Performances of advanced bread wheat (*Triticum aestivum* **L.) mutant lines for grain yield and some yield components**

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Abstract

This research was conducted to assess yield and some yield components of 65 advanced bread wheat mutant lines of M₆ generation selected from M₄ populations of 4 different bread wheat genotypes. They were sown using an augmented design consisting of five blocks of which every block had 13 mutant advanced lines and 4 check genotypes (parents) in the 2014-2015 growing season. According to the data obtained from the experiment, there was a great variation for plant height (69.15-119.56 cm), spike length (9.26-11.78 cm), number of grains per spike (30.59-59.84), grain weight per spike (1.43-3.13 g), harvest index (32.57-48.37%), thousand grain weight (34.13- 55.93 g) and grain yield (4712-9515 kg ha-1). The 33 lines for plant height, 22 lines for spike length, 21 lines for the number of grains per spike, 31 lines for grain weight per spike, 35 lines for harvest index, 30 lines for 1000 grain weight and 33 lines for grain yield have been found to perform better than check genotypes. According to the results of the study, MT35, MT14, MT27, MT19, MT20 and MT15 advanced mutant lines were superior to the parents in more than one trait. These mutant lines were selected for pre-registration yield trials, and they may prove useful as a parent for yield improvement in a wheat breeding program. Gamma irradiations with 100 and 200 Gy may be more beneficial for yield improvement in wheat mutation breeding.

Keywords: Bread wheat; mutant line wheat; augmented; grain yield; yield components

INTRODUCTION

Mutagenesis and hybridization are generally the most commonly used breeding methods to develop new superior varieties. Mutation breeding has some advantages compared to cross breeding. When comparing mutation and hybridization; the occurrence of even a few desirable mutations in high yielding varieties has the great advantage of becoming homozygous and expressing its superiority within a couple of generations after induction in M_2 or M as compared to F_6 or F_7 generations in case of hybridization (Chakraborty & Paul, 2013). Mutation breeding has become an appropriate option to improve plant characters when conventional breeding does not work, or the desired traits were recessive, or improving another character in an established plant variety, or improving one or two main

character(s) (Ahloowalia & Maluszynski, 2001; van Harten, 1998). Besides, mutagenesis can isolate mutants with multiple characters, as compared to transgenes where the only line can be introduced it's the major advantage of inducing mutations (Louali et al*.*, 2015). Mutation breeding can be applied to improve a specific character without changing other characters and it is possible to improve a single line without causing an important disturbance in the genome. The selection of tropical wheat mutant lines using selection index and multivariate analysis were considered effectively with a high determination value of 0.57 (57%). The results of the selection index show that seven lines were recommended for multi-location trials since they had a better selection index potential than the Dewata variety (Fadli et al., 2022). Furthermore, it may create a new character that did not belong to parental plants. The mutants developed in wheat have a great potential for direct release and inclusion in hybridization breeding programs (Sakin et al*.*, 2005). The released mutant cultivars in different crops had a great economic impact on agriculture and food production and added billions of dollars to the economy of many countries (Jain, 2006). More than 3000 varieties of different crops have been officially released by mutation breeding techniques. Mutation induction with radiation has been the most frequently used method to develop direct mutant varieties (64% with gamma-rays, 22% with X-rays), accounting for about 90% of obtained varieties (Ahloowalia et al., 2004). Gamma-ray mutagen was effective in broadening genetic variability and increasing the means of wheat cultivars for grain yield and its components, helping plant breeders to practice an efficient selection in the M_2 and next mutated generations (Khanna et al*.,* 1986; Al-Naggar et al*.,* 2007).

The mutant lines showed the capacity to biofortify wheat grain without negatively impacting crop productivity and this population offers promising donors for improving grain parameters such as GA, length, width and quality. The data presented showed how the genetic variation generated through radiation could be used to test the linkage between various important grain parameters (Miller, 2017). Mutant populations have now been created for many cereal crops, including rice (Suzuki et al*.*, 2008), durum wheat (Başer et al*.,* 1997; Sakin & Yildirim, 2004) and bread wheat (Slade et al*.*, 2005). Three seeds from each spike per plant of $M₁$ plants were collected from this M_2 , 17 desirable putative mutant plants which varied significantly with the mother were visually selected. Most of the mutant lines showed homogeneity for most of the characters studied. Eleven of these 17 lines were found to be promising in respect of days to flower, plant height (for semi-dwarf) and other traits including grain yield (Albokari, 2014).

