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GERMINATION, GROWTH AND YIELD PERFORMANCE OF FLAX (*LINUM USITATISSIMUM* L.) UNDER GAMMA IRRADIATION STRESS

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ABSTRACT. Gamma irradiation is a widely manipulated mutation breeding approach in agriculture for producing crops with desired agronomic traits. The technique is particularly advantageous to conventional breeding methods because of minimal labor and time requirement. Under laboratory and field experiments during 2013, seeds of *Linum usitatissimum* L. were irradiated with 2, 4, 8, 12, 16, 20, 24, 28 and 32 krad of gamma irradiation doses from Co-60 source for evaluating their effects on germination, seedling survival, radicle and plumule lengths, vegetative growth and productivity. It was noted that radiation doses caused significant changes in the studied traits of test plant. Germination, radicle and plumule lengths in lab study while shoot length, number of leaves and leaf area, number of fruits plant^{-1}, number of seeds fruit^{-1}, husk weight fruit^{-1}, number of branches plant^{-1}, fresh and dry biomass and moisture content of shoots under pot culture varied significantly under the applied radiation stress. In general, radiation doses up to 8 kr had stimulatory effects on the studied parameters while doses exceeding 8 kr negatively influenced germination, growth and productive attributes of flax. Results observed both stimulatory and inhibitory effects of the irradiation doses. The study suggests that radiation doses above 32 krad induced lethal effects on general growth of flax.

Keywords: mutation; metabolic abnormalities, free radicals; nuclear technology; agricultural productivity.

INTRODUCTION

Ionizing radiations are commonly applied in crop breeding because of their fewer disposal problems, simple use, high penetrability, greater mutagenic potentials and good reproductibility

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(Chahal & Gosal, 2002; Majeed *et al.,* 2017). Gamma irradiation technique is one of the most important methods used to create mutants with desired traits of agricultural significance (Sato *et al*., 2006). Gamma rays are powerful ionizing radiations, which interact with atoms/molecules of exposed plant materials for producing free radicals, highly reactive oxygen species in cells (Majeed *et al.,* 2017). Irradiated induced free radicals and reactive species of oxygen are capable of damaging or modifying the important cell components of the plant, which may potentially affect anatomical, biochemical, morphological, developmental and physiological traits of plants (Wi *et al*., 2005). The use of radiation to cause mutations or changes in genetic makeup has a lot of positive impacts on possible varietal development. This technique contributes to increased genetic diversity. Induced mutations in response to gamma irradiation have been widely used for the improvement of plants.

Flax (*Linum usitatissimum* L.) is an important plant cultivated for its seeds and fibers. Gamma irradiation effects on various variables of different plants, such as *Cicer arietinum* (Khan & Hussain, 2000), alfalfa (Fan *et al.,* 2004), *Foeniculum vulgare* (Zeid *et al.,* 2001), *Allium cepa* L. (Amjad & Anjum, 2002), *Vigna sesquipedalis* (Kon *et al.,* 2007), *Trigonella* (Al-Rumaih & Al-Rumaih, 2008), and *Ephorbia pulcherima* (Su *et al.,* 2009) have been widely studied; however, little is known about such studies on *Linum usitatissimum*. Therefore, this study was undertaken to study the effect of gamma irradiation on germination, plant growth and yield of *L. usitatissimum*.

MATERIALS AND METHODS

Plant materials and irradiation

Seeds of *L. usitatissimum* L. were obtained from local seed center in Peshawar, during 2013. Collected seeds were irradiated with nine different doses, *viz*. 0, 2, 4, 8, 12, 16, 20, 24, 28 and 32 krad (kr) of gamma rays from a Co^{60} source at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar. Non-irradiated seeds were used as a control.

Laboratory bioassay

Seed bioassay was performed in Botany Department, University of Peshawar. Fifty Petri dishes of equal size (9 cm diameter) were taken, washed with tap water and sterilized in an oven at 120^{o}C. The filter paper was cut according to the size of Petri dishes. Two folded filter paper was placed in each Petri dish and was used as a seedbed. Each treatment was replicated five times; 10 seeds were placed in each Petri dish. An equal amount of water (5cc) were provided to each Petri dish. Petri dishes were placed in an incubator at 25^{o}C. Data for germination %, plumule length (mm) and radicle length (mm) was noted after 96 hrs of incubation.

