured in 100 Gy treatments in both genotypes were almost identical to those in control. On the other hand, the increased gamma-ray doses gradually caused a significant reduction in growth parameters. The first leaf lengths measured in M_1 plants that completed their first development at four weeks after sowing the seeds under greenhouse conditions decreased significantly compared to those in control as the doses increased in both genotypes. The first leaf length decreased by 50% at 250 Gy dose in the cv. Yalin and 250 Gy and 300 Gy doses in the line YAA7050-14.

Due to increasing gamma-ray doses, significant decreases were detected in the seedling lengths measured in M_1 plants of both genotypes. Compared to the control, the seedling lengths obtained at 200 Gy doses in the cv. Yalin and 250 Gy in the barley line YAA7050-14 were approximately 50% less. Shoot fresh weight of M_1 seedling were gradually decreased by increasing irradiation doses. Shoot fresh weight of the cv. Yalin was 980 mg in control, while it was reduced to 240 mg at 250 Gy dose. The shoot dry weight of cv. Yalin gradually decreased by increasing irradiation doses from 171.7 mg at control to 104.5 mg at 150 Gy and drastically decreased to 41.3 mg when the dose was 200 Gy. On the other hand, the shoot dry weight of line YAA7050-14 showed gradual decreases with increasing gamma-ray doses from 222.9 mg to 22.5 mg. It should be noted that some destructive parameters such as shoot fresh weights, root fresh weights, shoot dry weights, and root dry weights were not able to be determined in cv. Yalin at 300 Gy treatment since very few seedlings of cv. Yalin treated with 300 Gy gamma-ray dose were able to survive.

The impact of gamma-ray doses on root parameters of hulless two-row barley genotypes was evaluated. The root length of cv. Yalin and line YAA7050-14 in control were 33.3 cm and 32.3 cm, while at the highest irradiation dose, it reduced to 12.2 cm and 16.8 cm, respectively. The root fresh weight of cv. Yalin gradually decreased from 710 mg in control to 180 mg in 200 Gy. However, the root fresh weight of line YAA7050-14 at 100 Gy and 150 Gy doses was identical to those in control, then dramatically reduction was observed with 440 mg and 150 mg at 200 Gy and 300 Gy irradiation doses, respectively. The root dry weight of both genotypes was significantly affected from the irradiation doses at 150 Gy, 200 Gy, and 300 Gy compared to those in control and respective irradiation doses. Contrary to all parameters, root/shoot dry weight ratios increased in parallel with irradiation doses. The lowest root/shoot dry weight ratios were observed in control with 0.44 and 0.42, while at 300 Gy, the highest ratios were 0.74 and 1.37 in cv. Yalin and YAA7050-14, respectively.

Table 2. Descriptive statistics for traits measured in M_1 plants of cv. Yalin

Doses		NOT	NOL	FLL	SL	RL	SFW	RFW	SDW	RDW
Control	min-max	1.0-3.0	2.0-8.0	3.7-8.4	10.5-28.9	21.8-42.5	300-1700	100-1000	47.2-317.9	22.1-135.0
	SD	0.41	0.83	1.01	2.59	4.40	212.3	160.5	44.53	21.54
	CV	19.8	15.7	16.6	11.3	13.2	21.6	22.7	25.9	28.5
100 Gy	min-max	1.0-3.0	2.0-7.0	1.4-7.4	5.5-24.5	12.2-37.8	100-1300	100-900	13.9-261.1	18.2-138.7
	SD	0.46	0.70	1.06	3.29	4.33	246.7	153.7	44.32	24.60
	CV	25.3	14.6	18.9	15.5	14.6	26.9	27.0	31.6	34.0
150 Gy	min-max	1.0-2.0	2.0-6.0	2.7-6.3	8.0-24.5	4.5-34.8	100-1100	100-700	19.4-171.0	10.5-86.8
	SD	0.47	0.65	0.86	3.71	5.32	219,1	146.2	33.92	17.25
	CV	35.6	14.8	17.6	19.7	21.4	35.6	36.4	32.5	33.7
200 Gy	min-max	1.0-3.0	1.0-7.0	1.0-7.0	1.0-20.4	6.0-27.5	10-800	10-500	6.4-92.7	5.7-58.4
	SD	0.55	1.31	1.06	5.28	5.12	212.9	130.7	23.47	11.00
	CV	42.3	27.7	29.9	51.2	27.5	72.5	72.2	56.6	44.1
250 Gy	min-max	1.0-3.0	1.0-7.0	1.0-4.8	1.0-20.5	4.5-28.2	10-700	10-400	9.9-90.2	8.4-47.0
	SD	0.45	1.28	0.83	4.20	4.93	182.3	106.7	18.78	9.08
	CV	38.5	28.2	27.2	58.4	26.4	74.1	58.7	53.7	36.2
300 Gy	min-max	1.0-1.0	1.0-5.0	1.0-3.3	1.0-9.6	6.7-18.8	-	-	-	-
	SD	0.00	1.47	0.78	2.24	4.97	-	-	-	-
	CV	0.0	67.0	47.6	99.0	40.5	-	-	-	-

NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio; SD Standard deviation; CV Coefficient of variation.

This study aimed to determine optimal gamma-ray doses for hulless two-row barley genotypes by comparing the mean values of the data collected from each treatment. On the other hand, we observed significant variation in terms of investigated parameters within each treatment. In order to reveal these variations, the standard deviation and coefficient of variation values of each parameter for each treatment were calculated and presented in Table 2. and Table 3. with the minimum and maximum values of the parameters measured. As it can be seen in Table 2. and 3. that, increased gamma-ray doses caused a higher coefficient of variation value, resulting in a wider variation within each treatment. In particular, the shoot lengths, one of the most representative parameters for determining optimal irradiation dose, ranged between 10.5 cm and 28.9 cm, 5.5 cm and 24.5 cm, 8.0 cm and 24.5 cm, 1.0 cm and 20.4 cm, 1.0 cm and 20.5 cm, and 1.0 cm and 9.6 cm in cv. Yalin at control, 100 Gy, 150 Gy, 200 Gy, 250 Gy, and 300 Gy gamma-ray doses, respectively. On the other hand, the calculated coefficient of variation values for shoot length of cv. Yalin were 11.3, 15.5, 19.7, 51.2, 58.4, and 99.0 at control, 100 Gy, 150 Gy, 200 Gy, 250 Gy, and 300 Gy gamma-ray doses, respectively. 200 Gy and over irradiation doses caused a wide variation within each treatment, resulting in higher CV values in parallel with our observations. Similar results were observed in the other genotype and the other parameters investigated in both genotypes.

Table 3. Descriptive statistics for traits measured in M_1 plants of the line YAA7050-14

Doses		NOT	NOL	FLL	SL	RL	SFW	RFW	SDW	RDW
Control	min-max	1.0-2.0	3.0-6.0	2.5-11.1	18.5-37.6	24.1-39.7	900-2500	500-1500	115.5-345.4	40.0-160.3
	SD	0.45	0.53	2.35	5.17	4.76	365.0	261.8	57.42	29.57
	CV	35.3	12.4	30.0	17.2	14.8	23.9	25.7	25.8	31.8
100 Gy	min-max	1.0-3.0	1.0-6.0	1.0-10.3	10.0-37.8	9.8-39.7	300-2900	50-1600	36.4-448.6	6.0-154.7
	SD	0.47	0.69	1.94	7.09	5.42	456	335.7	75.89	34.07
	CV	38.0	16.5	24.7	24.0	17.0	33.4	31.1	37.5	33.0
150 Gy	min-max	1.0-2.0	3.0-5.0	2.2-9.6	9.0-32.7	18.0-35.2	300-1800	200-1400	51.6-244.5	21.8-162.9
	SD	0.13	0.34	1.65	5.25	3.70	319.8	311.6	48.64	30.24
	CV	12.5	8.5	23.9	20.6	13.4	33.1	36.7	35.1	37.4
200 Gy	min-max	1.0-2.0	3.0-6.0	1.0-7.6	3.4-32.8	10.0-33.8	200-1300	10-1100	27.6-193.7	17.7-116.3
	SD	0.13	0.63	1.48	7.71	5.29	270.0	239.0	39.24	26.22
	CV	13.2	13.6	29.6	39.8	23.4	37.3	54.9	38.4	47.3
250 Gy	min-max	1.0-2.0	1.0-6.0	2.5-7.5	2.7-27.1	8.5-29.8	50-1300	10-1000	16.3-214.1	8.3-128.5
	SD	0.14	0.82	1.39	6.45	4.87	267.3	272.0	41.82	26.35
	CV	13.9	19.0	28.9	36.9	22.3	41.4	55.8	50.7	55.2
300 Gy	min-max	1.0-1.0	1.0-5.0	1.3-6.1	1.3-23.6	12.2-20.5	50-500	10-300	10.1-36.7	18.5-38.4
	SD	0.00	1.36	1.15	5.11	2.28	146.9	0.09	7.83	5.56
	CV	0.0	46.8	38.1	94.6	13.7	79.7	60.5	34.6	18.9