The goal of a wheat breeding program is to develop superior genotypes as a result of many years of selection. Early generation selection is based on visual observations of yield components (spike length and size, number of spike per plant, number of spikelets per spike), disease resistance, tillering potential, lodging resistance and seed quality. Efficient evaluation of such large numbers of entries in field experiments is laborious, expensive, and dif-

ficult to manage because of the confounding effects that genotype x environment interaction and soil heterogeneity. In the early stage of the selection process, there could be an insufficient seed of the new treatments' to undertake replicated experiments or the number of genotypes could be very large to manage in terms of resources. Therefore, augmented designs have been developed for the evaluation of genotypes in the early stages of a breeding program (Federer, 1956). Augmented designs consist of two kinds of treatments, the checks or the standard treatments and new or augmented treatments. The design presumes checks as fixed effects whereas the new entries as random effects. The new entries are usually not replicated owing to a large number of entries initially in a breeding program, especially when dealing with large germplasm sets. However, the checks are replicated to act as points of reference. More sophisticated augmented designs allow for the adjustment of test varieties by rows and columns (Federer & Raghavarao, 1975; Lin & Poushinsky, 1983). The efficiency of the analysis over another is usually measured in terms of reduced error variance, expected error mean square or average standard error of the difference between genotype means (Binns, 1987; Cochran & Cox, 1957; Magnussen, 1990). The average standard error of the difference (SED) was reported to be more appropriate since it is used for comparison among genotypes using the same scale as the traits (Binns, 1987; Cullis & Gleeson, 1991; Gleeson, 1997). This study, it was aimed to determine the yield and yield components of M 6 advanced bread wheat mutant lines selected from M ⁴ populations induced different gamma rays of 4 different bread wheat genotypes.

MATERIAL AND METHODS

Experimental site and growing conditions: This study was conducted at the University of Tekirdağ Namık Kemal, Faculty of Agriculture, Department of Field Crops, Turkey in the 2014-2015 growing season. Tekirdağ district locates at latitude 40° 36'- 40° 31' and longitude 26° 43'-28 $^{\circ}$ 08' and altitude is 10 m. The total precipitation was 435.1 mm and the average temperature was 11.5 °C during the 2014-2015 wheat growing season (November 2014-June, 2015) when the experiment was conducted. It is noted that the annual precipitation is about 30 mm lower than the long term average (466 mm) and the average temperature is similar to the long term average (11.5 \degree C). According to soil analysis results, the experimental area's soil was clay-loam, slightly acidic (pH 6.5), limeless, and poor (1.08%) in the organic matter.

Experimental materials, gamma irradiation and design: Four bread wheat (*Triticum aestivum* L.) genotypes, Avusturalya, Bezostaja 1, Kate A-I and IBWSN4 were used as the parent material. The moisture contents of seeds of the genotypes were 12.1% for Avustralya, 11.4% for Bezostaja 1, 11.7% for Kate A-I and 12.0% for IBWSN4.

Gamma treatment was obtained from ⁶⁰Cobalt, Ob-Servo Sanguis Co-60 Research Irradiator with isotope model, while the dose rate was 2.190 kGy h^{-1} before the 2009-2010 growing season sowing at the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, Turkey. Right after irradiation, the experiment was set up using a total of 20 M_0 (100, 200, 300, 400 and 500 Gy) combination seeds together with the un-irradiated (control) in the experimental field of the Field Crops Department of the Faculty of Agriculture of Tekirdağ Namık Kemal University during the growing season of 2009-2010. The seeds obtained from the harvested plants in M_1 generation were sown in 2010-11 (M₂), 2011-12 (M₃) and 2012-13 (M4) growing seasons as 20 cm row distance in 5 meters of a 6-row plot with 4 replicate and as 400 seeds in each row. The experiment was carried out

