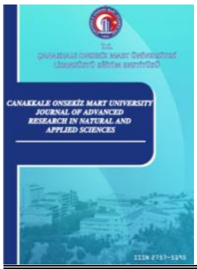
e-ISSN 2757-5195



Çanakkale Onsekiz Mart University

Journal of Advanced Research in Natural and Applied Sciences

Open Access

doi.org/10.28979/jarnas.1286891 2023, Vol. 9, Issue 4, Pages: 923-930 dergipark.org.tr/tr/pub/jarnas

Investigation of Chlorophyll Mutations in Gamma Irradiated Naked Barley Genotypes

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Article History
Received: 24.04.2023
Accepted: 10.07.2023
Published: 20.12.2023

Research Article

Abstract − The consumption of naked barley is significantly increasing as more people become aware of its beneficial role as a source of dietary fiber and β-glucan. As a result, breeding programs paid more attention to naked barley. Improvement of yield and quality of naked barley is hindered by the lack of available germplasm. Mutation breeding is an effective tool for generating variation for plant breeding. Chlorophyll mutations are often used as visual indicators in breeding research to determine the optimum mutagen dosage. The purpose of this study was to identify the types and frequency of chlorophyll mutations brought on by different gamma radiation doses in two genotypes of hulless barley and determine the effective dose (ED_{50}) based on the mutation frequency. Seeds of naked barley line YAA7050-14 and cv. Yalin that have been irradiated with doses of 100, 150, 200, and 300 Gy gamma rays delivered by a Cobalt-60 source. Chlorophyll mutations were observed in 8-day-old M_2 plants grown under greenhouse conditions. In the M_2 plants of cv. Yalin, the highest mutagen frequency was observed at 250 and 300 Gy, while in line YAA7050-14, the highest mutation frequency was found at 300 Gy. The rate of chlorophyll mutation rose in both genotypes as the gamma ray doses increased. The *albino* type of chlorophyll mutation was found in the cv. Yalin at the greatest rate, whereas the *xantha* type was found in the line YAA7050-14. The most common chlorophyll mutation type was *albino*, while the least common type was *viridis* when both genotypes were considered together. Based on the mutation frequency, 250-300 Gy doses could be used to effectively in further research to create mutations in the naked barley genotypes.

Keywords *– Chlorophyll mutations, gamma irradiation, M_2 plants, naked barley*

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated plants, which has been consumed as food for years (Badr et al., 2000, Lev-Yadun et al., 2000). Approximately, 3% of the barley produced worldwide is used as food in the world (Ullrich, 2011). Furthermore, the barley consumption is increasing day by day because functional foods are highly demandable by consumers. Barley is one of the functional ingredients since it is rich in dietary fibre and high beta-glucan (Bhatty, 1999). Naked (hulless) barley is preferred over hulled barley by food industry because (Newman & Newman, 2008) their hull and grain are easily separated from each other (Meints & Hayes, 2019) therefore grains are easily processed into food products. This technological characteristic plays the most important role in consuming naked barley as food (Meints et al., 2021).

The popularity of naked barley has encouraged breeders to increase their efforts to improve the yield and food quality of naked barley. Although it is possible to cross with naked barley in breeding studies, it is necessary to broaden the variation to obtain superior individuals in terms of yield and quality. One of the most effective

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ways of creating genetic variation required for naked barley breeding studies is mutation breeding (Chaudhary et al., 2019; Dyulgerova & Dyulgerov, 2022).

To achieve sufficient and effective variation in the plant species and cultivars in mutation breeding, it is important to determine the source of the mutagen to be used and the effective dose to be used in advance (Maluszynski et al., 2009) because the effective dose of mutagen depend on the plant species and cultivars (Nazarenko & Lykholat, 2020). In general, the rate of germinated or surviving plants (Ahumada-Flores et al., 2020), the rate of plant developmental retardation (Kodym et al., 2012) and the rate of chlorophyll mutations (Çiftçi & Şenay 2005) are used to determine effective doses and mutation frequencies. Chlorophyll mutations are successfully used as genetic markers in plant breeding programs to reveal the effects of mutagens and how the target genotype responds to the mutagen (Wani et al., 2011).