NOT Number of tillers; NOL Number of leaves; FLL First leaf length (cm); SL Shoot length (cm); RL Root length (cm); SFW Shoot fresh weight (mg); RFW Root fresh weight (mg); SDW Shoot dry weight (mg); RDW Root dry weight (mg); RSR Root/shoot dry weight ratio; SD Standard deviation; CV Coefficient of variation.

The effective doses (ED_{50}) for hulless two-row barley genotypes were determined by plotting growth reduction values of seedling lengths on the chart given in Figure 2. and 3., then the polynomial regression equations were calculated for each genotype. Effective dose 50 (ED_{50}) was accurately calculated for cv. Yalin (R^2=0.9529) and the line YAA7050-14 (R^2=0.9653) using polynomial regression equations. It is revealed that a 50% growth reduction in shoot lengths was reached at 214.1 Gy and 253.4 Gy for cv. Yalin and the line YAA7050-14, respectively. The survival rates of genotypes were also shown in Figure 2 and 3 to demonstrate the seedling's survival rate in response to increased gamma irradiation doses. At ED_{50}, the survival rates of the seedlings were over 70% and around 60% for cv. Yalin and the line YAA7050-14, respectively. Moreover, we also determined the ED_{30}, recommended in reduced background mutation frequencies. The ED_{30} for cv. Yalin and line YAA7050-14 were calculated as 163.8 Gy and 206.3 Gy, respectively.

