RAP CONFERENCE PROCEEDINGS, VOL. 9, PP. 57–62, 2024
ISSN 2737-9973 (ONLINE) | DOI: 10.37392/RAPPROC.2024.13
RAP-PROCEEDINGS.ORG

OPTIMIZATION OF X-RAY IRRADIATION DOSE FOR INDUCED MUTATION IN BREAD WHEAT VARIETIES CULTIVATED IN ALBANIA

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Abstract. *Induced mutagenesis is one of the most used techniques to improve the quality of agricultural products. One of the main problems of induced mutagenesis is the assessment of treatment doses interval of biological materials for their processing. Calculation of radio-sensitivity is the first step in induced mutagenesis to improve the traits of different varieties. Our study is based on the selection of four varieties of winter bread wheat cultivated in Albania: DAJTI, UBT-1, UBT-2 and KIA-1. Irradiation of bread wheat seeds was carried out with X-rays generated by the RS-2400Q irradiation unit for the evaluation of radio-sensitivity tests. Referring to IAEA protocols, we chose for irradiation six X-rays doses of 50, 100, 150, 200, 300 and 400 Gy. The quantity of seeds for each treatment was the same and the biological material was planted in greenhouse pots. Seeds treated with 6 doses of radiation and the control were planted in three replicates each. To obtain an effective mutation with high probability, the growth reducing dose (GR50) and the lethal dose (LD50) were used in the radio-sensitivity test. The high radiation doses of 300 Gy and 400 Gy for X-rays caused significant damage to the seeds of the four bread wheat varieties. The KIA-1 variety was less resistant and at the 200 Gy, more than 75% of the plants were lost. Following the radio-sensitivity test, to study abiotic and biotic stresses in the experimental field, the three most appropriate doses to irradiate the seeds of these bread wheat varieties were 50, 100 and 150 Gy.*

Keywords: *bread wheat, induced mutations, irradiation, LD50, LD30, radio-sensitivity test, X-rays*

1. INTRODUCTION

Wheat is one of the most widely used cereals for human consumption worldwide [1], [2]. Bread and durum wheats are one of the most important crops as a food product in Balkan region related to their large adaptability and ability to be grown in many different climatic conditions, and to high nutritional values [3], [4].

During least years the extreme weather patterns affecting recent seasons with hotter and drier summers, which has driven them to explore crop alternatives and new resilient varieties [3]. Climatic changes in the Mediterranean area, abiotic and biotic stresses make it necessary to increase the production of bread wheat. In addition, it is urgent to increase the resistance of different cultivars to diseases and parasites. Ensuring the food security in the coming decades will require a combination of improved varieties and agronomic practices warranting environmental sustainability [5].

Thus, the generation of new varieties tolerant or resistant to these new conditions is determined to ensure food security. Currently, the induction of mutations is one of the most used techniques to obtain improved varieties [6].

Currently, wheat production is negatively affected by several biotic (pests, pathogens, and soil degradation) and abiotic (drought, salinity, and increment of temperature) stress conditions [7], [8], [9].

Induced mutagenesis is one of the most used techniques to improve the quality of agricultural products [10], [11]. The application of nuclear techniques to induce mutations in cereals and legumes is very important to improve the sustainable development of agriculture in Albania. The use of X-rays is one of the ways of treating plant materials to improve their properties.

X-ray irradiation of cereal seeds is necessary to induce genetic variation for resistance to changed climatic conditions, high temperatures at the time of ear ripening and long droughts [10], [12], [13].

One of the main problems of induced mutagenesis is related with the calculation of irradiation doses of biological materials for their treatment. Radio-sensitivity assessment versus radiation is the first step in induced mutagenesis to improve the traits of different varieties. Our study is based on the selection of four varieties of winter bread wheat cultivated in Albania and we have selected four varieties of bread wheat: DAJTI, UBT-1, UBT-2 and KIA-1 [9].

The purpose of our work is to improve the characteristics of these varieties, such as their resistance to parasites during the ripening period, through induced physical mutagenesis.

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Growth Reduction Dose (GR50) and Lethal Dose (LD50) were used to obtain an effective and useful mutation with high probability, as part of the radio-sensitivity Test [10], [14]. Lethal dose (LD50) is the dose of radiation expected to cause death to 50 % of an exposed population and Growth Reduction Dose (GR50) is the dose at which 50% growth reduction is observed [15], [10]. The radio-sensitivity of the bread wheat DAJTI, UBT-1, UBT-2 and KIA-1 varieties was calculated from observations and measurements. Lethal dose (LD50) is the dose of radiation expected to cause death to 50 % of an exposed population within 30 days (LD50).

