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Impact of gamma radiation on the agronomic properties of naked barley genotypes

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Abstract
The usage of naked barley in the food industry is increasing day by day due to its health benefits. As a result, research on breeding naked barley have gained popularity. In these breeding studies, a wide variation in desired traits is needed to achieve higher success in selection. One of the best methods for obtaining genotypic variation, which is crucial for breeding studies on naked barley, is mutation. To obtain genotypic variation in certain agronomic parameters in naked barley genotypes, the impact of different gamma radiation doses on M_1 and M_2 plants of two naked barley genotypes was evaluated in this research. The seeds were treated with gamma irradiation using Cobalt 60 gamma source at six different doses, along with non-irradiated control samples. While the values at low doses were found to be comparable to the control in the majority of the traits, 250-300 Gy caused significant decreases in the majority of the traits in the M_1 generation of both genotypes. Plant height, number of spikelets per spike, and number of grains per spike at the M_2 generation were all negatively impacted by 250–300 Gy, although spike length, grain weight per spike, and thousand grain weight were positively impacted by the same doses. The mutant population generated by gamma irradiation of seeds of different naked barley genotypes was found to have suitable variation for the selection of desired traits. In addition, this material can be used to select individuals with outstanding agronomic characteristics.
Keywords: Naked barley, Mutation, Gamma irradiation, M_1 and M_2 plants, Variation

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cultivated crop that ranks second after wheat in terms of cultivation area and production amount among cool-season cereals. Currently, 146 million tonnes of barley grain are produced on 48 million hectares throughout the world (FAOSTAT, 2022). Globally, 60% of barley is used in animal feed, 40% in the malt industry, 5% as seed, and 3% for human consumption (Ullrich, 2011). Barley is an important source of feed and malt as well as an important food crop in some countries of the world. It is also the most abundant source of functional ingredient-rich cereals and the most available species for functional food crops. Barley is extremely rich in nutrients and functional components (Zeng et al., 2020). Depending on the separation of grains and husks after harvest, barley is classified into two main categories as hulled and naked. The recessive nud gene determines the naked caryopsis trait in barley (Duan et al., 2015).

Naked barley is grown mainly for food as it contains greater nutrients, including beta-glucan and total dietary fiber, and is easier to process than hulled types

(Meints et al., 2021). Despite its advantages as a food, the number of naked barley varieties released worldwide is still limited compared to its hulled counterpart. The neglect of naked barley in breeding programs and the limited genetic resources required for breeding studies are the two most prominent causes of this limitation (Dickin et al., 2012; Meints et al., 2021). A wide genetic diversity of genotypes provides for an efficient and successful breeding program for the development of new varieties. Crossing and mutation are the most common methods for creating genetic variation.

Mutations, which are sudden changes in the genome of plants, are commonly used in barley breeding and genetic research. The primary advantage of using mutations in breeding studies is the potential to improve one or two traits without affecting the rest of a well-adapted cultivar's genotypic structure (Dyulgerova & Dyulgerov, 2020). Mutant plants can be released as a new variety or used as a parent in crossing studies (Ahloowalia et al., 2004). In addition, mutation methods allow rapid generation of desired traits when genetic diversity or traits are unavailable in a germplasm collection (Maluszynski et al., 2009). Mutation breeding is one of the most effective ways for increasing genetic diversity for commercially important agricultural traits (Chaudhary et al., 2019).

Physical and chemical mutagens are utilized in mutant breeding studies to change the genetic structure of plants and increase variation. Gamma (γ) rays are the most popular physical mutagens used as mutation sources by the breeders because they are easy to use and can penetrate deep into a biological substance, are less harmful to the environment and humans, and are easily accessible (Suprasanna et al., 2015; Ulukapi & Nasircilar, 2015). In addition, gamma rays cause point mutations and small deletions that have less destructive effects on the organism than other physical mutagens (Suprasanna et al., 2015). As a gamma ray source, Cobalt 60 (^{60}Co), the radioactive isotope of Cobalt, is widely used in mutation breeding due to its short half-life and high energy (Shu et al., 2012).