Chlorophylls are essential pigments used by the plant during photosynthesis (Yu et al., 2014). Due to the inhibition of photosynthesis, chlorophyll mutations are often lethal to plants (Wani et al., 2011). Hence, they are generally not useful for plant breeding. However, research on chlorophyll mutations could be useful to determine suitable mutagens and effective mutagen doses to increase genetic variability and agriculturally important mutations in plant species. Therefore, chlorophyll mutation scores are reliable indices for assessing the genetic effects of mutagenic treatments (Goyal et al., 2019). Depending on how the pigments of chlorophyll are affected by mutagens, the occurrence of chlorophyll mutations varies. Gustafsson (1940), classified the chlorophyll mutations obtained as *albino, xantha, alboviridis, viridis, tigrina, striata, maculata*, unidentified mutation, and plasma mutations into nine categories. Although some researchers have modified this classification, it is still the most common classifications in determining the mutation frequency in barley and other cereals.

This study aimed to determine how gamma radiation affects the frequency and range of chlorophyll mutations in the M_2 generation of two naked barley genotypes and the effective dose of gamma radiation that could be used in naked barley breeding.

2. Materials and Methods

Naked barley cultivar (cv.) Yalin and the naked barley line YAA7050-14 developed by the Central Research Institute for Field Crops (CRIFC) were used as plant material. Gamma-rays obtained from the 381 Gray/hour Cobalt 60 (60Co) source in the Ankara Nuclear Research and Training Centre (ANAEM) were used as the physical mutagen source. For and each dose and control, healthy and having nearly 12% moisture, seeds of naked barley cv. Yalin and line YAA7050-14 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 separately in the experimental field of the CRIFC to grow M_1 plants. After the required measurements were taken from the M_1 plants that reached harvest maturity, the main spikes were harvested by hand. The seeds of the main spikes of M_1 plants obtained by gamma irradiation at different doses were used to establish M_2 populations. For each dose in both genotypes, 1000 carefully hand-threshed and selected seeds were sown in containers under greenhouse conditions.

On the 8th day after sowing, germinated and emerged plants were counted and then chlorophyll mutations were observed. Chlorophyll mutations were grouped according to Gustafsson (1940 and 1946) as *albino* (white), *xantha* (yellow) and *viridis* (light green) and counted. The images of plants belonging to different chlorophyll mutations observed in the study are given in Figure 1. The frequencies of chlorophyll mutations was determined according to Gaul (1964) by the following formula (2.1). Additionally, linear regression analyses were performed to illustrate the relationship between gamma ray doses and mutation frequency (Freund et al., 2006).

$$Mutation\ frequency\ (\%) = \frac{Number\ of\ mutant\ plants}{Total\ number\ of\ M2\ plants} \times 100 \tag{2.1}$$

Figure 1. Chlorophyll mutations in M_2 plants (a: *Albino,* b: *Xantha,* c: *Viridis,* d: Control)

3. Results and Discussion

The chlorophyll mutations and frequencies observed in M_2 plants of cv. Yalin are given in Table 1. The regression plot showing the relationship between different gamma-ray doses and chlorophyll mutation rates is given in Figure 2. As it is clearly seen in Table 1 and Figure 2, the incidence of chlorophyll mutations increased with increasing gamma ray doses. However, this increase in chlorophyll mutations was slow at first and then gradual. The total chlorophyll mutation rate was 0.74% at 100 Gy gamma dose. At 150 and 200 Gy gamma doses, a decrease was observed, and chlorophyll mutations were found at 0.35% and 0.24%, respectively. The rate of chlorophyll mutations started to increase again, especially at 250 and 300 Gy doses, and they were observed at a higher rate at these doses than at the others. The rate of *albino* plants was 0.39% and the rate of *xantha* plants was 0.77 % at the dose of 250 Gy in cv. Yalin. At 300 Gy dose, which is the dose with the highest rate of chlorophyll mutations, the rate of *albino* plants was 0.56 % and the rate of *xantha* plants was 0.97%.