Pot experiment

Before sowing the seeds in pots, the soil was prepared. Clay, sand, and organic matter were mixed in the ratio of 3:2:1, respectively. Pots of equal sizes (23 cm diameter) were selected. For each treatment, there were three replicates. Pots were kept in the net house of the Botany Department University of Peshawar under natural environmental condition of branches/plant, average leaf size/plant and no. of fruits/plant, fresh weight of roots, shoots and fruits/plants, no. of seeds and husk weight/fruits and moisture contents of roots, shoots, and fruits.

Statistical analysis

The raw data was transferred into means for statistical analysis. The data were statistically tested by applying ANOVA and complete randomized design (CRD) using the MSTAT-C software.

RESULTS

Germination

Data for germination (%) showed significant differences for various doses of gamma irradiation in laboratory and pot experiments. In lab assay, maximum (94.0%) germination was recorded at 2 kr, followed by 4 kr (92.0%) and control (90.0%), respectively. The minimum germination (74.0%) was recorded at the dose of 32 kr. Low doses increased germination percentage and high doses decreased germination percentage (*Fig. 1*).

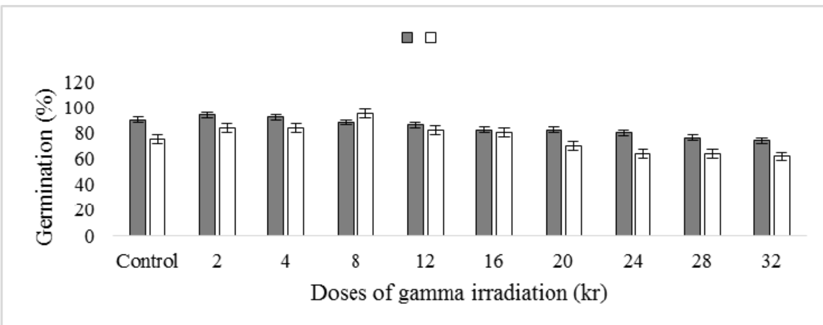

Figure 1 - Effect of different doses of gamma irradiation on germination of flax

Radicle and plumule growth

Results demonstrated that radicle and plumule growth was significantly influenced by applied doses of gamma irradiation. Maximum radicle length (16.3 mm) was recorded at 8 kr, while minimum (7.2 mm) was found at 32 kr. Compared to control, low doses increased radicle length. Similarly, maximum plumule length (16.8 mm) was noted for 2 kr and minimum plumule length (7.2 mm) was recorded at 32 kr. Stimulatory responses at low, but inhibitory effects on the studied attribute were found at higher doses of radiation (*Fig. 2*).

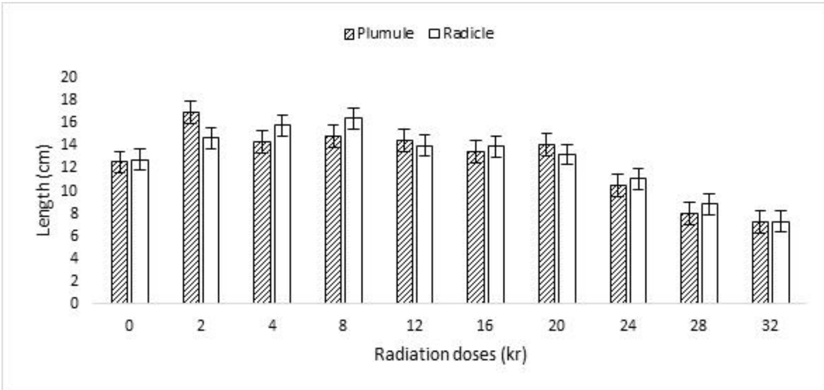

Figure 2 - Radicle and plumule length of flax as affected by gamma irradiation

Shoot length and no. of branches plant^{-1}

Shoot length was significantly affected by applied doses of gamma irradiation. Statistical analysis showed that maximum height (50.3 cm) was noted at 4 kr, followed by 8 kr (47.6 cm), 12 kr (45.9 cm), 20 kr (44.9 cm) and 4 kr (44.5 cm), while reduced shoot length was observed at 32 kr (38.5 cm) and 28 kr (40.4 cm) respectively. Highest applied dose (32 kr) resulted in a remarkable reduction of plant height, while lower doses increased it when compared to control. No. of branches per plant was also significantly affected. Mean values, which were statistically analyzed, revealed that maximum branches (2.83) were present at control, followed by 2 kr (2.80), 4 kr (2.73), 12 kr (2.57), while minimum (1.87) were observed at 32 kr. Generally, all doses decreased the number of branches/plant (*Table 1*).

