# EFFECTS OF GAMMA RADIATION ON GERMINATION AND PHYSIOLOGICAL PARAMETERS IN BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES

İrfan Öztürk[1], Ayşe Şen[2], Orkun Yaycılı[2], Adnan Tülek[1], Banu Tülek[1], Remzi Avcı[1]

[1]Trakya Agricultural Research Institute, Edirne, Turkey

[2]İstanbul University, Faculty of Science, Department of Biology, İstanbul, Turkey

## Abstract

*This research was carried out to determine the effects of gamma radiation on germination and physiological characteristics of bread wheat genotypes. Eight bread wheat genotypes were irradiated with 175, 200, 250 Gy gamma-ray. Physiological parameters were recorded in the M1 population for flag leaf area, days of heading, some stomata, chlorophyll content at three growth stages, and biomass (NDVI) at five growth stages. The results showed that flag leaf area ($cm^2$), biomass (NDVI), chlorophyll content (SPAD) decreased with increasing gamma irradiation doses but days of heading increased. The data showed that in comparison with the control population germination in mutagen-treated population was less than over the control population. NDVI was taken in the control treatment and M1 mutant lines at five growth stages and NDVI in the mutagen treated population was less than over the control population. Flag leaf area varies widely as a consequence of its interaction with the genotypes of treatment factors. The highest flag leaf area was determined in the control treatment (21.85 $cm^2$) and increasing in gamma irradiation level lowered flag leaf area (15.70 $cm^2$) in M250 Gy gamma irradiation. Control treatment had higher chlorophyll content and an increase in the dose of gamma rays reduced the chlorophyll content of genotypes scaled at Z30, Z45, and Z55 growth stages. It can be seen that days of heading increased with an increasing dose of mutagen treatments. Days of heading in the control treatment were 145.12 days and 154.75 days in mutagen 250 Gy gamma dosses. Based on mean value genotypes BBVD7-2014 had a higher flag leaf area (22.98 $cm^2$), followed by BBVD22-2016. The highest chlorophyll content was determined in BBVD22-2016 and Saban. Cultivars Anafarta and Abide had higher biomass (NDVI). In conclusion, gamma radiation could be useful for the alteration of physiological characters. Mutation breeding (gamma irradiation) is also a significant breeding tool that has been used successfully in several crops for breeding physiological and morphological traits.*

**Key words:** *Bread wheat, wheat breeding, gamma ray mutagenesis, physiological parameters*

## 1. INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world due to its adaptation to diverse environments from -35 ºC in the vegetative stage to 40 ºC during grain filling period (Shewry, 2009). Mutation breeding is relatively a quicker method for the improvement of crops (Ilirjana et al., 2007). Mutations induced artificially way are one of the tools used to increase genetic variation in populations where genetic variation has been shrinking especially due to various reasons one of which is domestication (Şen and Sarsu, 2018). Hundreds of useful mutants have been induced for various plant characters in a variety of crops including wheat through treatment with physical and chemical mutagens (ram Din et al., 2003; Morten et al., 2006). Induced mutation help to develop of many agronomically important traits such as shorter growing period, suitable for rotation, increased tolerance or resistance to abiotic and biotic stresses use in major crops such as wheat, rice, barley, cotton, peanuts, and beans (Ahloowalia et al., 2001; Maluszynski et al., 2002; Singh and Balyan, 2009). Gamma irradiation could be used successfully to develop mutants in wheat and abiotic stress-tolerant. Gamma rays have different effects on the morphological and biochemical characteristics of plants, such as stimulation of germination and seedling growth and increasing of proline content (Aly et al., 2018). Gamma irradiation is considered as physical mutagen imposing considerable effects on