Table 1
Chlorophyll mutations in M_2 plants of cv. Yalin irradiated with different gamma ray doses

Doses (Gy)	Number of total plants	Chlorophyll mutations							
		Albino	*Albino* (%)	*Xantha*	*Xantha* (%)	*Viridis*	*Viridis* (%)	Chlorophyll mutant plants	Mutation frequency (%)
Control	950	0	0	0	0	0	0	0	0.00
100	811	2	0.25	2	0.25	2	0.25	6	0.74
150	846	2	0.24	0	0.00	1	0.12	3	0.35
200	832	2	0.24	0	0.00	0	0	2	0.24
250	778	3	0.39	6	0.77	0	0	9	1.16
300	720	4	0.56	7	0.97	0	0	11	1.53
Total	4937	13	1.66	15	1.99	3	0.36	31	4.02

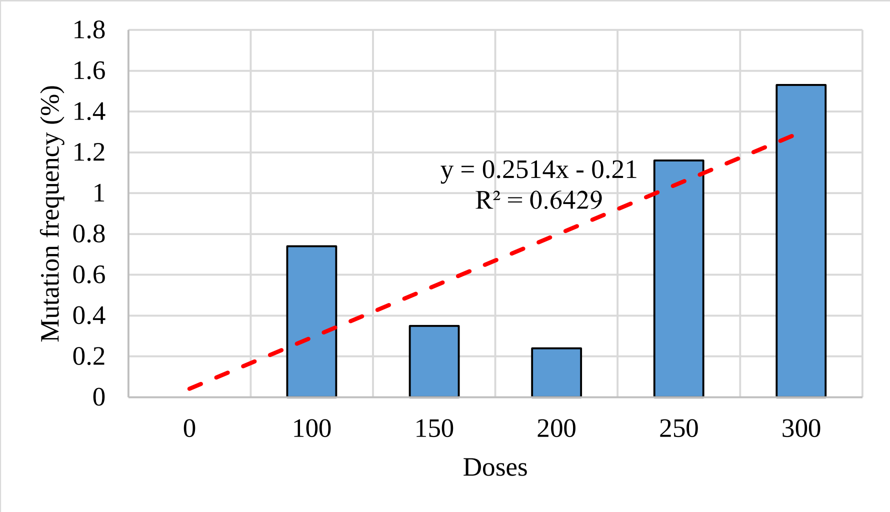

Figure 2. Regression graph between different gamma ray doses and chlorophyll mutation rate in M_2 plants of cv. Yalin

The relative distribution of chlorophyll mutations in naked barley cv. Yalin was the same for all three types (33.3%) at a dose of 100 Gy. *Albino* mutations occurred in 66.7% and viridis mutations in 33.3% at the 150 Gy dose. At the 200 Gy dose, all chlorophyll mutations were *albino* type. The highest rate of chlorophyll mutations was 66.7% and 63.6% for the 250 and 300 Gy doses, respectively. When a general evaluation was made for cv. Yalin, it was observed that the highest chlorophyll mutation rate occurred in the *xanth*a type with 48.4%. The *albino* type mutation rate was 41.9% and the *viridis* type mutation rate was 9.7% (Table 2).

Table 2
Relative spectrum of chlorophyll mutants M_2 plants of cv Yalin

Doses (Gy)	*Albino* (%)	*Xantha* (%)	*Viridis* (%)
100	33.3	33.3	33.3
150	66.7	0	33.3
200	100.0	0	0
250	33.3	66.7	0
300	36.4	63.6	0
Total	41.9	48.4	9.7

The chlorophyll mutations detected in M_2 plants of barley line YAA7050-14 grown under greenhouse conditions and their occurrence rates are given in Table 3. The regression graph of the relationship between different gamma doses and chlorophyll mutation rates is given in Figure 3. Similar to the cv. Yalin, the number and rates of chlorophyll mutations increased with the increase in gamma ray dose (Table 3, Figure 3). In the barley line YAA7050-14, the rate of chlorophyll mutation was determined as 0.96% at 100 Gy gamma ray dose, and similar to the cv. Yalin. A decrease in the rate of chlorophyll mutation was observed at 150 and 200 Gy gamma ray doses. At these doses, 0.21% and 0.25% chlorophyll mutations were detected, respectively. After the decrease, an increasing rate of chlorophyll mutations was observed at higher doses. Higher rates of chlorophyll mutation were observed at 250 and 300 Gy gamma doses (Figure 3).

In the barley line YAA7050-14, the rate of *albino* plants was 0.52% and the rate of *xantha* plants was 0.13% at 250 Gy dose. At the highest gamma ray dose (300 Gy) the rate of chlorophyll mutations observed was 1.62%. All the chlorophyll mutations in M_2 plants at 300 Gy were *albino* (Table 3, Figure 3).