This study aimed to evaluate the effect of six different gamma radiation doses on M_1 and M_2 plants of naked barley cv. Yalin and naked barley line YAA7050-14. In addition, genotypic variation in agronomic parameters affecting the yield of naked barley was obtained using gamma radiation. The differences between the impacts of the gamma ray doses compared to the non-irradiated control were determined by analysis of variance. The measurement of the variation created was based on the standard deviations and coefficients of variation of the traits.

MATERIALS AND METHODS

Materials

Elite seeds of the naked barley cv. Yalin and the naked barley line YAA7050-14 developed by the Central Research Institute for Field Crops (CRIFC) were used as plant material. As the physical mutagen source, gamma-rays obtained from the 381 Gray/hour Cobalt 60 (^{60}Co) source in the Ankara Nuclear Research and Training Centre (ANAEM) were used.

Methods

For each dose and control group, 500 seeds of naked barley cv. Yalin and line YAA7050-14, both healthy and having nearly 12% moisture, were prepared separately. The prepared seeds were irradiated with gamma rays at doses of 0 (Control), 100, 150, 200, 250, and 300 Gray (Gy). Seeds in the control group and irradiated at different doses were sown in a randomized complete block design with three replications separately in the experimental field of the CRIFC to grow M_1 plants.

The field trials were conducted in the Yenimahalle location in the first year (2017-2018 growing season) of the study for observations to be taken from M_1 plants, and seeding was done manually one day after irradiation. The plots consisted of four rows one meter in length, spaced by 30 cm. Twenty five healty seeds were sown in each row. The second-year (2018-2019 growing season) trials with M_2 plants were also done manually at the Ikizce location. Sowing was done in 1 m long rows with 30 cm row spacing in this trial. In each replication, the number of rows seeded was equal to the number of main spikes gathered in the M_1 generation.

Measurements and observations on M_1 and M_2 plants were made according to Senay & Ciftci 2005, Spencer-Lopes et al., 2018. Measurements related to height and length are given in centimeters (cm) and measurements related to weight are given in grams (g). During the field study, the data obtained from M_1 and M_2 plants were subjected to an analysis of variance according to the randomized complete blocks design, separately for the cv. Yalin and the line YAA7050-14. F test was used to determine the statistical significance level of the differences, and Duncan's multiple comparison method was used to group the means (Montgomery, 2013).

RESULTS AND DISCUSSION

Based on the results of variance analysis (ANOVA) performed on collected data from M_1 plants at different gamma-ray doses, significant differences in all attributes except spike length were detected between doses at 0.01 and 0.05 in both genotypes (Table 1).

While the control plant height in cv. Yalin was 114.3 cm, after 250–300 Gy gamma irradiation, plant height (PH) reduced by 15% to 97.0 cm (Table 2). The PH values in M_1 plants of line YAA7050-14 was decreased significantly at high gamma doses compared to the PH values in the control (Table 3). While the average PH was 108 cm in the control, the average PH was 96.2 and 94.0 cm at 250 and 300 Gy doses, respectively. When the results of similar

Table 1. ANOVA results for the traits of M_1 plants of cv. Yalin and line YAA7050-14.

	Yalin (M_1)				YAA7050-14 (M_1)			
	Replication	Doses	Error		Replication	Doses	Error	
DF	2	5	10		2	5	10	
	Mean square			CV	Mean square			CV
PH	50.18	177.85*	18.78	4.1	13.99	95.30**	11.98	3.4
NS	0.03	1.07*	0.029	3.0	0.56	3.65**	0.28	9.3
SL	0.19	0.38^{ns}	0.34	5.2	0.21	0.21^{ns}	0.72	2.7
NSS	2.17	6.47**	0.86	3.1	2.05	5.26**	0.43	2.2
NGS	1.00	31.16**	0.81	3.3	1.76	11.93**	0.38	2.4
FT	3.85	121.20**	4.12	2.8	0.77	27.03**	2.05	2.1
GW	0.01	0.11**	0.003	5.0	0.01	0.08**	0.004	7.4
SR	14.89	314.63**	14.47	5.4	1.82	188.00**	12.85	4.9