in augmented design with 5 blocks with 65 advanced bread wheat mutant lines of $M₆$ generation selected from M4 populations of 4 different bread wheat genotypes and their parents (Table 1) in the 2014-2015 growing season. Each block included 13 advanced bread wheat mutant lines and 4 check bread wheat genotypes (A, IB, K and B). Sown were made on Nov.7, 2014, by hand at the rate of 500 seeds per $m²$ and were 5 m in length, with 2 rows 0.2 m apart. Nitrogen and P_20_5 at 140 and 70 kg ha⁻¹, respectively, were incorporated into the soil as compound fertilizer (20-20-0) before sowing, urea during tillering and ammonium nitrate before heading. The crop was kept free of weeds by hand hoeing when necessary. Morphological and yield characters were recorded on 15 random and guarded plants to study the effect of irradiation doses on the studied genotypes on plant height (PH- cm), spike length (SLcm), the number of grain per spike (NGS-no), grain weight per spike (GWS-g), thousand grain weight (TGW-g), harvest index (HI-%), and grain yield (GY) was calculated as kg ha⁻¹.

The data set has been analyzed by the help of JUMP statistics package program in accordance with augmented experimental design was used. The characters averages that were statistically significant according to the variance analysis results have been compared to the Student-Newman-Keuls test $(p \le 0.01)$ and the alphabetical order of statistical significance groups obtained are shown in Tables 2 and 3 for each character (Gomez & Gomez, 1984).

Table 1. The experiment material of the study

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C1 (Bezostaja 1)	MT12 (400 Gy)	MT19 (200 Gy)	MT35 (400 Gy)	MT46 (300 Gy)	MT57 (200 Gy)					
MT1 (100 Gy)	MT13 (400 Gy)	MT20 (200 Gy)	MT36 (400 Gy)	MT47 (300 Gy)	MT58 (200 Gy)					
MT2 (100 Gy)	MT23 (500 Gy)	MT21 (200 Gy)	MT37 (400 Gy)	MT48 (300 Gy)	MT59 (200 Gy)					
MT3 (100 Gy)	MT24 (500 Gy)	MT22 (300 Gy)	MT38 (400 Gy)	MT49 (400 Gy)	MT60 (300 Gy)					
MT4 (100 Gy)	MT25 (500 Gy)	MT27 (300 Gy)	$C3$ (Kate A-1)	MT50 (400 Gy)	MT61 (300 Gy)					
MT5 (200 Gy)	MT26 (500 Gy)	MT28 (300 Gy)	MT39 (100 Gy)	MT51 (400 Gy)	MT62 (300 Gy)					
MT6 (200 Gy)	$C2$ (IBWSN 4)	MT29 (300 Gy)	MT40 (100 Gy)	MT52 (400 Gy)	MT63 (400 Gy)					
MT7 (200 Gy)	MT14 (100 Gy)	MT30 (300 Gy)	MT41 (100 Gy)	MT53 (400 Gy)	MT64 (400 Gy)					
MT8 (300 Gy)	MT15 (100 Gy)	MT31 (300 Gy)	MT42 (100 Gy)	C4 (Avustralya)	MT65 (400 Gy)					
MT9 (300 Gy)	MT16 (100 Gy)	MT32 (400 Gy)	MT43 (200 Gy)	MT54 (100 Gy)						
MT10 (300 Gy)	MT17 (100 Gy)	MT33 (400 Gy)	MT44 (200 Gy)	MT55 (100 Gy)						
MT11 (400 Gy)	MT18 (200 Gy)	MT34 (400 Gy)	MT45 (200 Gy)	MT56 (100 Gy)						
C: check genotype, MT: advanced mutant line										

RESULTS AND DISCUSSION

As a result of the analysis of variance, the differences between the means of the mutant lines were found to be statistically significant at 0.01 probability level for plant height, spike length, thousandgrain weight, the number of grains per spike, grain weight per spike and harvest index and grain yield. The check (parent) mean performances, the adjusted mean performances of the mutant bread wheat lines calculated taking these into account and the results of the significances test are given in Table 2 and Table 3 for the investigated characters.