Table 3
Chlorophyll mutations in M_2 plants of line YAA7050-14 irradiated with different gamma ray doses

Doses (Gy)	Number of total plants	Chlorophyll mutations							
		Albino	*Albino* (%)	*Xantha*	*Xantha* (%)	*Viridis*	*Viridis* (%)	Chlorophyll mutant plants	Mutation frequency (%)
Control	955	0	0	0	0	0	0	0	0
100	934	2	0.21	2	0.21	5	0.54	9	0.96
150	937	1	0.11	1	0.11	0	0	2	0.21
200	795	0	0	0	0	2	0.25	2	0.25
250	772	4	0.52	1	0.13	1	0.13	6	0.78
300	803	13	1.62	0	0.00	0	0	13	1.62
Total	5196	20	2.46	4	0.45	8	0.92	32	3.82

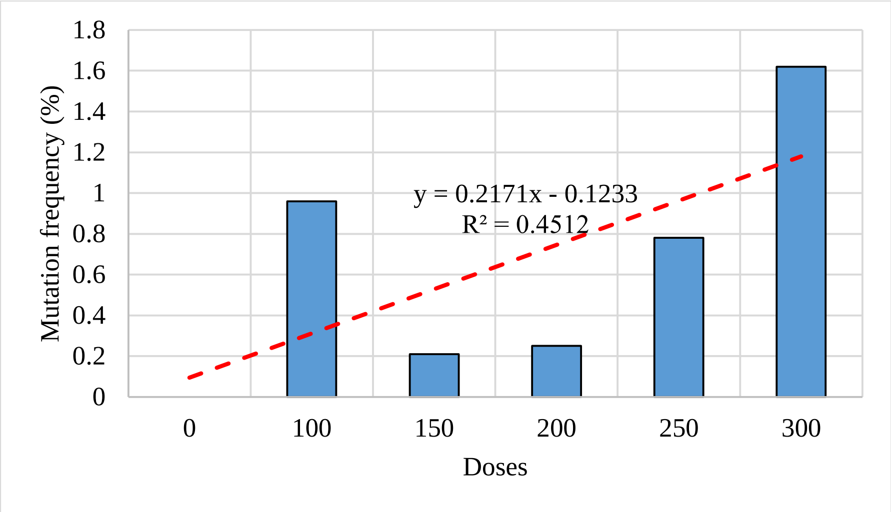

Figure 3. Regression graph between different gamma ray doses and chlorophyll mutation rate in M_2 plants of naked barley line YAA7050-14

In the naked barley line YAA7050-14, the highest chlorophyll mutation was seen in the *viridis* type with 55.6% at 100 Gy, while *albino* and *xantha* type mutations had the same rate with 22.2% at 100 Gy. *Albino* and *xantha* type mutations were found at equal rates (%50) at the 150 Gy dose. At 200 Gy dose, *viridis*-type chlorophyll mutation was observed in all mutant plants. The *albino* ratio was 66.7% and the *xantha* and *viridis* ratios were 16.6% at 250 Gy. At 300 Gy dose, *albino* type chlorophyll mutation was observed in all mutant plants. In the YAA7050-14 line, 62.5% of the total chlorophyll mutations were *albino* type, 25.0% were *viridis* type, and 12.5% were *xantha* type chlorophyll mutations (Table 4).

Table 4
Relative spectrum of chlorophyll mutants M_2 plants of line YAA7050-14

Doses (Gy)	*Albino* (%)	*Xantha* (%)	*Viridis* (%)
100	22.2	22.2	55.6
150	50.0	50.0	0
200	0	0	100
250	66.7	16.7	17
300	100.0	0.0	0
Total	62.5	12.5	25.0

When the results of chlorophyll mutations in 8-day-old seedlings of M_2 plants of both barley genotypes are evaluated together, the highest rate of chlorophyll mutation occurred at 300 Gy gamma ray dose in both the cv. Yalin and line YAA7050-14. Chlorophyll mutation was observed in 1.53% of the plants that emerged at this dose in cv. Yalin and 1.62% in the line YAA7050-14. The most common chlorophyll mutation type in both genotypes was *albino*, followed by *xantha* type chlorophyll mutations in cv. Yalin and *viridis* type in naked barley line YAA7050-14. In similar studies, it was reported that the rate of chlorophyll mutations increased in parallel with the increase in mutagen doses (Arain, 1974; Sing et al., 1977; Sakin & Sencar, 2001, Çiftçi & Şenay, 2005). Moreover, Ünver (1989) reported that *albino* and *xantha* type mutations increased in M_2 plants as the dose of EMS (Ethyl methanesulfonate) increased in barley cv. Obruk 86. Gustafsson (1946) reported that *xantha* type mutations were six times less frequent than *albino* mutants and both types of chlorophyll mutations increased as the dose increased, but this increase was not linear.