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ns Not statistically significant; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate; DF Degrees of freedom; CV Coefficient of variation (%)

studies were evaluated, it was reported that there was a decrease in plant height in barley (Ashmawy et al., 2016) at increasing mutagen doses. Possible reasons for this decrease in plant height include damage at DNA and chromosome levels (Stoilov et al., 2013), decreased photosynthetic activity and increased oxidative stress (Choi et al., 2021), negative effects on the synthesis and balance of plant hormones such as gibberellins, brassinosteroids, and strigolactones (Barboza et al., 2013; Marzec & Alqudah, 2018; Sarkar et al., 2004), damage to apical meristems (Sayed Hussien Elsayed et al., 2014), gas exchange in leaves, mineral uptake and utilization by roots (Singh et al., 2013). Increasing yield under irrigated conditions by shortening plant height, increasing stem robustness, and resistance to lodging through mutation breeding were the main objectives of many studies in barley and other cereals (Ahuja et al., 2014). As a result of these studies, dwarf and semi-dwarf mutant genotypes took their place in modern agriculture (Gruszka et al., 2011). The finding of shorter genotypes compared to the control in our study shows that the desired variation in plant height has occurred in the naked barley genotypes used as material.

In both genotypes, the decrease observed in plant height was not seen in spike length (SL). When all gamma doses were considered, although there was a slight decrease in SL only at 250 and 300 Gy doses, control and all other doses were in the same statistical group (Table 2 and Table 3). In similar studies conducted with different mutagen doses, it was found that there was no or very little difference in SL depending on the dose increase (El-Degwy & Hathout, 2014), while it is also possible to see results that SL decreased at high doses (Ashmawy et al., 2016). In our study, our results are in parallel with the findings of these researchers in terms of the presence of plants with longer spike and shorter spike plants together and similar to the control. SL in barley varies with the length and number of internodes on the spike axis. The variation observed at high doses is thought to be caused by differences in the length and number of internodes on the rachis. Mutant barley genotypes with lax spikelet arrangement (*lax*-a) with long internodes on the rachis or dense spikelet arrangement (*dsp*) with short internodes, in other words "*compact spikes*", are examples of sources of variation in spike length (Terzi et al., 2017). Disruption of cell division and growth in apical meristems after mutagen treatments and negative effects on the synthesis and release of plant hormones such as auxin and enzymes active in spike formation (Terzi et al., 2017; Wang et al., 2018) could be among the possible reasons for differences in spike length.

In M_1 plants of the cv. Yalin, higher values were obtained in the number of fertile spikes (NS) per plant counted at higher doses compared to the control (Table 2). While the average NS in the control was 5.3, the NS at the 300 Gy dose was 6.4. NS in M_1 plants of line YAA7050-14 was higher at high doses than in the cv Yalin. A decrease in surviving rate occurred after the 200 Gy dose in M1 plants (Table 3). One possible explanation for this occurrence is that the surviving plants are more tillering when the number of plants per unit area decreases at high doses. Barley with a high tiller number (*hnt1*) or no tillering (*uniculm2*) has been developed by mutation breeding (Okagaki et al., 2018; Ye et al., 2019). In our study, the coexistence of plants with less or more spike numbers per plant than the control shows sufficient variation in this material for selection for this trait.

The number of spikelets (NSS) counted in the main spike was around 31 in the control plants of cv. Yalin, however, the NSS decreased to 28 at the 300 Gy dose. The number of grains (NGS) counted in the same spikes showed a similar trend. The number of grains in the spike at doses of 250 and 300 Gy, however, was found to be 20% lower than the control, indicating that the adverse effects of high doses were more severe in this trait. The adverse effect might result from the decrease in the fertility rate

