Research Article

Gamma radiation induced mutation in M2 generation of Pea (*Pisum sativum* L.)

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Abstract

Distinctive measurements of gamma irradiations (6, 7, 8, 9, 10Krad and zero dosage as control) were used to assess different morphological and proximate parameters of *Pisum sativum*. An examination of the after effects of various treatments with control demonstrated that gamma radiations altogether influenced diverse morphological and proximate parameters. Days to germination stayed same when contrasted with control however 7Krad indicated least days to germination. Germination rate and seedling survival rate was 100 percent both in control and the irradiated seeds. Measurements of 7Krad took least days for bloom start when contrasted with control and different dosages. Flower initiation happened before in 7Krad as compared to control. Natural product start and organic product development was additionally before in 7Krad when contrasted with control. Plant tallness was fundamentally expanded in 10Krad when contrasted with control. In 7Krad number of pods per pot was high when contrasted with control. Pod length decreased in all measurements yet high pod length was seen in control. Greatest number of seeds per pod was recorded in control. Weight of 1000 seeds was high in 6Krad when contrasted with control. Proximate analysis i.e. ash demonstrated greatest sum in 9Krad when contrasted with control. Moisture was likewise high in 9Krad. Proteins were most extreme in 7Krad when contrasted with control. High fats were acquired in 8Krad as compared to control. Subsequently the ash, moisture, proteins, fats were expanded essentially with higher gamma radiation when contrasted with control.

Keywords: Biochemical parameter; Gamma irradiation; Morphological response; *Pisum sativum* L.

Introduction

Pisum sativum is an annual, self-pollinated and often climbing herb. It belongs to family Fabaceae. The expression "Pea" can be cited to little circular seed or to the pod. The name "Pea" is likewise used to depict other consumable seeds from the Fabaceae, for example, chickpeas (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), cow pea (*Vigna unguiculata*) and sweet pea (*Lathyrus* spp.) which are developed as

ornamentals. Starting point of pea is likely South Western Asia, conceivably Northwestern India, Pakistan and couple of contiguous territories of previous USSR and Afghanistan however, from there on spread to Europe [1] based on genetically assorted variety, four centers of beginnings, in particular, Abyssinia, Central Asia, the Near East, and the Mediterranean have been recognized [2]. Pea (*Pisum sativum* L.) is one of the world oldest agricultural

products. Archeological confirmation dates the presence of Pea back to 10000 B.C. in Near East and Central Asia. Pea is a feeble plant. It has ringlets which can help in help. It is made out of rachis finishing in a spread ringlet. Its stem is feeble, glabrous, interchange leaves, and terminal stretched ringlets, leaflets ovate or elliptic. Pea is one of the main developed harvest around 7000-6000 BC and is thought to have started from south-western Asia. Pea develop in an extensive variety of condition. Pea develop better in moderately cool atmospheres with normal temperatures in the vicinity of 7 and 24°C and in territories with 800-1000 mm yearly precipitation [3]. They can be found on a wide range of soils from sandy loams to heavy clays, provided the soil is well drained. The perfect pH of the soil is 5.5-6.5. A pH of 7-7.5 cannot hinder the development if the soil is not over limed and inclined to manganese shortage [4]. Acidic soils, high aluminum soils and waterlogged regions are injurious to pea development [3]. Hot climate and dry season pressure are especially harming to peas blooming time frame [4]. It is on rank fifth regarding significance nourishment vegetables in Turkey. It is rich in proteins, starch and absorbable supplement substance yet low in fiber substance which make it an amazing domesticated animal's feed. It is especially an essential vegetable grain in temperate regions with various nutrition (dry seed, vegetable) and nourishment (seed, feed) utilizations [5]. In the United States 550,000 MT are delivered commercially for sustenance every year and 200,000 MT of field pea for feed [6]. Pea is a high-yielding, short period crop with high proteins content. Pea is one of the prevalent trade crop in the world exchange and sum to around 40% of the aggregate exchange of all pulses [6]. Legumes are significant sources of sugars, dietary fibers, vitamins, minerals and proteins (17-40%) higher than grains (7-13%) however, circumstantially equivalents to the protein in meat (18-25%) [7-9] green and ripe fruits and seeds contain starch, proteins, oil,