While albinism in barley is determined by more than one gene region (Makowska & Oleszczuk, 2013), *xantha* mutations giving yellow color are usually governed by a single recessive gene (Motoyoshi, 1967; Liu et al., 2008). The combined use of physical and chemical mutagens in barley and wheat increases the frequency of chlorophyll mutation (Singh et al. 1977, Çiftçi and Şenay, 2005). The pigments involved in photosynthesis are called photosynthetic pigments and chlorophylls, the green pigments in plants, are the most active types among them. Chlorophylls are mostly found in chloroplasts in mesophyll cells in green leaves of plants and *chlorophyll*-a, and *chlorophyll*-b derivatives are found in higher plants (Kacar, 1996). Biochemical changes in these photosynthetic pigments (*chlorophyll*-a, *chlorophyll-b* and *xanthophyll*) and their deficiencies in plants are the main reasons for reduced viability at high mutagen doses (Marcu et al., 2013). While both green (chlorophyll) and yellow pigments are absent in *albino* mutants, only yellow pigments are present in *xantha* type mutants and green pigment is absent. In the *viridis* type, the proportions of yellow and green pigments are changed compared to normal plants (Gustafsson, 1946). Chlorophyll a and b deficiency causes the *chlorina*-type mutation that results in pale green leaf color, and there are different types defined by different gene regions (Simpson et al., 1985). In *albino*, *xantha*, and *chlorina* type mutations, plants mostly die 2-4 weeks after germination because they cannot photosynthesise, while in other types, the amount of chlorophyll approaches normal in time and they can continue their vitality until harvest maturity (Motoyoshi, 1967, Ahumada-Flores et al., 2021). Chlorophyll mutations, which are not of economic importance because they are generally lethal (Wani, 2017), are used as genetic, physiological, and biochemical markers and can be easily identified and examined in M_2 generation (Patial et al., 2017). Mutants deficient in chlorophyll could be easily detected as recessive alleles during germination (Ilhan, 2014). Chlorophyll mutations are one of the important parameters used to determine mutation frequency and mutation efficiency in M_2 generation (Sakin & Sencar, 2001, Çiftçi & Şenay, 2005). In mutation breeding studies, it is recommended to use the dose (effective dose) at which the highest chlorophyll mutation frequency is achieved (Çiftçi & Şenay, 2005). In our study, the highest chlorophyll mutation numbers were observed at 250 and 300 Gy doses in Yalin barley variety and at 300 Gy dose in YAA7050-14 barley line. Therefore, it can be said that the effective doses (ED_{50}) in these genotypes are close to 250-300 Gy gamma-ray dose levels.

4. Conclusion

The most common chlorophyll mutation in M_2 plants of cv. Yalin was *albino* type, while *xantha* type chlorophyll mutation was found in the line YAA7050-14. The highest chlorophyll mutations were found at 250 and 300 Gy gamma-ray doses in cv. Yalin and at 300 Gy gamma-ray dose in naked barley line YAA7050-14. Considering the chlorophyll mutations, it was determined that 250-300 Gy gamma-ray doses had the highest mutation frequency in naked barley genotypes. These doses could be utilized to generate mutant plants in naked barley breeding studies. Mutant plants have potential as gene sources for developing new naked barley varieties for human consumption.

Acknowledgement

The authors are grateful to Dr Ali Senay from the Ankara Nuclear Research and Training Centre for his help with gamma irradiation. We are also grateful to CRIFC for allowing us to use their greenhouse. This study is based on Namuk ERGÜN's PhD thesis completed at Ankara University, Graduate Scholl of Natural and Applied Sciences under the supervision of Saime ÜNVER İKİNCİKARAKAYA.

Author Contributions

Namuk Ergün: Conceptualization, running the experiments, collecting data, writing, and editing of original draft.

Güray Akdoğan: Investigation, writing-review and editing.

Saime Ünver Ikincikarakaya: Conceptualization and supervision.

Conflicts of Interest

The authors declare no conflict of interest.