(FT) of the spike. While the FT was around 95% at 0-150 Gy doses, this value decreased to 86.6% at 250 Gy dose and to 77.9% at 300 Gy dose. Due to the decrease in the NGS at high gamma-ray doses, the grain weights per spike (GW) also decreased at a similar rate (Table 2). NSS in the main spikes of YAA7050-14 and the NGS obtained from the same spikes were also negatively affected by high gamma-ray doses (Table 3). While there were approximately 32 spikelets in one spike in the control, the NSS decreased to 28 at the dose of 300 Gy. This decrease in the NGS at high gamma-ray doses was higher than the decrease in the NSS. The increase in the number of sterile spikelets in the spike at doses of 250-300 Gy caused the decrease in FT. While the FT of the control was approximately 91%, the FT decreased to 83% at high doses of gamma rays. Possible reasons for the decrease in the NSS according to increasing doses may include the negative effects on the synthesis and release of enzymes and auxin group plant hormones that are effective in the beginning of the formation of the spike draft in barley (Wang et al., 2019) and the disruption of cell division and growth in the apical meristems (*phytomere* 2) (Terzi et al., 2017) where the spike is formed after mutagen applications. In some similar studies conducted in barley, it was found that increasing mutagen doses decreased the number of grains in the spike (Ashmawy et al., 2016; Dyulgerova & Dyulgerov, 2020). The findings of our study are in parallel with the findings of these researchers. It can be clearly seen that the main reasons for the decrease in the NGS are the reduction in the total number of spikelets per spike and the lower fertility at high gamma doses in both genotypes. The most common cause of the fertility reduction after mutagenic applications is chromosomal abnormalities and the resulting non-functional gametes. In addition, defects in different stages of meiosis and reduced pollen fertility are among the main causes of reduced fertility (Lavinscky et al., 2017; Pagliarini, 2000). The results of similar studies (Choi et al., 2021; Gowthami et al., 2017; Nazarenko & Lykholat, 2020) on mutagen application in barley and other cereals reported a decrease in FT with increasing mutagen dose. The results of these researchers are similar to the results of our study and support our findings.

Average grain weight (GW) per spike in control cv. Yalin was 1.3 g, while GW in M_1 plants at 250 and 300 Gy doses was 0.9 g (Table 2). Whereas in the YAA7050-14 line, GW measured in the control was 1.0 g, it decreased to 0.7 and 0.6 g after irradiation at 250 and 300 Gy doses, respectively (Table 3) The decrease in the NGS caused a decrease in the GW. Previous studies conducted in barley (Akgün et al., 2019; Karakoca & Akgün, 2020), reported that the GW obtained after high-dose mutagen application was reduced. These researchers confirm the results of our study. The most important reasons for the decrease in GW at 250 and 300 Gy gamma ray doses in both naked barleys are the increase in the NGS in the spike at these doses. As a result, it is possible to accept the decrease in GW at 250-300 Gy doses as an indirect result of the decrease in FT and NGS in the spike at these doses.

The percentage of M_1 plants of the cv. Yalin that survived from germination to harvest (SR) decreased by roughly 30% as gamma-ray doses increased. While the SR was about 96% in the control and low gamma-ray doses, the SR was found 66% at 300 Gy (Table 2). The change in SR in the YAA7050-14 line was similar to the cv Yalin. While SR value was 97% in the control, it decreased to 72% at 300 Gy dose (Table 3). Results from previous studies (Ahumada-Flores et al., 2020; Nazarenko & Lykholat, 2020) indicate that high doses of mutagenic applications reduce SR in barley and wheat. The results of our study in terms of SR are consistent with the results of these studies. High mutagen doses increase the mutation frequency, however, they may also reduce the SR and, in some cases, it is not possible to reach the number of plants sufficient for M_2 generation (Rybinski et al., 2003). In this respect, SR (viability) is one of the important parameters used in determining the effective dose (ED_{50}) in mutagen applications(Ahumada-Flores et al., 2020). Plant death after mutagen treatments can occur at any time between germination and maturation and these plants are usually sterile (Nielen et al., 2018). Biochemical changes in photosynthetic pigments (*chlorophyll*-a, *chlorophyll*-b and *xanthophyll*) and the effect of free radicals (Marcu et al., 2013), adverse effects on the synthesis and balance of plant hormones (Bitarishvili et

Table 2. Means of the traits studied in M_1 plants of cv. Yalin at different gamma-ray irradiation doses