physiological and biochemical processes in plants (Begum and Dasgupta, 2011; Heidarieh et al., 2012; Kiong et. al., 2008). Mutation breeding is one of the important techniques to induce variation. More than 3000 varieties of different crops have been officially released by mutation breeding (Laghari et al., 2012). The irradiation of seeds may cause genetic variability that enables plant breeders to select new genotypes by improving characteristics such as precocity, tolerance to drought, grain yield, and quality (Ashraf et al., 2003). The mutants developed in wheat had great potential for direct release and to include them in the crossbreeding program (Sakin et al., 2005). Mutation breeding is a significant breeding tool that has been used successfully in several crops for breeding agronomically important traits (Maluszynski et al., 1995). Mutation breeding is one of the breeding tools based on artificially inducing hereditary changes in plants using either physical or chemical mutagen, which has been successfully used to develop diverse and valuable materials in several crops with agronomically important traits (Borzouei et al., 2010). In wheat, numerous studies have reported that gamma radiation of seeds causes physiological changes such as inhibition of seed germination and seedling growth (Borzouei et al., 2010; Melki and Marouani, 2010). Gamma rays, in particular, are well known physical mutagens and often used in mutation breeding program to induce desirable mutants with different genotypes (Konzak, 1987; Knott, 1991). A mutation is a heritable change in genes and chromosomes, and occurs at random, either spontaneous or induced with mutagenic agents (Şen et al., 2017). Induced mutations are necessary to enhance the rate of genetic variability since the spontaneous mutation rate is very low and that prevents breeders to exploit them in plant breeding programs. Selecting new cultivar(s) against tolerance to drought stress is generally evaluated using phenotypic observations. Thus, effective stress-related biomarkers are needed to identify and implement in breeding programs for the screening of drought-tolerant genotypes (Şen and Öztürk, 2018).

Gamma-ray mutagenesis had efficiently used in local bread wheat improving programs to develop mutant lines with superior characters, and 1000-kernel weight and test weight could be used as selection criteria for maintaining grain yield under divers rainfall conditions of Trakya Region (Öztürk et al., 2020). The objective of this study was to determine and compare the effect of different gamma radiation doses on the physiological and morphological traits to obtain mutant wheat populations and to improve some physiological characters of wheat genotypes.

## 2. MATERIAL AND METHODS

### *Experimental Design*

Eight winter bread wheat (*Triticum aestivum* L.) genotypes were subjected to 170, 200, and 250 Gy gamma irradiation. Treated and untreated seeds were planted to raise the M1 population. The untreated seeds of mother varieties (parental line/variety) were also compared with the M1 population. M1 plant rows were grown in three replications with check-rows of untreated varieties. The planting was implemented in the 2016-2017 growing season.

**Table 1.** Cross and pedigree number of the parent genotypes used in this study.

| No | Genotypes | Pedigree | Cross number |
|---|---|---|---|
| 1 | Saban | Trakia/3/MvC410-90/GKKalaka// MvC410-90/Ftm-II | TE6060-1T-1T-1T-0T |
| 2 | BBVD7-2014 | Admis//Milan/Ducula | CMSW01WM00331S-030YE-30E-1E-0E-1E-0E |
| 3 | BBVD25-2016 | Rumba//Chirya.3/GK Othalon | TE6627-0T-0T-20T-2T-1T-0T |
| 4 | ÖVD1-14-2016 | Tekirdağ//GK Kalasz/Beaubourg | TE6748-0T-0T-9T-1T-0T |
| 5 | BBVD22-2016 | Rumba//Chirya.3/GK Othalon | TE6627-0T-0T-4T-1T-3T-0T |
| 6 | Anafarta | 15.99/3/Pehl//Rpb8-68/Chrc/4/Chatelet | TE6411-2T-0T-9T-5T-3T-0T |
| 7 | Abide | Flm85/7/Kç66/Bez//Sup/3/Wrm/2*Hua /4/Dons/5/Kate/MvM/6/Presl | TE6295-2T-0T-13T-1T-6T-0T |
| 8 | Damla | 90Zhong150//Mex65/Momt/3/Trakia | TE6217-3T-1T-1T-3T-0T |

Trial plots were 3m x 0.30m size with 3 rows. The experimental plan was a randomized complete block design (RCBD) with 3 replications. Data on seed germination and surviving plants were recorded considering whole plots of the M1 population. Data on physiological parameters such as days of heading, flag leaf area, biomass (NDVI), chlorophyll content (SPAD), and the number of stomata were taken and comparison was made among them. The Zadoks Decimal Code (Z) was used to describe plant growth stages of cereals (Zadoks et al., 1974).

In this study, cultivars Saban, Anafarta, Abide, and Damla with four lines (Table 1) were irradiated with 170, 200, and 250 Gy gamma rays installed at İstanbul University, department of Biology in İstanbul. Irradiated seeds were grown to obtain the M1 mutant wheat populations in the experimental field of Trakya Agriculture Research Institute, Edirne, Turkey, which is located in the geographic latitude of 41°40’ N and longitude of 26°34’ E.