Plant height (PH) (cm): One of the most obvious and expected effects of mutagen applications on plants is the reduction in plant height. Considering this character, 65 advanced mutant lines averaged between 69.13-119.56 cm, while the averages of the checks varied between 92.86 - 117.74 cm (Table 2).

Among the check genotypes, the shortest and the longest plant height were measured in the IBWSN-4 line and the Australian variety respectively (Table 2). The twenty-three mutant lines gave a shorter plant height than the check genotype IBWSN-4, which has the shortest plant height. Among them, 13 lines were below the plant height value of 90.00 cm, which

is recommended for our region to avoid lodging problems. As a result, it is understood that these 13 advanced bread wheat mutant lines should be evaluated in the next generations in terms of plant height. Our results are supported by the findings of Shubhra et al*.* (2013), explaining that mutant lines with shorter plant lengths can be obtained as a result of gamma irradiation.

Spike length (SL) (cm): One of the most important yield components affecting wheat yield is the spike length and it is accepted as one of the most

Genotypes	PH (cm)	Genotypes	SL (cm)	Genotypes	NGS (no)	Genotypes	GWS(g)
C1	117.74 abc	C1	10.78 a-1	C1	$42.98 e^{-x}$	C1	1.91 k-u
C ₂	100.52 f-u	C ₂	$10.46 b-t$	C ₂	47.76 b-p	C ₂	2.12 g-t
C ₃	92.86 n-A	C ₃	10.51 a-s	C3	48.56 a-o	C3	$2.30 c-r$
C ₄	105.80 c-k	C ₄	$10.35 b-u$	C ₄	43.46 e-x	C ₄	2.11 g-t
MT64	119.56 a	MT14	11.78 a	MT61	59.84 a	MT14	3.13a
MT55	118.96 ab	MT35	11.45 ab	MT44	59.09 abc	MT31	3.02 ab
MT54	117.66 a-d	MT31	11.38 abc	MT30	56.44 a-d	MT28	3.01 ab
MT59	117.66 a-d	MT48	11.24 a-d	MT31	54.74 a-e	MT33	2.92 abc
MT13	113.78 a-e	MT36	11.20 a-e	MT59	54.24 a-e	MT30	2.87 a-d
MT ₅	110.18 a-f	MT27	11.07 a-g	MT28	54.04 a-f	MT27	2.85 a-e
MT61	110.05 a-f	MT17	10.96 a-h	MT27	53.44 a-g	MT32	2.81 a-f
MT10	109.88 a-g	MT25	$10.90a-k$	MT14	53.09 a-h	MT35	$2.72a-g$
MT12	109.88 a-g	MT40	10.86 a-1	MT42	52.19 a-1	MT37	$2.71a-g$
MT25	108.53 a-g	MT64	10.86 a-k	MT33	51.84 a-j	MT29	2.70 a-h
MT ₆	$108.08 b-g$	MT37	10.84 a-1	MT35	$51.44 a-i$	MT36	2.67 a-1
MT4	107.48 b-h	MT41	10.83 a-l	MT32	$51.30 a-i$	MT42	$2.58 a-j$
MT45	$107.23 b-1$	MT42	10.81 a-l	MT50	$51.19a-k$	MT44	$2.44 b-k$
MT40	$106.93 b - j$	MT18	10.70 a-l	MT47	50.89 a-k	MT7	$2.41 b-m$
MT3	106.48 c-1	MT52	10.68 a-m	MT29	50.54 a-m	MT ₆	$2.41 b-1$
MT11	105.08 d-l	MT28	$10.62 a - o$	MT36	50.44 a-o	MT19	$2.36c - o$
MT63	105.06 e-m	MT15	10.58 a-p	MT51	50.09 a-o	MT53	$2.36c - o$
MT51	104.63 e-n	MT33	10.57 a-p	MT53	$50.04a -o$	MT11	$2.33 c-p$
MT56	103.86 e-o	MT44	10.54 a-r	MT22	49.89 a-o	MT22	$2.31c-q$
MT9	103.18 e-p	MT38	10.53 a-s	MT54	49.24 a-o	MT38	2.27 d-s