Doses	PH	NS	SL	NSS	NGS	FT	GW	SR
Control	114.3 a*	5.3 b	11.2 a	31.3 a	29.9 a	95.2 a	1.3 a	95.7 a
100	110.6 a	5.2 b	11.8 a	30.7 ab	29.3 a	95.5 a	1.3 a	96.0 a
150	110.9 a	5.1 b	11.2 a	30.8 a	29.3 a	95.2 a	1.3 a	95.0 a
200	106.8 ab	6.4 a	11.0 a	30.2 ab	26.9 b	89.4 b	1.0 b	92.0 a
250	97.5 bc	5.4 b	11.5 a	27.7 c	24.0 b	86.6 b	0.9 b	76.3 b
300	95.5 c	6.4 a	10.8 a	28.3 bc	22.4 c	77.9 c	0.9 b	66.0 b
Mean	105.9	5.6	11.2	29.8	26.9	90.0	1.1	86.8

* Means indicated with the same letter belong to the same statistical group; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate

Table 3. Means of the traits studied in M_1 plants of line YAA7050-14 at different gamma ray irradiation doses

Doses	PH	NS	SL	NSS	NGS	FT	GW	SR
Control	108.0 a*	5.0 b	10.4 a	31.5 a	28.7 a	90.9 a	1.0 a	97.0 a
100	102.5 a	4.4 b	10.5 a	29.7 b	27.2 ab	91.4 a	1.0 a	96.0 a
150	106.8 a	5.4 b	10.2 a	30.4 ab	26.7 b	87.5 ab	1.0 a	92.7 a
200	103.4 a	6.8 a	10.0 a	29.5 b	26.5 b	89.6 a	0.9 ab	91.7 a
250	96.2 b	5.2 b	9.9 a	28.8 bc	24.3 c	84.1 b	0.7 bc	89.3 a
300	94.0 b	7.3 a	9.9 a	27.7 c	23.2 c	83.5 b	0.6 c	71.7 b
Mean	101.8	5.7	10.2	29.6	26.1	87.8	0.9	89.7

* Means indicated with the same letter belong to the same statistical group; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate

Table 4. ANOVA results for the traits of M_2 plants of cv. Yalin and line YAA7050-14.

	Yalin (M_2)				YAA7050-14 (M_2)			
	Replication	Doses	Error		Replication	Doses	Error	
DF	2	5	10		2	5	10	
	Mean square			CV	Mean square			CV
PH	3.05	35.83^{ns}	11.88	4.0	6.10	23.20^{ns}	10.42	4.0
SL	0.004	0.20^{ns}	0.08	2.6	0.09	0.50^{ns}	0.11	3.4
NSS	0.01	0.50^{ns}	0.17	1.4	0.95	2.00^{ns}	0.92	3.2
NGS	0.78	1.98**	0.22	1.7	1.97	0.52^{ns}	1.35	4.0
GW	0.001	0.003*	0.001	2.1	0.002	0.005^{ns}	0.005	5.3
TKW	0.16	1.50^{ns}	0.67	1.7	1.23	2.27^{ns}	0.80	1.9

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ns Not statistically significant; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate; DF Degrees of freedom; CV Coefficient of variation (%)

al., 2018) are among the most important reasons that decrease the SR at high doses. In addition, chromosomal and biological damage (Khah & Verma, 2015), disruptions in the division of somatic cells and inhibition of the activity of RNA polymerase and the effects of genes responsible for cell division (Ahumada-Flores et al., 2020) are the other most important reasons for this decrease. Furthermore, environmental stress factors experienced in field trials may also increase mortality rates after mutagen application (Nielen et al., 2018).

Table 4 shows the results of the analysis of variance on the data obtained from M_2 plants in the second year of the study (Table 4). The differences between the doses in PH, SL, NSS and TGW were not statistically significant in M_2 generation. In terms of NGS and GW, the differences between the doses were statistically significant in cv. Yalin and insignificant in line YAA7050-14. Although the differences between different gamma ray doses in most of the traits examined in M_2 generation were not statistically significant, it was determined that 250 and 300 Gy gamma ray doses decreased the PH, NSS, and NGS compared to the control and these effects were realized at different levels according to genotypes. On the other hand, SL, GW, and TGW values obtained from high doses such as 250 and 300 Gy were higher than the control and other doses. In addition, the variation obtained in most of the traits examined in M_2 plants was higher in 250 and 300 Gy doses compared to the control and other doses.