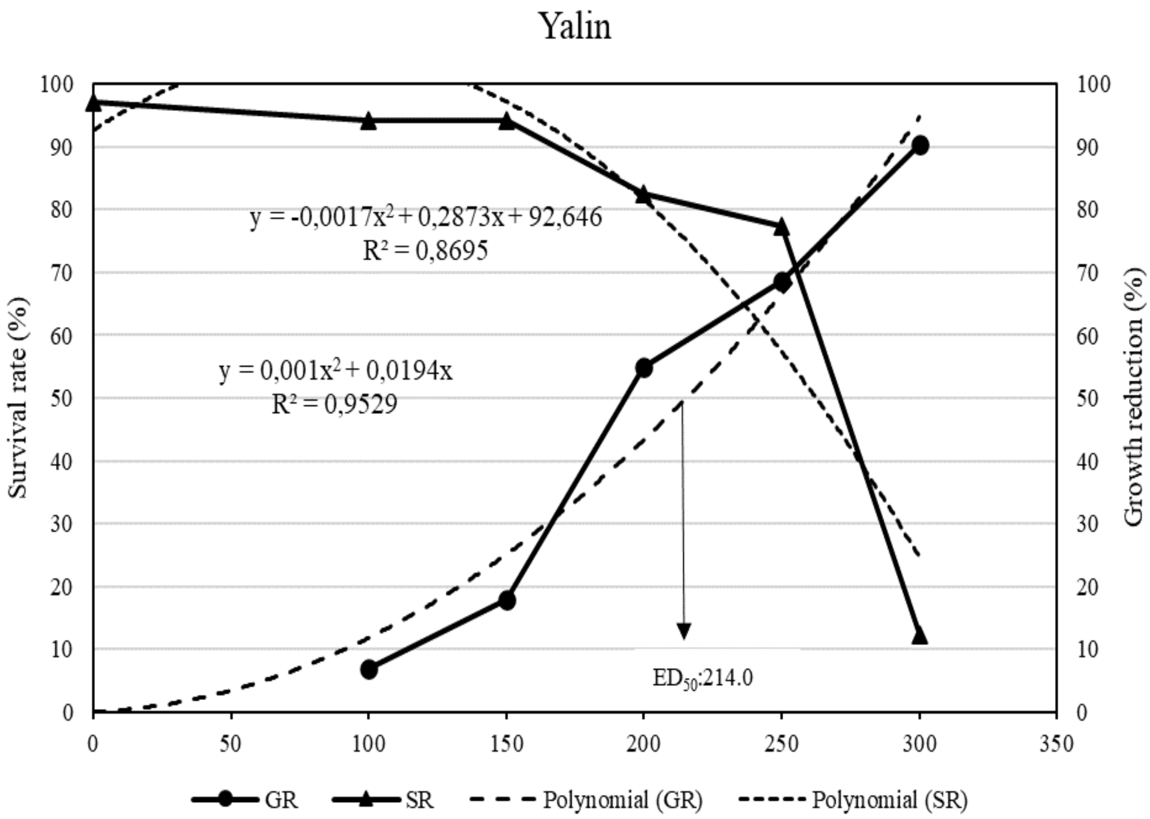

Figure 2. Polynomial regression graphic of survival rate and growth reduction of cv. Yalin.

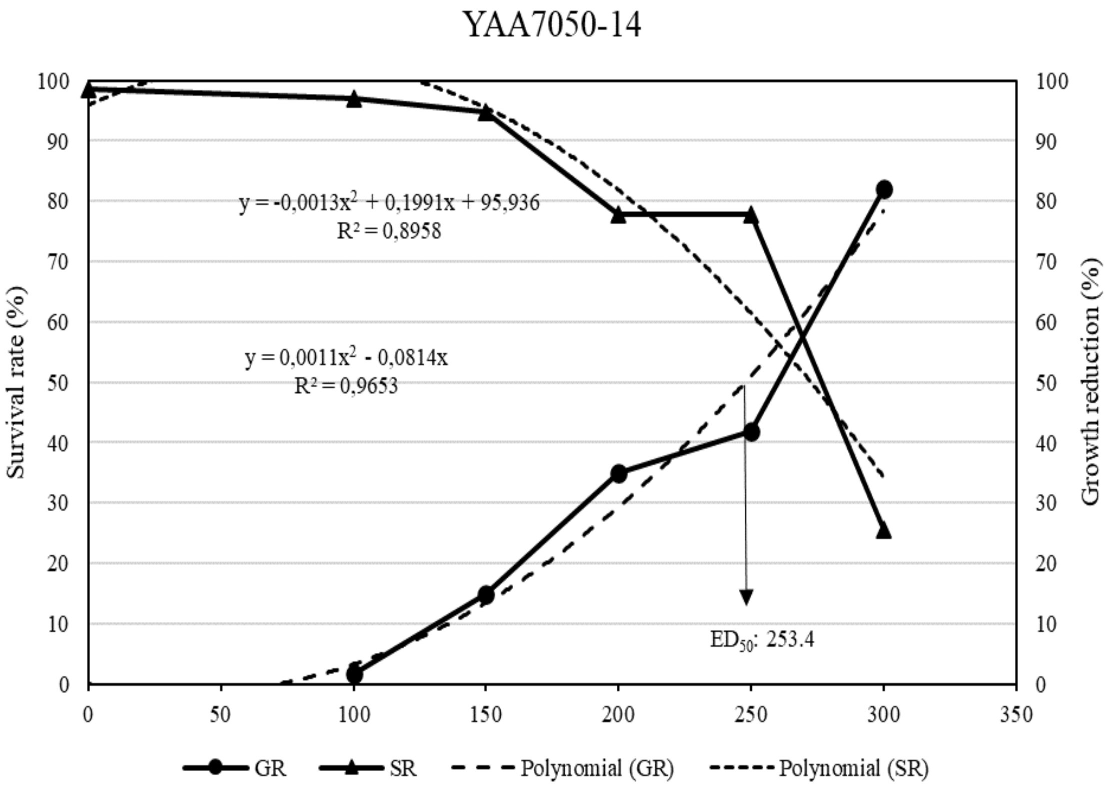

Figure 3. Polynomial regression graphic of survival rate and growth reduction of hulless barley line YAA7050-14.