***Measurement of Morphological and Physiological Parameters***

In the study, 10 flag leaves were randomly selected in each plot and their length (FLL) and width (FLW) were measured by a ruler. Flag leaf area (FLA) was then calculated using the following formula (Fowler and Rasmussen, 1969).

$FLA\ (cm^2) = (FLL \times FLW) \times 0.68$

Plant height was recorded at harvest and measured from the ground to the tip of the spikes, excluding awns. Days of heading were recorded from 1 January up to heading stage in each plot (when 50% spikes were seen in the parcels). The number of stomata was measured from flag leaf at Z30 and Z55 growth stages of the genotypes. The number of stomata was measured by the impression method using clear nail polish described by Wang and Clarke (1992). Samples were taken from flag leaves for each genotype. Four to 100 microscopic fields of view (0.04 $mm^2$) were randomly selected from the middle of the adaxial surface for each sample. Biomass (NDVI) and chlorophyll content (SPAD) was measured described by Jackson et al., 1981; Fisher, 2001; Babar et al., 2006 and Reynolds et al., 2001. For determining the biomass of the genotypes data was taken at five different plant growth

stages; at Z25, Z30, Z35, Z38, and Z55. Chlorophyll content (SPAD) was measured at Z30, Z45, and Z55 growth stages.

***Statistical Analysis***

Data were analyzed statistically for analysis of variance following the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a %5) test. The analysis of variance for each character was measured followed by LSD to test significance difference between means (Steel and Torrie, 1960), and simple correlation coefficient and path analysis, which is the direct and indirect effects of each character was performed as per the method of Dewey and Lu (1959). Coefficients of the regression equations ($R^2$) were calculated according to Finlay and Wilkinson, 1963; Eberhart and Russell, 1969, and Wright, 1934. Regression graphs were drawn to predict the adaptability of genotypes.

***Climate Parameters***

Meteorological data for the growing season from September 2016 to June 2017, monthly rainfall, mean humidity, minimum, maximum, and mean temperature were recorded and are given in Table 2. The average daily mean temperature during plant growth stages from September to June was 10.2 °C, absolute maximum and minimum temperature was 40°C, and -17.0°C., respectively. Total rainfall was 417.2 mm and, amount of the rainfall in November and December was less compared with a long year. Also, the mean humidity was 71.2% (Table 2).

**Table 2.** Climate condition in 2016-2017 growing years in the experimental area in Edirne.

| Months | Rainfall (mm) Long year | Rainfall (mm) | Humidity (%) | Temperature (°C) | | |
|---|---|---|---|---|---|---|
| | | | | Min. | Max. | Mean |
| September 2016 | 34.0 | 9.2 | 57.5 | 5.0 | 33.8 | 20.8 |
| October 2016 | 52.9 | 44.4 | 69.5 | 1.3 | 28.8 | 14.3 |
| November 2016 | 72.4 | 3.2 | 72.9 | -9.9 | 15.4 | 0.7 |
| December 2016 | 61.7 | 3.2 | 72.9 | -9.9 | 15.4 | 0.7 |
| January 2017 | 48.1 | 67.8 | 83.7 | -17.0 | 8.4 | -1.9 |
| February 2017 | 46.9 | 43.4 | 80.0 | -8.4 | 20.6 | 5.3 |
| March 2017 | 52.2 | 51.0 | 73.0 | -1.9 | 25.5 | 10.2 |
| April 2017 | 51.0 | 65.6 | 63.1 | -1.6 | 28.6 | 12.5 |
| May 2017 | 56.0 | 85.0 | 65.4 | 4.4 | 30.0 | 17.9 |
| June 2017 | 41.5 | 44.4 | 74.4 | 12.9 | 40.0 | 21.2 |
| Total/Mean | 516.7 | 417.2 | 71.2 | -17.0 | 40.0 | 10.2 |

## 3. RESULTS AND DISCUSSION

The ANOVA results for physiological and morphological characteristics of wheat genotypes were given in Table 3. Analysis of variance for the 2016-2017 seasons indicated statistically significant differences (P<0.01) for traits measured in the presented study except for stomata number and chlorophyll content at Z45 and Z55 growth stage (Table 3). The effect of gamma rays on morphological and physiological traits of wheat cultivars in M1 are shown in Tables 3, 4and 5. In this study, we have created the mutant wheat populations using 170, 200, and 250 Gy gamma irradiation. The data regarding the analysis of variance of eight genotypes for five quantitative traits are presented in Tables 4 and 5. In the M1 population, observations were recorded for flag leaf area, days of heading, stomata number, chlorophyll content at three growth stages, and biomass (NDVI) at five growth stages. Flag leaf area, chlorophyll content at three growth stages, and biomass at five growth stages decreased in all irradiation doses as compared to un-irradiated samples. The increase in the dose of gamma rays, the reduced germination percent was observed. Similar results were also being obtained on various crop plant species including wheat (Singh, 1993; Wiersinski, 1984). Mutant lines showed wide variation in various measured traits. The germination in M1 populations was less than over the control population. The data showed that in comparison with the control population the germination in the mutagen treated population was lower.