Descriptive statistics of the examined traits related to M_2 plants of cv. Yalin are given in Table 5. The control plants of cv. Yalin had an average plant height (PH) of 85.1 cm. The PH of the main stem in control ranged from 72.0 cm to 94.0 cm. In M_2 plants the highest variation in PH was observed at 200 and 250 Gy doses (Table 5). The lowest and highest PH values of 200 Gy were 65.0 cm and 102.0 cm, respectively. At 250 Gy dose, PH values varied between 59.0-94.0 cm. The highest coefficients of variation (CV) in plant height were 9.0% and 8.9% at 200 and 250 Gy doses, respectively. The coefficient of variation of the control for this trait was 6.6%. Coefficients of variation for spike length (SL) also increased after gamma irradiation. The CV of the control was 6.3%, while the CV of the 300 Gy dose was 8.3%. At 300 Gy dose, the variation around the average in SL increased, and the lowest and highest spike lengths varied between 9.7 cm and 13.5 cm, respectively. Control and M_2 plants obtained by gamma irradiation at different gamma doses had very similar mean and CV values for the NSS. However, the number of grains per spike (NGS) changed after gamma irradiation compared to the control. The

Table 5. Descriptive statistics of the traits examined in M_2 plants of cv. Yalin

Doses		PH	SL	NSS	NGS	GW	TGW
Control	min-max	72.0-94.0	9.6-12.7	25.0-34.0	21.0-33.0	0.98-1.62	40.0-53.1
	X-SD	85.1±5.58	11.0±0.70	30.1±2.30	29.3±2.51 a*	1.38±0.14 ab	46.9±2.98
	CV	6.6	6.3	7.6	8.6	10.4	6.3
100 Gy	min-max	80.0-104.0	8.9-13.0	25.0-35.0	18.0-35.0	0.77-1.73	35.8-52.4
	X-SD	91.3±6.08	10.9±0.79	30.5±2.23	28.5±3.54 a	1.33±0.22 bc	46.5±3.47
	CV	6.7	7.2	7.3	12.4	16.5	7.5
150 Gy	min-max	71.0-103.0	9.1-12.5	25.0-34.0	24.0-34.0	0.93-1.63	35.6-52.7
	X-SD	89.7±7.01	10.9±0.63	30.4±2.22	29.5±2.41 a	1.39±0.13 a	47.2±3.10
	CV	7.8	5.8	7.3	8.2	9.3	6.6
200 Gy	min-max	65.0-102.0	7.8-12.5	21.0-34.0	18.0-33.0	0.85-1.61	37.8-53.4
	X-SD	83.5±7.48	11.0±0.79	29.8±2.29	28.7±2.68 a	1.35±0.16 ac	47±3.16
	CV	9.0	7.2	7.7	9.4	11.7	6.7
250 Gy	min-max	59.0-94.0	9.8-13	25.0-33.0	19.0-33.0	0.88-1.68	39.7-52.6
	X-SD	83.0±7.38	11.4±0.82	29.8±2.08	27.2±3.90 b	1.3±0.22 c	47.8±2.85
	CV	8.9	7.1	7.0	14.3	17.2	6.0
300 Gy	min-max	67.0-99.0	9.7-13.5	27.0-34.0	20.0-34.0	0.94-1.65	40.9-52.8
	X-SD	84.6±7.08	11.4±0.91	30.8±1.84	28.4±3.60 ab	1.38±0.19 ab	48.5±2.38
	CV	8.3	7.9	6.0	12.6	13.4	4.9

* Means indicated with the same letter belong to the same statistical group; PH Plant height; SL Spike length; NSS Number of spikelets per spike; NGS Number of grains per spike; GW Grain weight per spike; TGW Thousand grain weight; X-SD Mean-Standard deviation; CV Coefficient of variation (%)

