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Research Article

Dose Dependent Effects of Gamma Radiation on Growth Parameters of *Lens culinaris Medikus subsp. Culinaris*

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Abstract

The assessment of treatment dose known as radio sensitivity test is the first step in any breeding program using radiation as physical mutagen. The aim of this preliminary investigation is to assess the dose that results in a 50% reduction of growth (LD50%) that should be enough to obtain lentil mutants for main treatment in a breeding program for resistance to *Stemphylium botryosum* which is our main goal in the furtherance of our project. Lentil dry seeds were exposed to a 60Co gamma source at doses ranging from 50 - 1000 Gy. For an irradiation dose between 50-400 Gy, results showed a significantly attaint of the growth parameters ($P \leq 0.05$) compared to untreated plants. While at doses over than 450 Gy an extremely significant difference ($P < 0{,}001$) of plant vegetative growth was recorded. At a dose above 450 Gy, a drastic reduction in the length of roots and shoots as well as in the total content of chlorophyll pigments was recorded. These observations confirm that exposure to lower doses has a simulative effect of physiological parameters, whereas it inhibits and have negative effects of these parameters at higher doses. The obtained results noticed that increased antioxidant capacity and polyphenols content of lentil plants were increased after application of γ-irradiation.

Keywords: Lentil; Gamma Radiation; Germination; Photosynthetic Pigments; Antioxidant Capacity

Abbreviations

AE: Ascorbate Equivalents; CNSTN: Tunisian National Center for Nuclear Sciences and Technology; DAP: Days After Planting; DPPH: 1, 1-diphenyl-2-picrylhydrazyl; d.wt: Dry Weight; FGP: Final Germination Percentage; GAEs: Gallic Acid Equivalents; GI: Germination Index; ICARDA: International Center for Agricultural Research in the Dry Areas; LSD: Least Significance Differences; TAC: Total Antiradical Capacity

Introduction

Researchers are challenged to develop new crop adaptation strategies to withstand the climate changes; at the same time there is the need of sustainable alternatives to our current agricultural model, intending to reduce the environmental impact. Crops may be improved by combining in them agronomically desired traits through laborious and time consuming selective hybridization and plant breeding techniques [1]. However this may take years to develop an agronomically important cultivar through conventional breeding procedures. Mutation breeding is robust, cost effective, quick and proven method to accelerate the development and selection of novel agronomic traits. Ionizing radiation has been widely applied with the aim of inducing genetic variability for genetic studies and selection. Mutagens used may break linkages genetic, cause changes in an organism and produce various new promising traits

for the amelioration and improvement of crop plants [2]. Gamma irradiation is an important physical mutagen which has proved to be an economical, effective and useful alternative approach for obtaining new combinations of desirable traits in agricultural crops [3]. The penetrating power of gamma rays helps its wider application in various plant amelioration techniques [4]. Once absorbed in biological material, the ionizing radiation can act directly on critical cell targets or indirectly through the free radicals generated by the interaction with molecules and atoms in the cell, particularly water, which can diffuse and damage different important components [5]. Researches studying the effects of ionizing radiation on plants have been carried out using many different indicators and parameters of plant growth responses. Phenotypic development changes and growth parameters have most commonly served as measurable responses to different ionizing radiation doses [6,7]. A number of researchers investigated the effect of gamma radiation on chlorophyll content since it's widely used as a good indicator of photosynthesis activity, stress and nutritional state, mutations, is of special significance to precision agriculture [8].

Lentil crop is an extremely nutritious afford-able grain legume with important mineral density and rich protein contents and thus playing a pivotal role in combating food in security and malnutrition. Being a self-pollinated crop with narrow genetic base, induction of genetic variability is prerequisite to initiate breeding program in lentil. This study was undertaken with the objectives of investigating the influence of various doses of gamma irradiation on some growth parameters of *Lens culinaris Medikus subsp. Culinaris* in order to initiate a breeding program in lentil for resistance to *Stemphylium botryosum*.