**Table 3.** Effect of gamma radiation on physiological and morphological parameters based on treatment.

| **Treatment** | **FLA** | **STN-Z30** | **STN-Z55** | **SPAD-Z30** | **SPAD-Z45** | **SPAD-Z55** |
|---|---|---|---|---|---|---|
| Control | 21.85 a | 42.76 a | 45.09 a | 54.79 a | 54.68 a | 56.48 a |
| M170 | 18.14 b | 40.05 a | 45.75 a | 54.33 a | 53.06 ab | 57.13 a |
| M200 | 16.14 c | 42.14 a | 45.11 a | 53.38 ab | 52.65 ab | 55.51 a |
| M250 | 15.70 c | 41.26 a | 49.79 a | 51.79 b | 52.20 b | 55.60 a |
| Mean | 17.96 | 41.55 | 46.43 | 53.56 | 53.15 | 56.18 |
| LSD (0.05) | 1.96** | 6.2 | 9.80 | 1.65 | 2.15 | 1.8 |
| F value | 8.61** | 0.31ns | 0.45ns | 5.41** | 2.11ns | 1.52ns |
| **Treatment** | **DH** | **NDVI-Z25** | **NDVI-Z30** | **NDVI-Z35** | **NDVI-Z38** | **NDVI-Z55** |
| Control | 145.12 c | 0.45 a | 0.78 a | 0.82 a | 0.79 a | 0.74 a |
| M170 | 150.00 b | 0.27 b | 0.61 b | 0.78 ab | 0.78 ab | 0.74 a |
| M200 | 151.12 b | 0.23 c | 0.56 b | 0.74 b | 0.74 b | 0.73 a |
| M250 | 154.75 a | 0.17 d | 0.34 c | 0.64 c | 0.61 c | 0.71 b |
| Mean | 150.3 | 0.28 | 0.57 | 0.75 | 0.73 | 0.73 |
| LSD (0.05) | 1.57** | 0.023 | 0.067 | 0.07 | 0.035 | 0.0099 |
| F value | 54.95** | 219.44** | 65.46** | 10.43** | 47.77** | 22.42** |

*, ** Significant at p<0.05 and p<0.01 respectively. ns: non-significant, M: Mutant, FLA: Flag leaf area ($cm^2$), DH: Days of heading, NDVI: Biomass, SPAD: Chlorophyll content, STN: Number of stomata; Z: Zadoks scale

Flag leaf area varies widely as a consequence of its interaction with the genotypes of treatment factors. Gamma-ray imposed a significant impact on the flag leaf area of the genotypes. To compare control treatment, the highest flag leaf area (FLA) was determined in the control treatment (21.85 $cm^2$) and increasing in gamma irradiation level lowered flag leaf area and minimum FLA (15.70 $cm^2$) determined in M250 Gy gamma irradiation (Table 3).

Treatment with gamma radiation also did not affect the number of stomata in genotypes at both the growth stage (Z30 and Z55). Based on chlorophyll content (SPAD) the highest data was determined in the control treatment and the increase in the dose of gamma rays reduced the chlorophyll content of genotypes scaled at Z30, Z45, and Z55 growth stages. The mean performance of chlorophyll content (SPAD) in mutants M250 was significantly less (51.79) compared to the mean SPAD of the control (54.79) at the Z30 growth stage. Also, similar results were obtained at the Z45 growth stage (Table 3).

Days of heading in the control treatment and mutagen treatment were given in Table 3. The treatment combined with the higher concentration (170, 200, and 250 Gy gamma dosses) caused an increase in days of heading in genotypes. It can be seen that the days of heading increase with an increasing dose of mutagen treatments. Days of heading in control treatment were 145.12 days and 154.75 days in mutagen 250 Gy gamma dosses.