Table 2. Mean performance of checks and mutant bread wheat lines for PH, SL, NGS and GWS

The identical letters indicate statistical groups of identical values with 0.01 confidence level by the Student-Newman-Keul Test (SNKT)

important selection criteria in wheat breeding as well. Previous studies have shown that mutagen applications cause significant changes in spike length in wheat (Mohammad et al., 2004; Farag & El-Khawaga, 2013). In the study, the spike length of 65 advanced bread wheat mutant lines ranged from 7.61 to 11.78 cm, and the spike length of check genotypes ranged from 10.46 to 10.78 cm (Table 2). Among the mutant lines examined in the experiment, 13 lines (MT14, 35, 31, 48, 36, 27, 17, 25, 64, 40, 37, 41 and 42) formed longer spike than all check genotypes. These results are similar to those of Githinji & Birithia (2015), who also reported highly significant differences for spike length and mutant bread wheat lines produced the longest spike as compared to the parent wheat variety.

The number of grains per spike (NGS) (no): In the case of wheat improvement, the number of grains per spike is one of the selection criteria considered as the most important main yield component in wheat breeding studies. It has been determined that 65 advanced bread wheat mutant lines tested in the M6 genome exhibit a wide variation in the number of seeds per spike from 30.59 to 59.88, while it is as low as 42.98 to 47.76 no (Table 2). Among the tested lines, 21 lines (MT61, 44, 30, 31, 59, 28, 27, 14, 42, 33, 35, 32, 50, 47, 29, 36, 51, 53, 22, 54 and 48) was found to have a higher number of grains per spike than the check IBWSN-4 genotype, giving the highest mean and other parents. These results are similar to the results of Anter (2021), who explained that 22% of the mutant lines have more grains per spike than the parents.

Grain weight per spike (GWS) (g): One of the most important yielding factors affecting wheat yield is the grain weight per spike and it is accepted as one of the most important selection criteria in wheat breeding as well. In terms of this character, means of the grain weight per spike for the 65 advanced bread wheat mutant lines in the M6 generation ranged from 1.43 to 3.13 g, while the check genotypes ranged from 1.95 to 2.30 g indicating variation was wider in lines than that of check genotypes (Table 2). Among them, 19 mutant lines (MT14, 31, 28, 33, 30, 27, 32, 35, 37, 29, 36, 42, 44, 6, 7, 53, 19, 11 and 22) were a higher grain weight per spike than the IBWSN-4 check genotype, which gave the highest averages. These results suggest that these 19 mutant lines should be considered as a priority for grain weight per spike and agree with the results of Sari et al. (2016) who have reported that 32% of the mutant lines have more grain weight per spike than the parents.

Harvest index (HI) (%): The harvest index, which is the ratio of economic yield to biological yield, is an important selection criterion. It is desirable that the harvest index be as high as possible (even closer to 50%) in wheat breeding. The harvest index values of sixty-five advanced bread wheat mutant lines ranged between 32.57% and 48.37%, and between 35.39% and 40.60% in check genotypes. 35 mutant lines (MT33, 37, 30, 47, 11, 29, 42, 50, 44, 48, 46, 27, 32, 28, 21, 35, 31, 51, 4, 19, 17, 20, 7, 6, 14, 52, 39, 15, 10, 62, 58, 36, 22, 43 and 9) were the highest harvest index value compared to check genotypes (Table 3). Fourteen mutant lines (MT33, 37, 30, 47, 11, 29, 42, 50, 44, 48, 46, 27, 32 and 28) performed well with harvest index averages of over 45% and the currently accepted harvest index value is close to 50%. The findings of Rahimi & Bahrani (2011), which explains that lines with higher harvest index averages than the germplasm can be developed with gamma irradiation, proves our results.