4. Discussion

One of the most restricting factors for developing new varieties is the absence of variation in hulless barley breeding research; therefore, the mutation approach can be a potential strategy for developing new varieties in breeding research (Dyulgerova and Dyulgerov, 2020). The most critical step of mutation approach is the determination of the optimum dose of mutagen agent (Maluszynski et al., 2009). In this study, gamma rays at different doses (Control, 100, 150, 200, 250 and 300 Gy) applied to the seeds of hulless barley cv. Yalin and hulless barley line YAA7050-14. Germination, seedling, and root characteristics of both hulless barley genotypes were affected by increasing the dose of gamma rays. However, the effects of gamma rays varied based on the dose, genotype and the characteristics of plants.

According to our results, most of the investigated parameters in both hulless barley genotypes were found to be almost identical statistically at 100 Gy irradiation dose compared to those in controls. Moreover, the emergence rate of plants at the dose of 100 Gy was higher than that of control which could be the stimulating effect of gamma rays at 100 Gy. Researches demonstrated that low doses of gamma irradiation can break dormancy (Beyaz et al., 2016; Volkova et al., 2019), increase germination and emergence rate (Rozman, 2015), and have a growth-stimulating effect on seedling and root development in plants (Geras'kin et al., 2017; Volkova et al., 2019; Gorbatova et al., 2020). The positive effect of 100 Gy on plants can be explained by the hormesis phenomenon. Hormesis is known as an adverse effect on plant growth and development; nevertheless, it has a stimulating influence of low doses of harmful chemicals on plant growth and development (Małkowski et al., 2020; Jalal et al., 2021).

Low irradiation doses in all variables evaluated under greenhouse conditions were mainly close to the control, while 250 and 300 Gy doses negatively influenced germination, seedling, and root traits in both genotypes. The statistically significant differences in emergence and survival rate were found in M_1 plants at 250 and 300 Gy doses. Similar research on barley indicated that when mutagen doses increased, germination and emergence rates decreased (Sarduie-Nasab et al., 2010; Rozman, 2015; Navid et al., 2021). Possible reasons for the decrease in germination and emergence rates at higher gamma-ray doses can be structural damage to the embryo (Wang and Yu, 2011), disruption of cell differentiation resulting from mutations in DNA during germination (Daran, 2013), abnormalities at the chromosome and DNA level (Stoilov et al., 2013; Hong et al., 2022), changes on the plant hormones and DNA synthesis (Hong et al., 2022), disruptions in the synthesis and balance of plant growth regulators such as auxins and cytokinins that play a role in cell division and differentiation (Mok and Mok, 2001).

In addition to germination characters, above-ground plant parts were also negatively affected by high gamma-ray doses. The number of tillers, number of leaves, first leaf length, shoot length, fresh and dry weight of the shoot all reduced as the gamma-ray dose increased. Our results have been supported by other researchers showing that higher doses of mutagens diminish the number of tillers (Khah and Verma, 2015) and the number of leaves (Başer et al., 2005). DNA and chromosome damage (Stoilov et al., 2012) and damage to the lateral (axillary) meristems are two potential causes for the decline in tillers and leaves in M_1 plants grown in greenhouses (Hussien et al., 2014; Ye et al., 2019). Possible other explanations include disturbances in the synthesis and balance of hormones active in tillering in barley, such as auxin and cytokinin group hormones (Marzec and Alqudah, 2018) and enzymes (Bitarishvili et al., 2018). Gamma-ray doses over 150 Gy demonstrated a negative effect on leaf characteristics. Previous studies show that as the mutagen dose increased, the length of the first leaf and the shoot decreased (Borzouei et al., 2010; Ahumada-Flores et al., 2020; Navid et al., 2021). Leaves are formed from cells located in the periphery and are suitable for differentiation. Auxin plays an important role in the initiation of leaf formation by identifying these cells (Du et al., 2018). High doses of mutagen applications can cause disturbances in the synthesis and release of auxin group plant hormones (Bitarishvili et al., 2018; Hong et al., 2022.). In addition, mutagens at high doses have genotoxic and mutagenic effects, which may cause a decrease in mitotic index (İlbaş et al., 2006). The reductions in shoot length at high doses might be explained by the reduction of the rate and amount of cell division in apical meristems by mutagens (Oney-Birol and Balkan, 2019), reduction in DNA content (Yamaguchi et al., 2008), and disturbances in the synthesis and balance of auxin group plant hormones (Bitarishvili et al., 2018). The decrease in seedling wet and dry weights at increasing doses occurred as an indirect consequence of decreases in the number of tillers and leaves and seedling height. In other words, abnormalities and damages at chromosome and DNA levels at high mutagen doses (Kiong et al., 2008; Stoilov et al., 2013), disruption of cell division and growth in apical meristems (Oney-Birol and Balkan, 2019), adverse effects on the synthesis and release of enzymes and plant hormones (Bitarishvili et al., 2018; Wang et al., 2018), carbon exchange in the leaves and negative effects on mineral uptake and utilization in the root (Singh et al., 2013). The exact reasons can be listed among the causes for the decrease in shoot wet and dry weights in the study.