Materials and Methods

Plant material

'Kef' lentil variety of Lens culinaris Medikus subsp. culinaris developed at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, and released in 2003 by the Institute Agronomy Research of Tunisia, Ministry of Agriculture, Tunisia, for commercial cultivation in Tunisia [9].

Gamma irradiation

Gamma irradiation was conducted using a 60Co [Cobalt-60] gamma source at a dose rate of 9.836 Gray/minutes (Gy/min) at the Tunisian National Center for Nuclear Sciences and Technology (CNSTN). The doses of exposure were 50, 100, 150,200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 Gy.

Seed germination test

Three replicates of 25 seeds each/treatment were surface-sterilized for 30 minutes, with 10% calcium hypochlorite, and then rinsed three times with sterile water. After the cleaning and sterilization step, the lentil seeds were placed in petri dishes (containing sterile filter paper soaked in distilled water) and allowed to germinate in the dark at 28 ° C for 10 days. The lentil seeds not treated with ionizing radiation served as controls. The lentil seeds samples were considered as germinated ones when they exhibited a radical extension of > 0.1 cm. Counts of germinated lentil seeds were carried out on a daily basis for 7 days in order to determine and subsequently calculate both the final germination percentage and the germination index. The (FGP) final germination percentage was calculated by virtue of Anjum and Bajwa (2005) study [10] as follows (1):

$$FGP=\frac{N_T \times 100}{N} \quad (1)$$

Where NT = proportion of germinated lentil seeds in each treatment for the final measurement; and N = number of lentil seeds used in bioassay Germination index is a quantitative expression of germination that relates the daily germination rate to the maximum germination value. The germination index (GI) was calculated as described by the Association of Official Seed Analysts [11] by the following formula (2):

$$GI = \frac{N_1}{1}+\frac{N_2}{2}+\frac{N_3}{3}+\cdots+\frac{N_n}{n} \quad (2)$$

Where N1, N2, N3, Nn represents the number of lentil seed which germinated in day 1, 2, 3..., n. For all these measurements, the results represent an average of three repetitions.

The notations of roots and hypocotyls lengths for either control samples or irradiated ones were measured on the 7th and 14th days after the experiment beginning. Shooting and rooting were defined as protrusion of the shoot and root to the extent of at least 0.5 cm.

Physiological and biochemical parameters

The ionizing radiation treated and untreated lentil seeds, were sown in pots in order to study the development of established

plants under greenhouse conditions at 25°C with a 16h photoperiod. After 1, and 2 weeks days after planting (DAP), the following growth criteria were recorded (root and hypocotyl length), using six random plants from all treatments. Fresh leaves from each treatment were collected at 28 DAP and analyzed for chlorophyll contents (following the method of Lichtenthaler (1987)) [12], total polyphenol content and antioxidant activity. The recorded data was statistically analyzed by analysis of variance and least significance differences (LSD) test.

Determination of total phenolic content by Folin-Ciocalteu method: Total phenolic content was determined using the Folin-Ciocalteu reagent and reported as gallic acid equivalents (GAEs) mg^{-1} d.wt by reference to a standard curve [13]. A volume of 20 mL leaf extract was mixed with 1.58 mL of deionized H_2O and with the 100 mL of Folin-Ciocalteu reagent. The sample was incubated for 1 - 8 min and the reaction was then neutralized with 300 mL of a (Na_2CO_3, 20%) sodium carbonate solution (w/v). The sample was incubated at 40°C in the dark for 30 min. The blue colour resulting absorption was measured at λ = 765 nm, using a spectrophotometer (Genesys 5, France.). A Gallic acid (50 - 400 mg L^{-1} range) calibration curve was built. The specific antioxidant capacity (SAC) was expressed as µg AE mg^{-1}GAE (the ratio between the total antiradical capacity and the total phenolic content) [14].