No. of leaves/plant and leaf area

Among various treatments of gamma irradiation, significant differences were observed for leaf number and leaf area. Least significant difference test showed that maximum leaves/plant (157.7) were present at control, which is statistically followed by 2 kr (151.5) and 20 kr (134.4), while minimum (115.1) leaves were observed at 8 kr. Mean values showed that maximum leaf area/plant (0.58 mm^2) was present at 2 kr, which was followed by 4 kr (0.54 mm^2) and 8 kr (0.51 mm^2), respectively, while minimum leaf area (0.39 mm^2) was recorded at 32 kr. Generally, low doses increased leaf area/plant, but high doses decreased the studied parameter (*Table 1*).

Table 1 - Shoot length, no. of branches, no. of leaves and leaf area (mm^2) of *Linum usitatissimum* L. as influenced by gamma irradiation doses

Treatments	Plant height (cm)	No. of branches	No. of leaves	Leaf area (mm^2)
Control	41.27 ^{efg} (100)	2.83 ^{a} (100)	157.70 ^{a} (100)	0.420 ^{e} (100)
2 kr	41.83 ^{defg} (101.35)	2.80 ^{ab} (98.93)	151.50 ^{b} (96.06)	0.577 ^{a} (137.38)
4 kr	50.33 ^{a} (121.95)	2.73 ^{ab} (96.46)	117.10 ^{e} (74.25)	0.540 ^{ab} (128.57)
8 kr	47.57 ^{ab} (115.26)	2.50 ^{abc} (88.33)	115.10 ^{e} (72.98)	0.510b ^{c} (121.42)
12 kr	45.77 ^{bc} (110.90)	2.56 ^{abc} (90.45)	131.10 ^{c} (83.13)	0.510 ^{bc} (121.42)
16 kr	44.53 ^{bcd} (107.89)	2.46 ^{abc} (86.92)	129.50 ^{cd} (82.11)	0.450 ^{de} (107.14)
20 kr	44.90 ^{bcd} (108.79)	2.36b ^{c} (83.39)	134.20 ^{c} (85.09)	0.427 ^{de} (101.66)
24 kr	43.27 ^{cdef} (104.84)	2.26 ^{cd} (79.85)	134.40 ^{c} (85.22)	0.400 ^{e} (95.23)
28 kr	40.40 ^{fg} (97.89)	2.16 ^{cd} (76.32)	133.40 ^{c} (84.60)	0.400 ^{e} (95.23)
32 kr	38.53 ^{g} (93.36)	1.86 ^{d} (65.72)	124.40 ^{d} (78.88)	0.397 ^{e} (94.52)

LSD value at alpha = 5% for plant height = 3.382, no. of branches = 0.4571, no. of leaves = 5.397 and leaf size = 0.05425.

No. of fruits/plant, no. of seeds/fruit

Table 2 lists data regarding no. of fruits and seeds. Average numbers of fruits/plant were significantly affected by different doses of gamma irradiation. The least significant test showed that maximum fruits/plant (17.7) were present at 20 kr, which is statistically followed by 16 kr (16.1) and 12 kr (15.4), while minimum (10.8) were observed at 32 kr. Medium doses increased fruit/plant significantly and both lower and high doses decreased no. fruits/plant. Results documented that maximum (8.4) seeds/fruit were present at 12 kr, which was followed by 0 kr (8.1) and 4 kr (8.0), while minimum (4.9) were observed at 20 kr. All doses decreased seeds/fruit (*Table 2*).

Seeds and husk weight/fruit (g)

The analysis of variance (ANOVA) demonstrated that both seed and husk weight varied significantly under different doses. Highest seed weight (0.045 g) was present at 12 kr, which was statistically followed by control (0.044 g), 4 kr (0.039 g) and 8 kr (0.038 g), while minimum (0.023 g) was observed at 32 kr. Maximum husk weight (0.019g each) were present at 12 kr and control, which is statistically followed by 20 kr (0.017g), 24 kr (0.016g), while minimum (0.012 g) was observed at 8 kr (*Table 2*).