Biomass (NDVI) was taken in the control treatment and M1 mutant lines at five growth stages (Z25, Z30, Z35, Z38, and Z55), and there were statistically significant differences ($P<0.01$) among treatment and genotypes. The data were taken from genotypes showing that in comparison with the control population the NDVI in the mutagen treated population was less than over the control population. Biomass scaled in genotypes after gamma radiation revealed that mean biomass decreased with increasing irradiation dose for all genotypes (Table 3). In this experiment, the biomass (NDVI) of gamma-irradiated wheat displayed a gradual decrease from 170 up to 250 Gy dose. A maximum decrease in biomass (NDVI) was observed with 250 Gy treatments at all growth stages (Table 3).

**Table 4.** The mean value of the genotypes in terms of physiological characters.

| Entry | Genotypes | FLA | STN (Z30) | STN (Z55) | SPAD (Z30) | SPAD (Z45) | SPAD (Z55) |
|---|---|---|---|---|---|---|---|
| 1 | Saban | 16.91 cd | 39.28 a | 39.83 b | 54.80 b | 56.28 b | 57.48 b |
| 2 | BBVD7-2014 | 22.98 a | 42.68 a | 43.28 b | 53.30 bc | 51.50 c | 53.75 c |
| 3 | BBVD25-2016 | 19.35 bc | 46.08 a | 58.35 a | 51.53 c | 50.98 c | 57.90 b |
| 4 | ÖVD1-14-2016 | 16.65 cd | 40.03 a | 44.90 ab | 55.15 b | 52.33 c | 53.65 c |
| 5 | BBVD22-2016 | 20.71 ab | 41.85 a | 41.60 b | 57.80 a | 60.28 a | 65.18 a |
| 6 | Anafarta | 16.50 d | 40.38 a | 51.15 ab | 51.13 c | 50.88 c | 53.75 c |
| 7 | Abide | 16.04 d | 40.43 a | 43.08 b | 51.78 c | 51.25 c | 54.13 c |
| 8 | Damla | 14.56 d | 41.73 a | 49.30 ab | 53.08 bc | 51.70 c | 53.60 c |
| Mean | | 17.96 | 41.55 | 46.43 | 53.56 | 53.15 | 56.18 |
| LSD (0.05) | | 2.77 | 8.7 | 13.9 | 2.35 | 3.06 | 2.56 |
| C.V (%) | | 10.5 | 14.4 | 20.5 | 3.0 | 3.9 | 3.1 |
| F value | | 8.61** | 0.50ns | 1.65ns | 7.84** | 10.34** | 21.16** |

*, ** Significant at $p<0.05$ and $p<0.01$ respectively. ns: not-significant, FLA: Flag leaf area ($cm^2$), STN: Number of stomata; SPAD: Chlorophyll content, Z: Zadoks

Table 4 and 5 were based on the mean value of the genotypes. Gamma-ray imposed a significant impact on the flag leaf area of the genotypes. The mutant lines of BBVD7-2014 had a higher flag leaf area (22.98 $cm^2$), followed by BBVD22-2016. The highest chlorophyll content was determined from the mutant lines of BBVD22-2016 at all growth stages (Z30, Z45, and Z55). Cultivars Anafarta and Abide and their M1 mutant lines had higher biomass (NDVI).

**Table 5.** The mean value of the genotypes in terms of physiological characters.

| Entry | Genotypes | DH | NDVI (Z25) | NDVI (Z30) | NDVI (Z35) | NDVI (Z38) | NDVI (Z55) |
|---|---|---|---|---|---|---|---|
| 1 | Saban | 151.0 c | 0.28 a | 0.53 ab | 0.72 abc | 0.74 bc | 0.73 c |
| 2 | BBVD7-2014 | 143.5 f | 0.26 a | 0.50 b | 0.67 c | 0.67 d | 0.70 de |
| 3 | BBVD25-2016 | 146.0 e | 0.29 a | 0.55 ab | 0.69 bc | 0.68 d | 0.69 e |
| 4 | ÖVD1-14-2016 | 156.8 a | 0.27 a | 0.61 a | 0.78 ab | 0.78 ab | 0.76 b |
| 5 | BBVD22-2016 | 145.3 ef | 0.29 a | 0.59 ab | 0.79 a | 0.68 d | 0.70 de |
| 6 | Anafarta | 156.5 a | 0.29 a | 0.62 a | 0.80 a | 0.80 a | 0.78 a |
| 7 | Abide | 154.3 b | 0.28 a | 0.59 ab | 0.76 abc | 0.78 ab | 0.77 ab |
| 8 | Damla | 148.8 d | 0.27 a | 0.59 a | 0.76 abc | 0.71 cd | 0.71 d |
| Mean | | 150,3 | 0.28 | 0.57 | 0.75 | 0.73 | 0.73 |
| LSD (0.05) | | 2.21 | 0.033 | 0.093 | 0.099 | 0.049 | 0.014 |
| CV (%) | | 1.0 | 7.1 | 11.1 | 9.1 | 4.7 | 1.3 |
| F value | | 46.94** | 0.84ns | 1.72ns | 2.11ns | 9.21** | 45.72** |