Antioxidant activity: Leaf extract was prepared as described by Li., *et al.* (2008) [15]. Lentil plant samples (60 mg) were reduced to fine powder using a pestle and a mortar thereafter recovered by adding 1.2 mL of acetone: water (70:30; v/v) and switched to a microtube and incubated at 23 °C overnight under non-brutal shaking. Afterward, the sample was centrifuged and the supernatant was recovered and stored at -20°C. The lentil leaf extracts free radical-scavenging activity was calculated as done by Braca., *et al.* (2001) [16]. The 100 mL of extract was added to 3 mL of a solution containing 100 mM of DPPH dissolved in methanol. The reaction has been done at room temperature in the dark and for 20 min. For each sample, absorbance at λ=517 nm was measured using a Genesys 5 spectrophotometer. DPPH reduction was calculated from the following equation [16]:

$$\left[\frac{A0 - A1}{A0}\right] \times 100 \quad (3)$$

Where: A0 represent the control absorbance without seed extract and A1 represent the extract/standard absorbance. Using ascorbic acid (0.1-1.5 mM range) a standard curve was built and the total antiradical capacity (TAC) was expressed as ascorbate equivalents (AEs) mg^{-1}d. wt.

Results and Discussion

The effect of different doses of gamma irradiation (50 - 1000Gy) on final germination percentage and germination index of Lentil culinaris is illustrated in figure 1. The exposure of seeds to lower doses (50 - 250 Gy) did not alter significantly these parameters. However, the parameters under consideration were negatively influenced by a dose level of 700 Gy. The present study revealed drastic effects of higher gamma doses (700 up to 1000Gy) on final germination percentage and germination index of *L. culinaris* which exhibited decreasing trend with increasing dose intensity level. Larger doses have been widely shown to induce drastic changes and variations in various quantitative and qualitative agronomic traits of different crops, while lower doses of ionizing radiation induce a growth stimulating effect. It has been widely proved that higher doses induces drastic changes for different qualitative and quantitative agronomic traits of different crop while lower doses of gamma irradiation causes growth stimulatory effect [17].

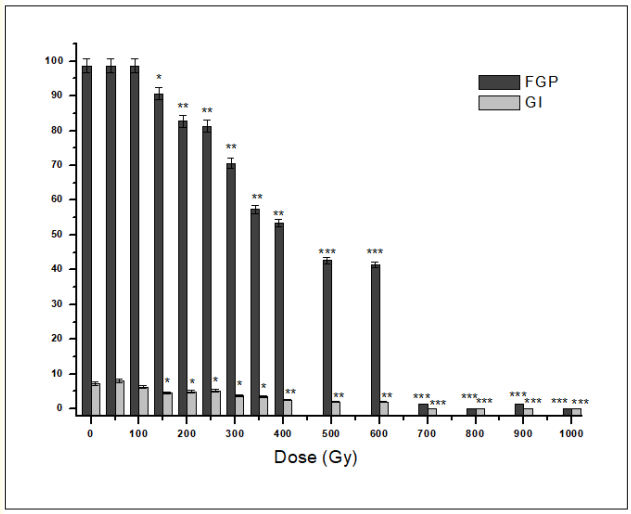

Figure 1: Effect of gamma irradiation on final germination percentage (FGP) and germination index (GI) of *Lens culinaris Medikus subsp. culinaris* (Each bar represents Mean ± SD [Standard deviation] for average of n = 3 independent experiments). Data are the means of three replicates± SD/*Significant difference (P < 0, 05), **highly significant difference (P < 0, 01), ***extremely significant difference (P < 0,001).

The biometric measurements of roots emerged from irradiated lentil seeds (50 - 1000 Gy) (Table 1) showed a significant inhibition and decrease of lentil root length after 14 days from the start of the experimentation. At an absorbed dose of 50, 100 and 150 Gy, the root length decreased by 14.5%, 19.35% and 34.64%, respectively. The maximum decrease of 74.79% was recorded at 450 Gy, while at a higher doses; the radicular system is totally inhibited compared with the control. Following exposure to gamma radiation, hypocotyl lengths was significantly inhibited over 50Gy.