Thousand grain weight (TGW) (g): Thousand grain weight (g) or seed index which is an indicator of grain size and flour yield in wheat is an important main yield contributing trait. In our study, the thousand grain weight means ranged from 34.13 to 55.93 g for the 65 advanced bread wheat mutant lines ranged from 36.9 to 47.04 g for the check genotypes. Among the bread wheat mutant lines, 30 lines (MT43, 41, 5, 49, 21, 46, 65, 39, 8, 7, 20, 29, 52, 40, 19, 31, 28, 30, 36, 38, 6, 23, 24, 18, 9, 37, 12, 35, 25 and 53) were a higher 1000-grain weight than the check genotype. These results show that a wide variation can be achieved with the application of mutagen for 1000 grain weights. Fifteen mutant lines (MT43, 41, 5, 49, 21, 46, 65, 39, 8, 7, 20, 29, 52, 40 and 19)

draw attention with an average of thousand grain weight above 50 g (Table 3). Our results are similar to the results of Singh & Balyan (2009) who illustrated mutant lines with larger grains than the parents can be obtained as a result of gamma irradiation, while it is contradictory to the findings by Öztürk et al. (2020) who the mutant lines have a lower thousand-grain weight than their parents.

Grain yield (GY) (kg ha⁻¹): The main purpose of plant improvement is to increase grain yield obtained from the unit area. Grain yield is polygenic

The identical letters indicate statistical groups of identical values with 0.01 confidence level by the Student-Newman-Keul Test (SNKT)

and is highly influenced by cultivar, environment and cultivar x environment interactions. The average performance of grain yield $(kg ha⁻¹)$ of all genotypes is depicted in (Table 3). In the study, the grain yield of advanced bread wheat mutant lines ranged from 4712-9415 kg ha⁻¹, while the grain yield of check genotypes ranged from 6074-8556 kg ha (Table 3). Considering the other characteristics examined, it is seen that there is a higher variation in grain yield between mutant genotypes obtained by mutagen application. Results regarding the trait revealed that a higher grain yield was observed in 9 mutant lines (MT53, 19, 33, 35, 14, 20, 42, 40 and 27) as compared to the other genotypes. Ayub et al*.* (1989) stated that the effect of different doses of gamma rays irradiation on the grain yield of wheat varieties responded differently in different varieties. Our results are in confirmation with those of Öztürk et al. (2020) who explained that 33% of the

mutant lines have more grain yield than the parents and check genotypes. As a result, it can be said that it would be appropriate to take these 9 mutant lines into the pre-registration yield trials in terms of grain yield.

CONCLUSION

Regarding plant height, among the 65 mutant lines, 13 mutant lines below 90 cm are recommended for our region to avoid lodging problems. Thirteen mutant lines gave a long spike than 10.5 cm and 1 mutant line gave a spike longer than 11.0 cm. In total, 21 lines were higher the number of grains per spike, which is one of the important yield criteria than the parents, and of them14 mutants gave 50 grains, 3 mutant lines gave more than 55 grains. When grain weight per spike values of mutant lines are examined, it is shown that 19 mutant lines were a higher grain weight per spike than the parent which gave the highest averages, among them 9 mutant lines were more than 2.5 g, 2 mutant genotypes were above 3.0 g. Fourteen mutant lines gave well with harvest index averages of over 45% and the currently accepted harvest index value is close to 50%. Among the advanced bread wheat mutant lines, 30 lines were a higher 1000-grain weight than the parent genotypes. The higher grain yield was recorded in 9 mutant lines as compared to the other mutant genotypes and their parents, of the 4 mutant lines yielded higher than 850 kg ha-1 and 6 lines yielded more than 900 kg ha⁻¹. The mutant MT53, MT19, MT33, MT35, MT14, MT20, MT42, MT40 and MT27 were out yielded than the other mutant lines and parent varieties. Among them, MT35, MT14 and MT27 were over-performed for all yield components except thousand grain weight. As a result, MT35, MT14, MT27, MT19, MT20 and MT15 should be included in further breeding programs for exploitation of its genetic potential in new cross combinations. The fact that the majority of selected and promising mutants originated from populations irradiated with 100 and 200 Gy gamma rays (Table 1), suggests that these gamma doses may be more beneficial for yield increase in wheat mutation breeding.

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