References

Ahumada-Flores, S., Briceño-Zamora, M., García-Montoya, J., López-Cázarez, C., Pereo-Galvez, A., Parra-Cota, F. & de los Santos-Villalobos, S. (2020). Gamma radiosensitivity study on wheat (*Triticum turgidum* ssp. *durum*). *Open Agriculture*, *5*(1), 558-562. https://doi.org/10.1515/opag-2020-0057

Ahumada-Flores, S., Pando, L. R. G., Cota, F. I. P., de la Cruz Torres, E., Sarsu, F., & de los Santos Villalobos, S. (2021). Gamma irradiation induces changes of phenotypic and agronomic traits in wheat (Triticum turgidum ssp. durum). *Applied Radiation and Isotopes*, *167*, 109490. https://doi.org/10.1016/j.apradiso.2020.109490

Arain, A. G. (1974). The effect of ethyl methanesulfonate treatment on floret sterility and chlorophyll mutation rate in barley. *Radiation Botany*, *14*(4), 347-350. https://doi.org/10.1016/S0033-7560(74)80027-X

Badr, A., Rabey, H. E., Effgen, S., Ibrahim, H. H., Pozzi, C., Rohde, W., & Salamini, F. (2000). On the origin and domestication history of barley (Hordeum vulgare). *Molecular biology and evolution*, *17*(4), 499-510. https://doi.org/10.1093/oxfordjournals.molbev.a026330

Bhatty, R. S. (1999). The potential of hull-less barley. *Cereal Chemistry*, *76*(5), 589-599. https://doi.org/10.1094/CCHEM.1999.76.5.589

Chaudhary, J., Deshmukh, R., & Sonah, H. (2019). Mutagenesis Approaches and Their Role in Crop Improvement. Plants (Basel, Switzerland), 8(11), 467. https://doi.org/10.3390/plants8110467

Çiftçi C.Y. & Şenay, A. (2005) Effect of seperate and combined treatments of different doses of gamma rays and EMS on durum wheat (Tritium durum Desf.) in M1 generations. *Journal of Field Crops Central Research Institute 14*(1-2), 41-49. (*in Turkish*) https://www.biotechstudies.org/uploads/pdf_329.pdf

Dyulgerova, B., & Dyulgerov, N. (2022). Mutagenic effect of sodium azide on winter barley cultivars. *Agriıcultural Science and Technology, 14*(2), 27-33. https://doi: 10.15547/ast.2022.02.016

Gaul, H. (1964). Mutations in plant breeding. *Radiation Botany, 4*(3), 155–232. https://doi:10.1016/s0033-7560(64)80069-7

Goyal, S., Wani, M.R. & Khan, S. (2019) Frequency and Spectrum of Chlorophyll Mutations Induced by Single and Combination Treatments of Gamma Rays and EMS in Urdbean. *Asian Journal of Biological Sciences, 12*, 156-163. https://scialert.net/abstract/?doi=ajbs.2019.156.163

Gustafsson, Å. (1940). The Mutation system of the chlorophyll apparatus. *Acta Universitatis Lundensis, 36*(11), 1-40.

Gustafsson, Å. (1946). The origin of albina and xantha mutations in barley. *Acta radiologica*, *27*(3-4), 300–307. https://doi.org/10.3109/00016924609135187

Ilhan, D. (2014) Mutagenicity tests used in plants. *Kafkas University Institute of Natural and Applied Science Journal*, 7(1), 29-47. (*in Turkish*) https://dergipark.org.tr/en/pub/kujs/issue/30901/334479

Kacar, B. (1996). Plant Physiology 4th Edition, Ankara University Faculty of Agriculture Publications, Publication No: 1447, 424, Ankara (*in Turkish*).

Kodym A., Afza, R., Forster, B.P., Ukai, Y & Nakagawa, H. (2012) Methodology for physical and chemical mutagenic treatments. In: Shu Q.Y., Forster, B.F. & Nakagawa, H. (eds), *Plant mutation breeding and biotechnolog*y. CABI, FAO, Oxfordshire, UK pp. 169–180

Lev-Yadun, S., Gopher, A., & Abbo, S. (2000). The cradle of agriculture. *Science, 288*(5471), 1602-1603. https://doi.org/10.1126/science.288.5471.1602

Liu, Z. L., Yuan, S., Liu, W. J., Du, J. B., Tian, W. J., Luo, M. H., & Lin, H. H. (2008). Mutation mechanism of chlorophyll-less barley mutant NYB. *Photosynthetica, 46*, 73-78. https://doi.org/10.1007/s11099-008-0013-0

Makowska, K. & Oleszczuk, S. (2013) Albinism in barley androgenesis. *Plant Cell Reports, 33*, 385-392. https://doi.org/10.1007/s00299-013-1543-x

Maluszynski, M., Szarejko, I., Bhatia, R., Nichterlein, K. & Lagoda, P.J.L. (2009) Mutation techniques. In: Guimares, E., Ceccarelli, S., Weltzein, E. & Rajendran, P.G. (eds) *Plant breeding book*. FAO, Rome, pp 159-194.