Table 2 - No. of fruits/plant, no. of seeds/fruit, seeds weight/fruit (g) and husk weight/fruit (g) of *Linum usitatissimum* L. under radiation exposure

Treatments	No. of fruits/plant	No. of seeds/fruit	Seeds weight/fruit	Husk weight/fruit
Control	12.43 de (100)	8.10 ab (100)	0.043 a (100)	0.0187 a (100)
2 kr	12.23 de (98.39)	5.43 cd (67.03)	0.034 d (79.06)	0.0160 bc (85.56)
4 kr	12.70 de (102.17)	7.79 ab (96.17)	0.039 b (90.70)	0.0147 cd (78.60)
8 kr	13.39b cd (112.04)	5.53 cd (68.27)	0.038 b (88.37)	0.0123 e (65.77)
12 kr	15.37 abc (123.65)	8.36 a (103.20)	0.044 a (102.23)	0.0193 a (103.20)
16 kr	16.13 ab (129.76)	6.68 bc (82.46)	0.036 c (83.72)	0.0133 de (71.12)
20 kr	17.70 a (142.39)	4.94 d (60.98)	0.029 e (67.44)	0.0167 b (89.30)
24 kr	13.43 cd (108.04)	6.23 cd (76.91)	0.033 d (76.74)	0.0163 bc (87.16)
28 kr	11.97 de (96.29)	5.50 cd (67.90)	0.024 f (55.81)	0.0157 bc (83.95)
32 kr	10.83 e (87.12)	5.00 d (61.72)	0.023 f (53.48)	0.0160 bc (85.56)

LSD value at alpha = 5% for fruits/plant = 2.592, no. of seeds = 1.442, seeds weight = 0.001715 and husk weight per fruit = 0.001715.

Fresh and dry biomass of root per plant (g)

Significant differences were observed for root fresh weight and dry weight (g) by applying various doses of gamma irradiation. The least significant test showed that maximum fresh weight (0.29 g) was noted at 24 kr, which is statistically followed by 2 kr (0.27 g), 20 kr, 28 kr (0.26 g each), while minimum weight (0.23 g) was observed at 4 kr. All doses, except 4 kr, increased root fresh weight/fruit. Results showed that at 24 kr maximum (0.25 g), shoot dry weight (g) was present, which is statistically followed by 20 kr (0.23 g) and 2 kr (0.22 g), while minimum weight (0.19 g) was observed at control. The results showed that all doses significantly increased dry weight, as compared to control (*Table 3*).

Root moisture contents/plant (%)

It was found from the data that significant differences were present for various doses of gamma irradiation on moisture content. Maximum moisture content (25.3%)

was present at control, which is statistically followed by 8 kr (22.7%) and 28 kr (20.3%), while minimum moisture content (6.1%) was observed at 4 kr. Generally, all doses decreased moisture content, but low doses have pronounced effect, as compared to other doses (*Table 3*).

Table 3 - Response of root fresh and dry weight/plant (g) and moisture contents/plant (%) of *Linum usitatissimum* L. to applied doses of gamma irradiation

Treatments	Fresh weight/plant	Dry weight/plant	Moisture contents/plant
Control	0.233 [g] (100)	0.187 [h] (100)	25.20[a] (100)
2 kr	0.267 [b] (114.59)	0.223 [c] (119.25)	9.70 [d] (38.40)
4 kr	0.230 [h] (98.71)	0.217 [d] (116.04)	6.08 [d] (24.06)
8 kr	0.253 [d] (108.58)	0.207 [g] (110.69)	22.70 [ab] (89.86)
12 kr	0.250 [e] (107.29)	0.210 [f] (112.29)	19.07 [abc] (75.49)
16 kr	0.250 [e] (107.29)	0.210 [f] (112.29)	19.30 [abc] (76.40)
20 kr	0.257 [c] (110.30)	0.227 [b] (121.39)	13.17 [cd] (52.13)
24 kr	0.290 [a] (124.46)	0.253 [a] (135.29)	14.46 [bcd] (57.24)
28 kr	0.257 [c] (110.30)	0.213 [e] (113.90)	20.34 [abc] (80.52)
32 kr	0.237 [f] (101.71)	0.217 [d] (116.04)	9.26 [d] (36.65)

LSD value at alpha = 5% for roots fresh weight = 0.001715, dry weight = 0.001715 and moisture content = 9.087.

Shoot fresh and dry weight/plant (g)

Maximum root fresh weight (0.94 g) was present at 12 kr, which is statistically followed by 16 kr (0.90 g), 2 kr (0.87 g), while minimum (0.63 g) was observed at 32 kr. Higher doses uniformly decreased shoot fresh weight/fruit. Least significant difference test showed that maximum root fresh weight (0.84 g) was present at 12 kr, which is statistically followed by 16 kr (0.80 g), 2 kr (0.76 g) and 24 kr (0.75 g), while minimum (0.57 g) was observed at 4 kr. Results showed that both low and higher doses decreased the dry weight of root/plant, while medium doses increased shoot dry weight (*Table 4*).