*, ** Significant at $p<0.05$ and $p<0.01$ respectively. ns: not-significant, DH: Days of heading, NDVI: Biomass, Z: Zadoks

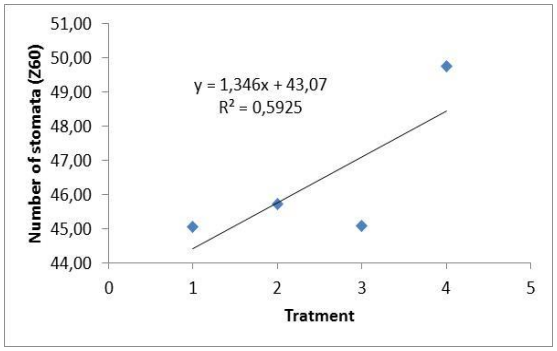
The correlations between the physiological and morphological parameters were determined by Pearson's correlation analysis (Table 6). Physiological parameters showed varying degrees of relationship. Days of heading was generally negatively correlated with flag leaf area, stomata number at the Z30 growth stage, and chlorophyll content at Z30, Z45 (r= -0.973*), and Z55 growth stages. Early maturing genotypes had higher biomass so, days of heading were generally negatively correlated with biomass (NDVI) at all growth stages. Flag leaf area in genotypes was shown a positive correlation with chlorophyll content scaled at Z30, Z45 (r=0.990*), and Z55. Flag leaf area (FLA) positively associated with chlorophyll content scaled at Z30, Z45 (r=0.990*), and Z55. The correlations between flag leaf area (FLA) and biomass (NDVI) (r = 0.985*, r = 0.893, r = 0.838, r = 0.711, and r = 0.722) were positive significant and medium significant at all growth stages (Table 6).

**Table 6.** Correlation coefficients among physiological and morphological parameters.

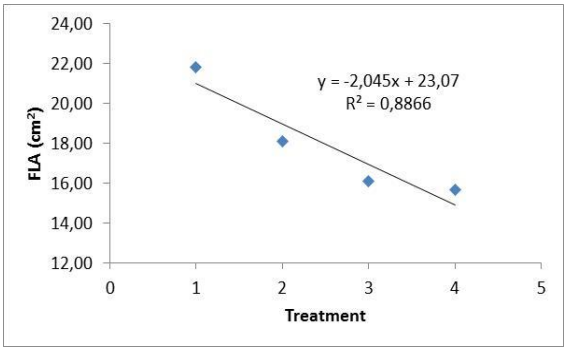
| Traits | DH | FLA | STN Z30 | STN Z55 | SPAD Z30 | SPAD Z45 | SPAD Z55 | NDVI Z25 | NDVI Z30 | NDVI Z35 | NDVI Z38 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FLA | -0.950 | | | | | | | | | | |
| STN-Z30 | -0.473 | 0.406 | | | | | | | | | |
| STN-Z55 | 0.781 | -0.554 | -0.296 | | | | | | | | |
| SPAD-Z30 | -0.925 | 0.830 | 0.158 | -0.878 | | | | | | | |
| SPAD-Z45 | -0.973* | 0.990* | 0.516 | -0.618 | 0.834 | | | | | | |
| SPAD-Z55 | -0.541 | 0.597 | -0.475 | -0.405 | 0.734 | 0.505 | | | | | |
| NDVI-Z25 | -0.980* | 0.985* | 0.525 | -0.643 | 0.845 | 0.999** | 0.499 | | | | |
| NDVI-Z30 | -0.986* | 0.893 | 0.399 | -0.870 | 0.968* | 0.921 | 0.576 | 0.932 | | | |
| NDVI-Z35 | -0.945 | 0.838 | 0.241 | -0.899 | 0.996** | 0.854 | 0.673 | 0.866 | 0.984* | | |
| NDVI-Z38 | -0.862 | 0.711 | 0.131 | -0.946 | 0.980* | 0.731 | 0.671 | 0.747 | 0.934 | 0.979* | |
| NDVI-Z55 | -0.853 | 0.722 | 0.058 | -0.913 | 0.985* | 0.729 | 0.738 | 0.743 | 0.923 | 0.976* | 0.996** |