For crop grains related saved settings, low-dose irradiation will not influence germination rate and will not totally inhibit plant growth and plant development (up to 400 - 450 Gy), whereas higher dose ionizing irradiation will induce apparent plant morphological aberration and deterioration, which will be more accentuated along dose gradient and can lead even to lethal and sub-lethal effects (500 Gy and higher).

Shoot and root length: In this examination, effect ionizing irradiation dose variation on root and shoot length was adverse and inhibitory as is evident from data in the table 1. Lentil seeds exposed to higher doses produced dwarf plants with considerably reduced and inhibited roots. This inhibitory effect of gamma rays on shoot and root length of lentil plants was even more acute for high doses (Table 1).

The research carried out by Shakoor and collaborators (1978) and Khalil and collaborators (1986) [18,19] assigned declined root and shoot lengths at higher doses of ionizing irradiation to attenuated mitotic activity in meristematic tissues and regressed moisture contents in seeds respectively. Lessen grow in root and shoot lengths of a number of crops has previously reported by Thimmaiah., *et al*. (1998) [20].

Photosynthetic pigment content: Chlorophyll contents of *L. culinaris* analyzed at 28 days after planting were found to be sig-

	7th day		14th day	
Dose (Gy)	**Root length (cm)**	**shoot length (cm)**	**Root length (cm)**	**shoot length (cm)**
0	1,8 ± 0,11	3,6 ± 0,19	4,96 ± 0,27	7,6 ± 0,24
50	1,77 ± 0,025	2,14 ± 0,27***	4,24 ± 0,42**	7,06 ± 0,83*
100	1,73 ± 0,04*	1,12 ± 0,08***	4 ± 0,34**	5,73 ± 0,51***
150	1,6 ± 0,02**	0,6 ± 0,09***	3,24 ± 0,37***	4,8 ± 0,32***
200	0 ± 0,00***	0 ± 0,00***	2,61 ± 0,43***	5,81 ± 0,19***
250	0 ± 0,00***	0 ± 0,00***	3,11 ± 0,13***	5,25 ± 0,26***
300	0 ± 0,00***	0 ± 0,00***	2,29 ± 0,34***	5,26 ± 0,54***
350	0 ± 0,00***	0 ± 0,00***	1,79 ± 0,36***	5,6 ± 0,22***
400	0 ± 0,00***	0 ± 0,00***	1,45 ± 0,43***	5,68 ± 0,22***
450	0 ± 0,00***	0 ± 0,00***	1,25 ± 0,24***	4,86 ± 0,13***
500	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***
600	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***
700	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***
800	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***
900	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***
1000	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***	0 ± 0,00***

Table 1: Effects of ionizing irradiation on root and shoot mean length (cm) of *L.culinaris* at 1, and 2 weeks days after planting (DAP) Data are the means of three replicates ± SD / *Significant difference (P < 0, 05), **highly significant difference (P < 0, 01), ***extremely significant difference (P < 0,001).

nificantly ($P \leq 0.05$) affected by various levels of gamma irradiation doses. Figure 2 presents the content of assimilatory pigments in 28-days-old lentil leafs, both for the control and irradiated samples. The statistical analysis illustrated that chlorophyll a level had significantly enhanced in lentil plant leaves acquired from dry lentil seeds exposed at doses between 50 and 500 Gy. Compared with the control, the chlorophyll (a+b) content increased by 37.26% at 50 Gy and the maximum increase was recorded at 200 Gy of 44.22%. A remarkable decline in chlorophyll 'a' was observed in plantlets subjected to doses over than 500Gy (not showed results).

Figure 2: Effect of irradiation dose variation on the content of photosynthetic pigments in 28 days lentil leaf (Each bar represents Mean ± SD [Standard deviation] for average of n = 3 independent experiments).