In cereals, radio-sensitivity is genotypic dependence. It is highly desirable to establish a radiosensitive curve and determine LD50 of mutagen for treating seeds, for the induction of mutations in cereals and others. LD50 is defined as the dose at which highest frequency of mutation occurs [10], [15].

2. Materials and Methods

This study was carried out in a greenhouse to determine the effective doses for mutation breeding of four bread wheat during the October - November 2023.

We undertook the analyze of radiosensitivity results on wheat varieties under the influence of soft ionizing radiation (X–rays) generated by the RS2400Q device and we selected seeds of four bread wheat varieties DAJTI, UBT-1, UBT-2 and KIA-1.

The seeds of the wheat varieties were obtained from the Genetic Bank, Agriculture University of Tirana, and the plant materials belong to 2023. The seeds were selected in advance by choosing healthy seeds and of the same size. The seeds must be clean, with a high germination capacity and stabilized as genetic material. This is necessary for the successful mutation breeding experiment, once the target variety/genotype has been chosen.

In the Induced Mutagenesis Laboratory of the Department of Biotechnology, University of Tirana, the seeds of the four varieties were tested for their germination ability in Petri dishes before radiation treatment.

The wheat seeds were observed for their ability to germinate in the laboratory for 7 days. The seeds must have a very good development and a good germination ability more than 90%. Before X-ray treatment, the seeds of bread wheat varieties must have a moisture content of up to 14%. The seeds were placed in a desiccator with 60% glycerin for a duration of 4-6 days to ensure 14% moisture content [10], [15].

The seeds were observed for their ability to germinate in the Induced Mutagenesis Laboratory, Department of Biotechnology, University of Tirana, for 7 days. The seeds have a very good development and a good germination ability. It was observed that their germination ability was at 98% for the four varieties as is shown Figure 2.

After preparing the seeds of bread wheat of four varieties with 14% moisture according to the protocols, the seeds were treated with X-rays generated by RS2400Q Unit for evaluation of the radio-sensitivity Test, in the Institute of Applied Nuclear Physics in Tirana.

RS-2400Q Irradiator [16] is a Biological Irradiator developed as a Cesium137 replacement device for the research irradiation of small animals, cells, tissue, insects and more. It has a cylindrical, rather than a point X-ray source, which yields higher dose rates. The X-ray beam is uniform within 10% at the surface of the X-ray tube over a wide range of voltages. RS-2400Q does not need a shielded room & added safety cost.

Figure 1. RS-2400Q X-Ray
Irradiator Designed for Life Science

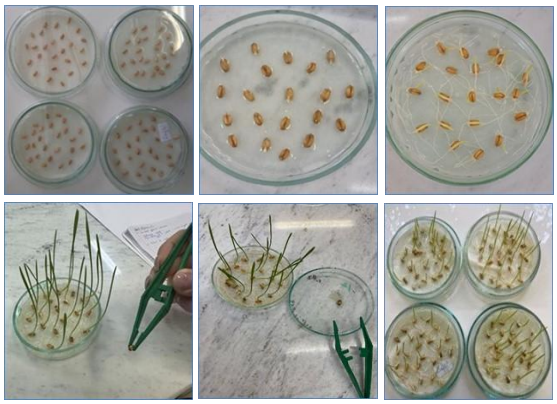

Figure 2. The different stages of the germination ability of the seeds of the four bread wheat varieties DAJTI, UBT-1, UBT-2 and KIA-1

RS-2400Q irradiator has a Carousel Circulation Technology. It has a high capacity, single chamber design with an integrated multi-canister carousel technology. In our case the average Dose Rate in the canister center is 16.47 Gy/min and the Dose uniformity is 1.45.

Referring to IAEA protocols and different publications, we chose for irradiation six X-rays doses of 50, 100, 150, 200, 300 and 400 Gy [17]. The quantity of seeds for each treatment was the same and five days after seed irradiation, the biological material was planted in greenhouse pots. Seeds treated with 6 doses of radiation and the control were planted in three replicates each. The seeds planted in the greenhouse were watered every two days. The percentage of seed germination was assessed by counting the number of seedlings 7, 14 and 21 days after sowing. Survival percentages were assessed by counting viable plants 30 days after sowing.