Significant at * P<0.05 and ** P<0.01 levels, respectively. DH: Days of heading, FLA: Flag leaf area ($cm^2$), STN: Number of stomata; SPAD: Chlorophyll content, NDVI: Biomass, Z: Zadoks

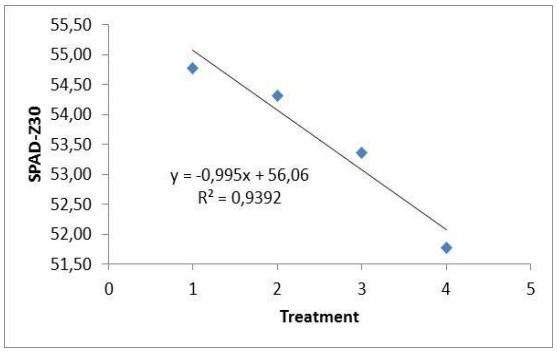
The number of the stomata at the Z55 plant growth stage in genotypes was a generally negative association with chlorophyll content (at Z30, Z45, and Z55) and biomass (at Z25, Z30, Z35, Z38, and Z55). Chlorophyll content (at Z30, Z45, and Z55) was positively related to biomass (at Z25, Z30, Z35, Z38, and Z55). There was a medium and positive correlation between chlorophyll content and biomass (NDVI) at all growth stages. Chlorophyll content at Z45 and biomass at the Z25 growth stage showed strong associations (r = 0.999). Chlorophyll content at Z30 was significantly associated (r = 0.996**) with biomass at Z35.



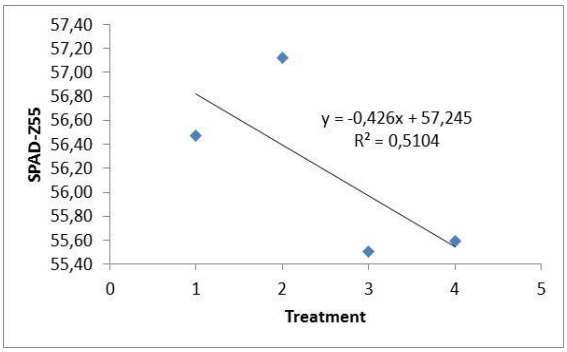

1a: Number of stomata at Z60 and treatment




1b: Flag leaf area (FLA) and and treatment

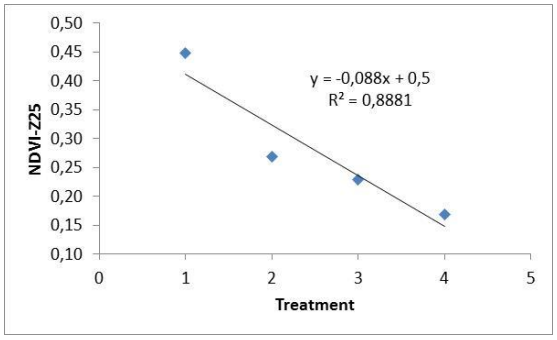



1c: Chlorophyll content (SPAD) at Z30 and treatment

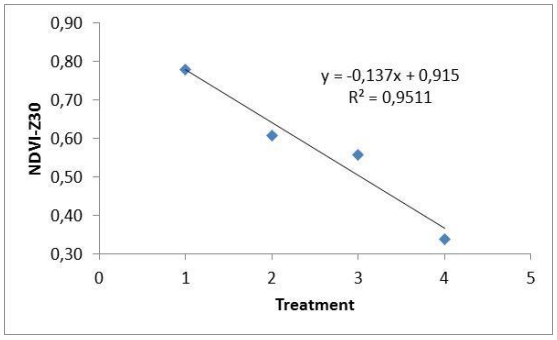



1d: Chlorophyll content (SPAD) at Z55 and treatment

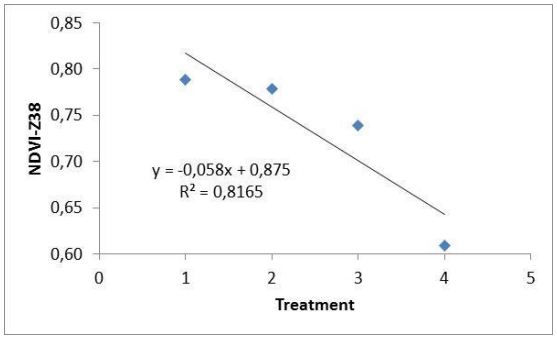
**Figure 1.** Regression analysis among mutant treatment and physiological parameters