Tablo 6. Descriptive statistics of the traits examined in M_2 plants of line YAA7050-14

Doses		PH	SL	NSS	NGS	GW	TGW
Control	min-max	71.0-89.0	8.5-10.7	20.0-36.0	19.0-35.0	0.85-1.64	36.8-51.5
	X-SD	80.5±4.24	9.6±0.46	30.0±2.75	29.3±3.02	1.36±0.16	46.5±3.10
	CV	5.3	4.8	9.2	10.3	11.9	6.7
100 Gy	min-max	65.0-88.0	8.3-11.5	22.0-38.0	19.0-38.0	0.81-1.71	30.3-52.3
	X-SD	80.5±4.74	9.8±0.63	30.5±2.73	29.9±3.95	1.33±0.22	45.6±4.79
	CV	5.9	6.4	9.0	13.2	16.5	10.5
150 Gy	min-max	71.0-95.0	8.0-10.7	25.0-35.0	23.0-34.0	0.99-1.64	34.2-51.4
	X-SD	81.1±5.26	9.3±0.67	29.7±2.22	29.1±2.36	1.33±0.15	45.6±3.74
	CV	6.5	7.2	7.5	8.1	11.1	8.2
200 Gy	min-max	51.0-90.0	7.7-11.5	21.0-36.0	19.0-35.0	0.82-1.73	37.9-51.7
	X-SD	77.3±7.19	9.6±0.76	29.8±3.17	29.1±3.45	1.37±0.20	47±3.14
	CV	9.3	8.0	10.6	11.9	14.5	6.7
250 Gy	min-max	49.0-91.0	8.5-11.2	21.0-36.0	15.0-36.0	0.75-1.73	37.5-50.9
	X-SD	74.7±8.66	9.8±0.60	30.5±3.33	28.7±4.62	1.32±0.23	46.1±2.81
	CV	11.6	6.2	10.9	16.1	17.3	6.1
300 Gy	min-max	62.0-89.0	8.0-12.7	26.0-38.0	20.0-34.0	0.9-1.83	32-51.7
	X-SD	78.2±6.45	10.5±0.85	31.9±2.56	29.0±3.16	1.43±0.21	47.9±3.11
	CV	8.2	8.1	8.0	10.9	15.0	6.5

PH Plant height; SL Spike length; NSS Number of spikelets per spike; NGS Number of grains per spike; GW Grain weight per spike; TGW Thousand grain weight; X-SD Mean-Standard deviation; CV Coefficient of variation (%).

coefficients of variation increased relative to the control at 250 and 300 Gy gamma-ray doses, reaching 14.3% and 12.6%, respectively. At 300 Gy gamma-ray dose, 34 grains per spike were observed, indicating a positive variation in this trait. A similar situation was observed in grain weight per spike (GW), which is closely correlated with NGS. The lowest and highest GW in control were 0.98 g and 1.62 g, respectively. The highest CV for this trait among M_2 plants was found at 250 Gy with 17.2%. For this dose, the lowest and highest GW values were 0.88 g and 1.68 g, respectively. The following highest CV was found at 100 Gy. At the 100 Gy dose, a 1.73 g spike grain weight value was reached. The coefficients of variation in TGW were found to be similar in control and M_2 plants obtained after gamma irradiation at different doses. The highest CV for this trait was found at 100 Gy with 7.5%. The highest mean value in terms of TGW was determined at 300 Gy gamma-ray dose with 48.5 g.