3. Results and Discussion

Referring to the literature data, we chose for treatment with X – rays 6 doses of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 300 Gy and 400 Gy [10], [12], [15]. Each package was labelled with the variety/genotype name, the dose (Gy), the irradiation time, the source of irradiation, and the irradiation date. The number of seeds for each treatment was the same [10].

Five days after seeds irradiation, the biological materials were planted in greenhouse pots in three repetitions for each dose treated as well as for the control (Co). 12 pots (6 different irradiated seeds and Co) were used for X-rays experiment.

The seeds treated with 6 radiation doses (50, 100, 150, 200, 300, 400 Gy) and the control were planted with three replicates each. The soil preparation of the pots in which the radiosensitivity experiment was carried out was done according to IAEA protocols [15], [18].

To obtain effective and useful mutation at high probability, growth reduction dose (GR50) and lethal dose (LD50) were used, as part of radio-sensitivity Test. Radio-sensitivity of bread wheat was calculated from the observations and the results of the measurements. The treated/ irradiated seeds and untreated control seeds were sown in trays in the greenhouse. Based in observations, measurements and calculated data, the LD50 was determined for the seeds irradiated separately with X-ray we used GR50 to find the optimal doses for the seeds of four varieties Dajti, UBT-1, UBT-2 and KIA-1.

In each row we planted 20 seeds. The seeds planted in the greenhouse were watered every two days with irrigation system in the form of rain. The germination percentage of seeds were evaluated by counting the number of seedlings at 7, 14, and 21 days after sowing.

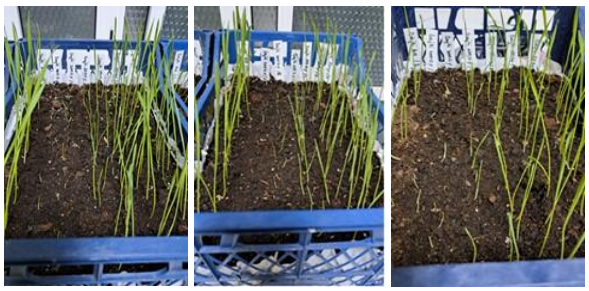

Figure 3. The three replicates of the DAJTI variety in the third week of their development in the greenhouse

The figure shows the development of bread wheat DAJTI variety treated with X-radiation in the third week, and for the two maximum doses of 300 Gy and 400 Gy, there are almost no plants developed.

The survival percentages were assessed by counting the viable seedlings at 30 days after sowing. In each pot, the good plants were counted, their germination and growing phases were carefully observed, photographed (Figures 1 - 6), and we kept detailed notes. The body heights and root length were measured at the end of 30 days. To obtain effective and useful mutation at high probability, growth reduction dose (GR50) and lethal dose (LD50) were used, as part of radio-sensitivity Test. Radio-sensitivity of breads wheat was calculated from the observations and the results of the measurements.

From the observations, the high doses such as 200 Gy, 300 Gy and 400 Gy we have noticed the reduction of the number of surviving plants after four weeks. The KIA-1 variety had no plants in the 200 Gy dose. For the DAJTI variety, there are less than 50% of the surviving plants in the greenhouse and these are very little developed and with a very weak root system.

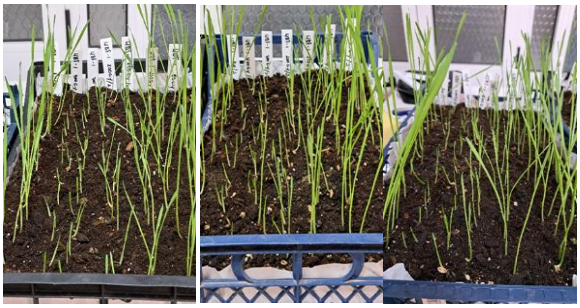

Figure 4. The three replicates of the UBT-1 variety plants in the third week of their development in the greenhouse

Figure 5. The three replicates of the UBT-2 variety plants in the third week of their development in the greenhouse

Figure 6. The three replicates of the KIA-1 variety plants in the third week of their development in the greenhouse

Table 1. The measurement for DAJTI M1 plants

Applied dose (Gy)	Plant height (cm)	% height over control	% height reduction
0	21.81	100	0.00
50	19.6	89.86	10.14
100	17.44	79.96	20.03
150	13.34	61.44	38.56
200	4.14	18.98	81.11
300	0.51	2.33	97.67
400	0.54	2.36	97.64