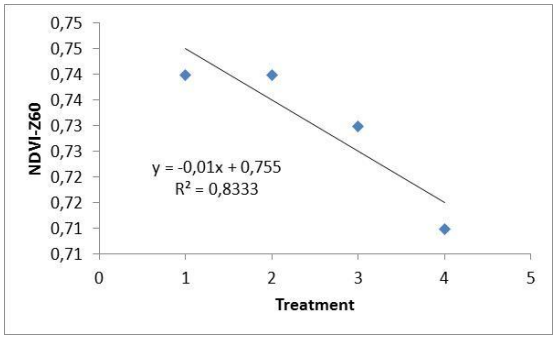

2a. Biomass (NDVI) at Z25 and treatment




2b. Biomass (NDVI) at Z30 and treatment




2c. Biomass (NDVI) at Z38 and treatment




2d. Biomass (NDVI) at Z60 and treatment

**Figure 2.** Relation among mutant treatment and biomass (NDVI) at various growth stages.

The results of the Regression equation ($R^2$) were given in Figures 1 and 2. In Figure 1 the number showed that 1: Control, 2: Mutant 170 Gy, 3: Mutant 200 Gy, and 4: Mutant 250 Gy doses. The higher gamma irradiation dose increased in the number of stomata. So, in the study, significant association was found between mutant treatment and the number of stomata at the Z60 growth stage ($R^2$=0.592) (Figure 1a). Regression analysis indicated that higher doses of gamma irradiation, applied to the seed, resulted in reduced flag leaf area (FLA) in genotypes, and there was a negative association between gamma-ray treatment and FLA ($R^2$=0.886) (Figure 1b). The results showed that an increase in gamma-ray decreased chlorophyll content in genotypes and there was a negative relation between treatment and chlorophyll content (SPAD) at Z30 ($R^2$=0.939), and Z55 ($R^2$=0.510) growth stages (Figure 1c and 1d). The higher doses of gamma irradiation led to a decrease of biomass

in all growth stages at Z25, Z30, Z38, and Z60. In this study, it was found a negative relationship between treatment and biomass (NDVI) at Z25 ($R^2$=0.888), at Z30 ($R^2$=0.951), at Z38 ($R^2$=0.816), and Z60 ($R^2$=0.833)(Figure 2a, 2b, 2c, 2d).

## 4. CONCLUSIONS

This research was carried out to determine the effects of gamma radiation on the physiological and morphological characteristics of bread wheat genotypes. The data showed that in comparison with the control population the germination in the mutagen treated population was less. The data indicated that all treatments reduced the morphological and physiological traits except the number of stomata. In general, it has been reported that by increasing gamma-ray irradiation caused a decrease in physiological parameters in genotypes. Flag leaf area, chlorophyll content at three growth stages, and biomass at five growth stages decreased in all irradiation doses as compared to un-irradiated samples. Biomass scaled in genotypes after gamma radiation (170, 200, 250 Gy) revealed that mean biomass was decreased with increasing irradiation dose for all genotypes. In this study, the biomass (NDVI) of gamma-irradiated wheat displayed a gradual decrease from 170 up to 250 Gy dose. A maximum decrease in biomass (NDVI) was observed with 250 Gy treatments at all growth stages. Flag leaf area varies widely as a consequence of its interaction with the genotypes of treatment factors. The highest flag leaf area was determined from the control treatment and increasing in gamma irradiation level lowered flag leaf area. Control treatment had higher chlorophyll content and the increase in the dose of gamma rays reduced the chlorophyll content of genotypes scaled at Z30, Z45, and Z55 growth stages. In conclusion, gamma radiation can be useful for the alteration of physiological characters. Mutation breeding is also a significant breeding tool that has been used successfully in several crops for breeding physiological and morphological traits.

## ABBREVIATIONS

M: Mutant, FLA: Flag leaf area, DH: Days of heading, NDVI: Biomass, SPAD: Chlorophyll content, STN: Number of stomata; Z: Zadoks scale

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