Several reports on other crops such as, *Vigna radiata L.* [21], *Medicago sativa L.* [22], *Oryza sativa L.* [23], *Abelmoschus esculentus* [24], *Paulownia tomentosa T.* [25], *Capsicum annuum L.* [26] demonstrates that chlorophyll contents increases at lower doses, which can be correlated with photosynthesis stimulation, and decreases at higher level of gamma irradiation [27]. It was demonstrated that low-intensity Magnetic field and gamma irradiation application increased protein and chlorophyll levels in onion plant [28], and it increased chlorophyll a, b and total chlorophyll levels in Paulownia.

Significant increase in total phenolic contents was caused by applied doses of γ-irradiation (Table2). The maximum increase of this parameter was observed at dose of 1 kGy (2.83 times for total phenols content compared to control test). Guo., *et al*. (1997) revealed that augmentation in total phenolic content was of value for antioxidant properties of seeds due to polymerization of phenolic constituents and also cross-linking and fragmentation, which were the fundamental reactions controlling the properties of macromolecules such as proteins [29].

DPPH radical scavenger capacity (DPPH RSC) was increased corresponding to dose of γ-irradiation (up to 5.71 times). Maximum was observed at the highest irradiation dose (Table 2). Polynomial relationship between DPPH RSC and γ-irradiation dose ($y = 0.564x2 -1.31 x+54.593$; $R^2 = 0.94584$. It was also reported that γ-irradiation doses up to 5 kGy enhanced DPPH RSC of soybean butanolic extracts [30]. DPPH RSC is the measure of non-enzymatic antioxidant activity. Higher levels of DPPH antiradical activity were in correlation with no enzymatic antioxidants, especially plant polyphenols [31] what was also in agreement with results given in table 2.

Dose Gy)	Total polyphenol content (mg Gallic acid/100g leaves)	Antioxidant activity SAA (µg AAE mg^{-1}GAE)
0 (control)	1.91 ± 0.14^{d}	58.235 ± 6.22^{e}
50	1.85 ± 0.11^{d}	57.227 ± 5.28^{e}
100	1.95 ± 0.04^{d}	57.944 ± 1.45^{e}
150	1.75 ± 0.07^{d}	53.434 ± 3.22^{e}
200	2.29 ± 0.20^{c}	54.075 ± 6.19^{e}
250	2.23 ± 0.16^{c}	64.175 ± 4.92^{d,e}
300	2.53 ± 0.16^{c}	72.235 ± 7.38^{d}
350	2.28 ± 0.11^{c}	85.25 ± 7.39^{d}
400	3.53 ± 0,20^{c}	82.432 ± 7.28^{d}
450	4.40 ± 0.24^{b}	83.333 ± 6.22d^{c}
500	4.50 ± 0.20^{b}	128.41 ± 21.5^{b}
600	5.34 ± 0.20^{b}	119.89 ± 2.83^{c}
700	5.28 ± 0,11^{b}	139.72 ± 26.5^{a,b}
800	7.6 ± 0,40^{a}	155.53 ± 2.53^{a}
900	9.3 ± 0,20^{a}	161.99 ± 58.0^{a}
1000	10.62 ± 0,40^{a}	165.24 ± 3.39^{a}

Table 2: Effects of gamma radiation dose on Total polyphenol content and Antioxidant activity in *L.culinaris.* Values are means of at least four replications ± standard deviation. Numbers succeeded by the same letter are not significantly different.

Conclusion

Results of this study reveal that higher doses of gamma irradiation exhibited strong detrimental effects on final germination percentage, germination index, root length and shoot length and pigments content of L. culinaris. However, higher irradiation dose (greater than 500Gy) resulted in significant increase in total polyphenols contents and antioxidant activity. Likewise, the DPPH radical scavenging activity indicated that lentil plants issued from treated seeds with gamma rays possessed excellent antioxidant properties. Our results indicate that the mutagen dose which killed half of the tested population (LD50) was 400 Gy for L. culinaris, so we recommended that 350 - 400 Gy should be enough to obtain lentil mutants for main treatment in a breeding program for resistance to *Stemphylium botryosum* which is our main goal in the furtherance of our project.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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