Marcu, D., Damian, G., Cosma, C., & Cristea, V. (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays*). *Journal of biological physics*, *39*, 625-634. https://doi.org/10.1007/s10867-013-9322-z

Meints, B., Vallejos, C., & Hayes, P. (2021). Multi-use naked barley: A new frontier. *Journal of Cereal Science*, *102*, 103370. https://doi.org/10.1016/j.jcs.2021.103370

Meints, B. & Hayes, P.M. (2019) Breeding naked barley for food, feed, and malt. In: Goldman, I. (ed) *Plant Breeding Reviews*, 43 (1). John Wiley & Sons, Inc., pp. 95-119.

Motoyoshi, F. (1967). Chlorophyll formation in several chlorophyll mutants of sand oats and barley cultured on nutrient media. *The Japanese Journal of Genetics*, *42*(5), 291-297. https://doi.org/10.1266/jjg.42.291

Nazarenko, M. M., & Lykholat, T. Y. (2020). Variability at winter wheat varieties first generation which obtained mutagen action. *Ecology and Noospherology*, *31*(2), 77-81. https://doi.org/10.15421/032012

Newman, R.K. & Newman, C.W. (2008) Barley for food and health: Science, technology, and products. John Wiley & Sons, Inc., 243, New Jersey.

Patial, M., Thakur, S. R., Singh, K. P., & Thakur, A. (2017). Frequency and spectrum of chlorophyll mutations and induced variability in ricebean (Vigna umbellata Thunb, Ohwi and Ohashi). *Legume Research-An International Journal*, *40*(1), 39-46. https://doi.org/10.18805/lr.v0iOF. 10757

Sakin, M.A. & Sencar, Ö. (2001). Makarnalık buğday (*Triticum durum* Desf.) çeşitlerinin mutagenlere tepkileri. *Journal of Agricultural Faculty of Gaziosmanpaşa University*, 18(1), 89-93. https://dergipark.org.tr/tr/pub/gopzfd/issue/7349/96160 (*in Turkish*)

Simpson, D. J., Machold, O., Høyer-Hansen, G., & Von Wettstein, D. (1985). Chlorina mutants of barley (Hordeum vulgare L.). *Carlsberg Research Communications*, *50*, 223-238. https://doi.org/10.1007/BF02907148

Singh, R. M., Singh, J., & Srivastava, A. N. (1977). Mutagenic effects of gamma rays, EMS, and HA in barley. *Barley Genetics Newsletter*, *7*, 60.

Ullrich, S.E. (2011). Significance, adaptation, production, and trade of barley. In: Ullrich, S.E. (ed.). *Barle*y, Blackwell Publishing Ltd., , Chichester, UK. pp. 3-13

Ünver, S. (1989). The effect of different doses of EMS and the temperatures and periods of post washing applied on the some characters in M1 and M2 plants of barley (*Hordeum vulgare* L.) (*in Turkish*) (Unpublished PhD thesis). Ankara University, Ankara, Turkey.

Wani, M. R., Khan, S., & Kozgar, M. I. (2011). Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mungbean. *Frontiers of Agriculture in China*, *5*, 514-518. https://doi.org/10.1007/s11703-011-1126-y

Wani, M.R. 2017. Induced chlorophyll mutations, comparative mutagenic effectiveness and efficiency of chemical mutagens in lentils (*Lens culinaris* Medik). Asian J. Plant Sci., 16: 221-226. https://doi.org/10.3923/ajps.2017.221.226

Yu, K., Lenz-Wiedemann, V., Chen, X., & Bareth, G. (2014). Estimating leaf chlorophyll of barley at different growth stages using spectral indices to reduce soil background and canopy structure effects. *ISPRS Journal of Photogrammetry and Remote Sensing*, *97*, 58-77. https://doi.org/10.1016/j.isprsjprs.2014.08.005