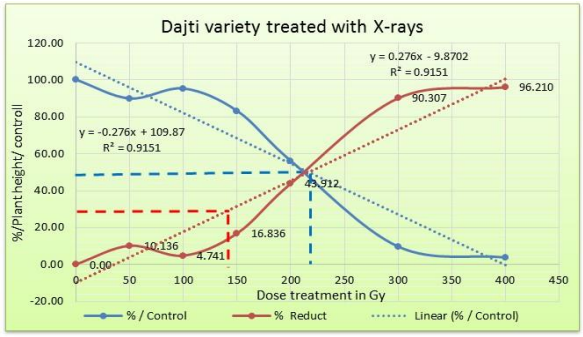

Figure 7. % plant height / control and % height reduction for the DAJTI M1 plants from seeds treated with X - ray in different doses during their growth in the greenhouse, GR30 and GR50

To obtain effective and useful induced mutations in bread wheat varieties with high probability, a growth reducing dose (GR50) and a lethal dose (LD50) were used in the radio-sensitivity test. The radio-sensitivity of bread wheat was calculated from observations and measurement results. To evaluate and calculate the irradiated doses for each variety applied in experimental field, we used two indicators GR30 and GR50.

From the data in the Table 1, we generated the linear equations and evaluated that for DAJTI bread wheat variety, GR50 dose was 224 Gy and GR30 was 148 Gy. For more the LD 30 dose, that shows that only 30% of the plants did not germinate after irradiation was 173 Gy. From the height of the plants in the table, for the DAJTI variety GR50 was starting from the dose of 200 Gy, 300 Gy and 400 Gy.

Table 2. The measurement for UBT-1 M1 plants

Applied dose (Gy)	Plant height (cm)	% height over control	% height reduction
0	21.97	100	0
50	22.21	101.09	-1.09
100	20.67	94.08	5.92
150	16.10	73.28	26.72
200	6.53	29.72	70.28
300	3.33	15.16	84.84
400	2.28	10.38	89.62

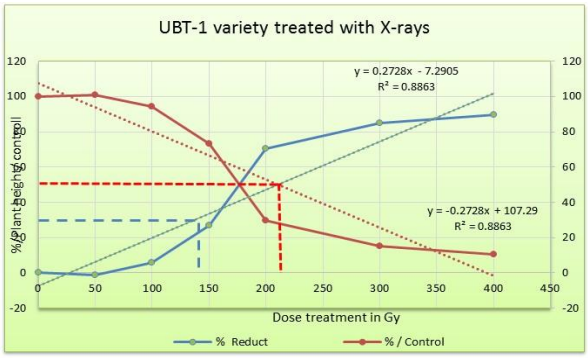

Figure 8. % plant height / control and % height reduction for the UBT-1 M1 plants from seeds treated with X - ray in different doses during their growth in the greenhouse, GR30 and GR50

From the data in the Table 2, we created the linear equations and evaluated for UBT-1 bread wheat variety, GR50 dose, that shows growth reduction of 50% of the plants irradiated with X-ray, was (205 – 210) Gy as well as the GR 30 dose, that shows that only 30% of the plants did not germinated after radiation treatment, was (145 – 151) Gy. It might be more appropriate to use the interception with the trend line (dots) where GR50 is more than 200 Gy and that of GR30 is less than 150Gy. On the other hand, the LD50 dose, that shows that 50% of the plants did not survive was 178 Gy as well as the LD 30 dose, that shows that only 30% of the plants did not germinate after irradiation was 151 Gy.

Table 3. The measurement for UBT-2 M1 plants

Applied dose (Gy)	Plant height (cm)	% height over control	% height reduction
0	22.160	100	0
50	20.130	91.2	8.8
100	19.279	91.01	8.99
150	19.900	90	10
200	18.900	81.14	18.86
300	7.663	33.6	66.4
400	1.360	9.3	90.7