Shoot moisture contents/plant (%)

Significant differences were noted for various doses of gamma irradiation affecting shoot moisture content/plant. Mean values revealed that maximum moisture contents (42.7%) were present at 12 kr, which is statistically followed by control (24.4%) and 8 kr (21.6%), while (4.9%) minimum was observed at 32 kr. Results showed that all doses significantly decreased moisture content in shoots (*Table 4*).

Table 4 - Shoot fresh and dry weight/plant (g) and shoot moisture contents/plant (%) of *Linum usitatissimum* L. as affected by gamma irradiation

Treatments	Fresh weight/plant	Dry weight/plant	Moisture contents/plant
Control	0.837 abc (100)	0.673 bcd (100)	24.44 b (100)
2 kr	0.867 abc (103.58)	0.757 abc (112.48)	42.72 a (174.79)
4 kr	0.813 abc (97.13)	0.573 d (85.14)	20.00 bc (81.83)
8 kr	0.770 bcd (91.99)	0.637 cd (94.65)	21.55 bc (88.17)
12 kr	0.943 a (112.66)	0.843 a (125.26)	15.01 bcd (61.41)
16 kr	0.900 ab (107.52)	0.803 ab (119.31)	12.82 cd (52.45)
20 kr	0.833 abc (99.52)	0.683 bcd (101.48)	11.90 cd (48.69)
24 kr	0.763 abc (91.15)	0.747 abc (110.99)	11.42 cd (46.72)
28 kr	0.727 cd (86.85)	0.637 cd (94.65)	6.57 d (26.88)
32 kr	0.633 d (75.62)	0.603 d (89.59)	4.89 d (20.00)

LSD value at alpha = 5% for shoot fresh weight = 0.1534, dry weight = 0.1329 and moisture content = 11.56.

DISCUSSION

The results showed that low dosed increased germination percentage and higher dosed decreased germination percentage, as compared to control. The increase in germination with radiation doses was recorded for *F. vulgare* L. (Zeid *et al.,* 2001), while a decrease in germination percentage under irradiation treatment has been found in alfalfa (Fan *et al.,* 2004), and groundnut (Mokobia & Anomohanran, 2005), hence negate the present work. The increase in germination may be due to the increased membrane permeability or changes in cellular oxidation potentials of membranes, which may correspond to breaking seed dormancy (Wang & Yu, 2000; Majeed *et al.,* 2016).

Plant height was significantly affected by gamma irradiation. The result showed that lower doses increased plant height and higher doses decreased plant height. The maximum plant height (50.3 cm) was recorded at 2 kr. The irradiation favored plant height. The increase in plant height with radiation was also found by Nassar *et al.* (2004) (chamomile) and Asmahan & Nada (2006) (tomato hybrid). The increase in plant height is due to poor translocation of both macro and micronutrients to reproductive parts due to damage of gamma irradiation, as a result these are stored in the stem and thus promote plant height (Singh & Data, 2010; Majeed *et al.,* 2017). Similarly, the number of branches decreased significantly by various doses of gamma irradiation, which are in agreement with previous studies carried out for chickpea genotypes (Khan *et al.,* 2005). Our results regarding number of leaves, leaf area, 50% flowering, number of fruits, seeds/fruit, root and shoot fresh and dry weight are in agreement with studies of Widiastuti *et al.* (2010), Muthusamy & Jayabalan (2011) and Majeed *et al.* (2016), who documented similar results in *L. sativum*, *Garcinia mangostana, Gossypium hirsutum* and *Pisum sativum*, respectively. The decrease in the studied parameters at higher doses, while stimulation at lower doses of gamma irradiation might be due to premature abscission, enhanced production of ethylene or inhibition of auxin activity after gamma irradiation or hermetic responses of the tested plant to vital metabolic and enzymatic activities (Majeed *et al.,* 2016, Majeed, 2017)

CONCLUSION

It is concluded from the results of this study that lower doses (2-8 kr) had stimulatory effects on some parameters, such as germination, radicle, and plumule length, while higher doses > 8 kr significantly reduced germination, growth, flowering and productive traits of flax under lab and pot experiments. The highest dose (32 kr) particularly was found lethal in effects on the studied parameters of the tested plant. Thus, doses of gamma irradiation exceeding 8 kr are not promising for inducing positive mutations in flax.

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