Table 6 shows the descriptive statistics of the characteristics examined in M_2 plants of the naked barley line YAA7050-14. The main stem PH of the control ranged from 71.0 cm to 89.0 cm. In M_2 plants obtained after gamma irradiation, the greatest variation in PH was observed at 250 Gy dose with decreased 9.0% (Table 6). At 250 Gy, 49.0 cm and 91.0 cm were the lowest and highest PH values, respectively. The CV of the control group for this characteristic was 5.3 %. Gamma irradiation also increased the coefficient of variation for spike length. The CV of the control was 4.8%, while the CV of the 300 Gy dose was 8.1%. At 300 Gy SL varied between 8.0 cm and 12.7 cm. The coefficient of variation of NGS increased compared to the control at 250 and 100 Gy, reaching 16.1% and 13.2% respectively. At 100 Gy gamma dose, 38 grains per spike were observed, indicating a positive variation in this characteristic. A similar trend was observed for the grain weight per spike. The lowest and highest GW measured in the control were 0.85 g and 1.64 g respectively. The highest CV for this characteristic in M_2 plants was found at 250 Gy with 17.3%. At this dose, the lowest and highest GW values were 0.75 g and 1.73 g, respectively. In M_2 plants, the maximum GW value was found at 300 Gy dose with 1.83 g. The coefficients of variation for TGW were found to be similar in control and M_2 plants obtained after gamma irradiation at different doses. The highest CV for this characteristic was found at 100 Gy with 10.5% (Table 6).

At high gamma ray doses, plants close to the control were observed in the M_2 generation as well as plants with much shorter height than the control at these doses. The presence of genotypes shorter than the control indicates that the desired variation in terms of plant height has occurred in the naked barley genotypes used as material and that short and lodging resistant genotypes can be selected from this variation. When the results of similar studies on mutagen application in barley were evaluated (Ashmawy et al., 2016), similar results were reported that PH decreased with increasing doses.

A closer evaluation of the spike length data in this study shows that plants with short and long spikes were found together compared to the control, i.e. the variation in spike length at high doses was higher than at the control and other doses (Tables 5 and 6). In similar studies investigating the effects of mutagen applications on spike length, it was found that there was no or very little difference in spike length depending on the dose increase (El-Degwy & Hathout, 2014), while it is possible to see the results that spike length decreased at high doses (Ashmawy et al., 2016). The presence of genotypes with longer spike length compared to the control in the material obtained from the study shows that there is a very promising variation for increasing the yield potential.

In M_2 generation of both naked barley genotypes, 250 and 300 Gy doses increased the number of sterile spikelets in the spike and caused a decrease in the NGS. Although the NGS decreased at high gamma ray doses in M_2 generation, plants with grain numbers close to the control and higher grain number were also observed (Tables 5 and 6). In other words, it can be said that there was enough variation in both genotypes as a result of gamma ray application to make a selection in the desired direction for this trait. Increasing the NGS is among the main objectives of mutation breeding in terms of its positive relationship with yield (Ahuja et al., 2014).

The results show that high doses of gamma radiation, such as 250-300 Gy, induce a variation with higher TGW in these genotypes, and it is possible to select plants with higher values than the control from this material. In many studies, it has been reported that TGW decreases with increasing mutagen doses (Dyulgerova & Dyulgerov, 2020; El-Degwy & Hathout, 2014; Singh & Datta, 2010), while in some studies, similar to our results, it has been reported that there is no difference with mutagen applications or an increase in TGW can be achieved (Cheng et al., 2015). The presence of plants with higher TGW compared to the control, which is both one of the most important yield factors (Ataei, 2006), as well as one of the most important quality parameters in barley (Jilal et al., 2013), indicates that there is sufficient variation in this material to make target-oriented selection.

The negative effects of high gamma ray doses and the decrease in M_2 generation compared to the control were not as effective and significant as in M_1 generation. The reason for this could be the somatic effects of gamma irradiation and the non-heritable morphological differences in the plants (Raina et al., 2022) and the DNA repairs after mutagen application (Viana et al., 2019).

CONCLUSION

The results of this study showed that within the mutant population created by gamma irradiation of the seeds of naked barley cv. Yalin and naked barley line YAA7050-14, a variation suitable for selection in the desired direction was formed with respect to plant height, spike length, number of grains per spike, grain weight per spike, and thousand grain weight characteristics. In addition, it is possible to select individuals with superior characteristics to the controls from this material. Gamma irradiation at 250 Gy caused the highest variation in both barley genotypes in this study, followed by 300 Gy. These doses could be recommended for the creation of genetic variation in naked barley. Consequently, it can be said that gamma irradiation is an effective way to create variation for use in naked barley breeding studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

All authors declare that they have no conflicts of interest

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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