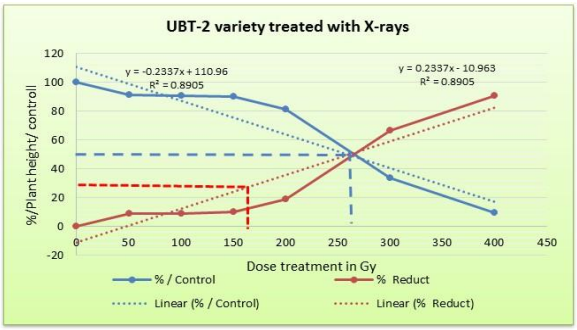

Figure 9. % plant height / control and % height reduction for the UBT-2 M1 plants from seeds treated with X - ray in different doses during their growth in the greenhouse, GR30 and GR50

From Table 3 we generated the linear equations of % height over control and % height reduction for the UBT-2 and evaluated that for UBT-2 variety. The GR50 dose was 263 Gy and GR30 was 165 Gy. For more the LD50 dose, that shows that 50% of the plants did not survive was 263 Gy as well as the LD 30 was 222 Gy. From the graphic presentation, Figure 9, we evaluated GR50 and GR30 where the lines of the respective doses intercept with the trend line (dots).

Table 4. The measurement for KIA-1 M1 plants

Applied dose (Gy)	Plant height (cm)	% height over control	% height reduction
0	20.5	100.00	0.00
50	22.25	108.55	-8.55
100	21.1	88.32	11.67
150	20.19	85.51	14.50
200	11.44	75.35	24.65
300	2.25	10.99	89.01
400	0.97	4.75	95.25

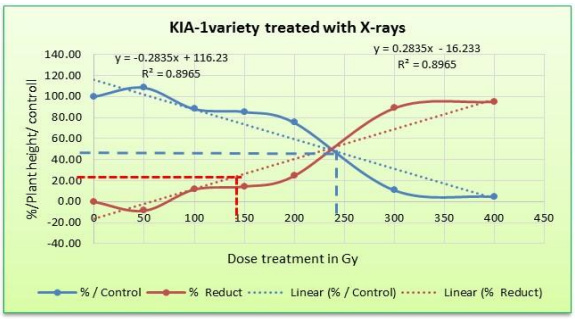

Figure 10. % plant height / control and % height reduction for the KIA-1 M1 plants from seeds treated with X - ray in different doses during their growth in the greenhouse, GR30 and GR50

From the graphic presentation, Figure 10, we prepared the linear equations and evaluated that for KIA-1 bread wheat variety, GR50 was 235 Gy and GR30 was 145 Gy for KIA-1 bread wheat variety. On the other hand, LD50 dose was 235 Gy as well as the LD30 was 215 Gy.

The high radiation doses of 300 Gy and 400 Gy generated from X – rays, caused significant damage to the bread wheats seeds of DAJTI, UBT-1, UBT-2 and KIA-1 varieties. The albin plants were observed in pots with seed treated with 200 Gy, which significantly reduced the number of plants that survived. In the plants of Dajti, UBT-1, UBT-2 that belonged to the 200 Gy radiation dose, both for irradiation with X-rays, the number of plants that survived after 30 days was no more than 9 - 12 developed plants. The variety KIA-1 was less resistant and at the dose of 200 Gy, more than 75% of the plants were lost. Following the radio-sensitivity test, the three most appropriate doses to irradiate the seeds of these bread wheat Dajti and KIA-1 varieties of were 50, 100 and 150 Gy for the experimental field.

This choice of doses was made after the plants in the greenhouse were observed for a longer period then four weeks and the plants with 200Gy radiation lose their vitality for the variety DAJTI and KIA-1.

4. Conclusion

Bread wheat variety KIA-1 is more sensitive to X-rays and for application of induced mutagenesis, low doses should be used, not exceeding the limit of 150Gy.

For the variety DAJTI in the dose of 50 Gy a greater growth of the control plants is observed, this shows that this is a stimulating dose. while the dose of 200 Gy in greenhouse conditions caused the number of plants to increase GR50 by more than 50%. This shows that this dose is not optimal to be used for this variety of bread wheat.

For the varieties UBT-1 and UBT-2, a dose of 200 Gy can be used for irradiated the seeds.

Based on the results of this study, the field experiment will be carried out with the 4 varieties of wheat and with the results that will be achieved after the 5th generation, we will select the mutant materials with improved features.

Acknowledgements: *The paper is a part of the research done within the project "Developing the use of X-ray Irradiation Unit as safe alternative for Induced Mutation in plants for sustainable production ", supported by Office of Radiological Security, NNSA and Sandia National Laboratories, Albuquerque, New Mexico, USA.*

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