Like the seedling traits in this study, root characters also showed a dramatic decrease at gamma-ray doses of 250-300 Gy. A significant negative effect of high irradiation doses on root length was found. Other researchers also found that root lengths decreased parallel with the increase in mutagen doses (Alghamdi et al., 2010; Olgun et al., 2012). Moreover, high mutagen doses reduced root fresh and dry weight of cv. Yalin and the hulless barley line YAA7050-14. The results of previous studies (Borzouei et al., 2010; Grover and Khan, 2014; Navid et al., 2021) also indicate that high mutagen doses have a negative effect on root fresh and dry weight. High gamma irradiation doses decreased root wet and dry weight, which was indirectly caused by a reduction in the number of tillers and root lengths. As gamma-ray doses increase in barley, the balance in plant hormones such as indoleacetic acid, indolebutyric acid, abscisic acid, and zeatin is disrupted, and the effect of growth-promoting hormones is lost (Bitarishvili et al., 2018). In addition, high gamma-ray doses reduce cell division in root tip meristems (Oney-Birol and Balkan, 2019) and mitotic index (Eroğlu et al., 2007). Since adventitious roots did not grow when tillering did not occur in M_1 plants at high doses, this might cause weaker root system development (Geçit, 2016).

Both seedling and root dry weight decreased significantly at high irradiation doses compared to the control and doses less than 200 Gy. However, the reduction in seedling dry weight was greater than the decrease in root dry weight. While both traits declined, the root/shoot dry weight ratio increased compared to the control and other doses. It can be assumed that the negative effect of high doses on root dry weight was less effective than on shoot dry weight. Furthermore, the coefficients of variation in all seedling and root characteristics were higher at the highest doses of 250 and 300 gray compared to control plants and plants irradiated with lower doses. In other words, high gamma-ray doses increased the deviations of the traits studied from their mean values.

Overall, the effect of different doses of gamma rays applied to the seeds on the seedling and root characters varied based on the hulless barley genotypes. Gorbatova et al. (2020) found that the genetic structure of the varieties was an important factor in their response to low doses of gamma rays. Some other research results indicate that the effects of mutagens may vary according to varieties (Nazarenko and Lykholat, 2020; Hong et al., 2022).

Conclusion

Hulless barley is in demand on the market because it has desirable nutritional properties such as high beta-glucan and total dietary fiber content. The number of released hulless barley varieties is limited compared to hulled barley. Wide genetic diversity helps breeders create new cultivars; nevertheless, germplasm shortages restrict the number of hulless barley varieties released. This research investigated different gamma ray doses (0, 100, 150, 200, 250, and 300 Gy) for hulless two-row barley genotypes (Yalin and YAA7050-14) to determine optimum irradiation doses before executing large-scale mutagenesis. The optimal gamma-ray irradiation dosages were determined via constructed dose-response curves. Based on the results, the effective doses (ED_{50}) for the hulless barley genotypes examined in the study ranged from 214.1 to 253.4 Gy gamma rays. At these gamma ray doses, where the mutagenic effect is optimum, the number of surviving plants around 60-70% may help to increase the chance of reaching the desired genotypes in following selection studies.

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