galactolipids, alkaloids, trigonelline and pipartine, essential oil and dissolvable sugars [10]. Cis-trans and Trans, trans-xanthoxin found in roots [11]. Seeds yield trypsin and chymotrypsin. 100 grams of consumable part of crisp sweet pea pods contain: 67 kcalories, water 82.4 g, protein 3g, fat 0.4g, sugar 12.8g, dietary fiber 2.1g, ash 1.4g, calcium 92mg, phosphorus 48mg, iron 1.2mg, vitamin-A 52.0µg, thiamin 0.16mg, riboflavin 0.09mg, niacin 1.0mg, ascorbic acid 67.0mg. Leaf, petiole, tendril, and stems yielded kaempferol-3-triglucoside, quercetin-3-triglucoside and their p-coumaric esters. Growing pea seedlings yield high amount of D-alanine. Free homoserine has been identified in the seeds and pods.

Mutation breeding is potential, fast and profitable way for bringing quantitative and qualitative variability in crop plants. Mutation breeding is a process by which a gene undergoes a structural change or exchange of one nucleotide for another. Numerous crops with enhanced qualities have been obtained utilizing induced mutation [12]. Mutagenic operators, for example chemicals, x-rays, electron beam light, γ-beams and so on are typically used to induce mutation artificially. This procedure produces mutants [13, 14] which could then facilitate the identification, isolation and cloning of genes for designing crops with quality yield and other quality attributes [15]. Crop mutation to modify their genetic makeup and look for desired changes to enhance the yield potential and certain fascinating characters is a typical component of research work everywhere throughout the world. There are distinctive sorts of ionizing radiation (viz., X beams, gamma beams, protons, neutrons, alpha and beta particles) yet gamma beams are generally utilized for genetic transformation as they have shorter wave length and have more energy per photon than X beams and infiltrate profound into the tissue [16, 17]. Other than the financial advantages, a few mutants additionally assume an imperative part in the

investigation of hereditary qualities and plant advancement. A few positive changes have been made in farming yields by utilizing gamma illuminations. Yields have effectively been produced with enhanced attributes by mutagenic acceptances [18, 19]. Gamma illumination has drawn the consideration as a fast and new technique to enhance the subjective and quantitative characters of numerous yields. Gamma light has been utilized widely as a powerful physical mutagenic specialist [20]. For the modification of physiological characters' gamma radiation can be valuable [21]. Gamma rays are known to impact plant development and advancement by affecting cytological, morphogenetic changes in cells and tissue [22].

Materials and methods

The M2 seeds of pea plant were chosen for the current investigation. These seeds were obtained from M1 plants in 2013-14. Gamma irradiation utilized was created from the Cobalt-60 source, at Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan. Pea seeds (M0) were irradiated with 6, 7, 8, 9, and 10 Krad while non-irradiated seeds of every variety were kept as control. An investigation was executed in 2013-2014; M0 plants brought about M1 seeds which were subjected to analyze in the present examination. This investigation was planned to assess the impact of gamma beams in M2 generation.

Field Experiment

A field experiment was completed in greenhouse, Department of Botany, Islamia College Peshawar, in 2014-2015. The M2 Seeds of each dosage were sown on 21 November 2014 in pots; all pots were similarly separated with parallel soil substance in each pot. Total pots were thirty in number. The design was totally random with each measurements having 5 replicas. Equal number of seeds were sown in all pots. The pots were checked frequently for water requirements.

Parameters

The parameters i.e. germination rate, days to germination, flower initiation, flower

maturation, seedling survival rate, fruit initiation, fruit maturity, number of pods per plant, pod length, number of seeds per pod, plant stature, 1000 seed weight, moisture rate, ash, fats and proteins were considered in this investigation.

Statistical investigation

Exploratory information was factually examined with the Analysis of variance (ANOVA) and least significance difference (LSD) at $\alpha = 0.05$ utilizing Statistics 10.0 programming.

Proximate investigation

Proximate investigation of seeds was done at National Institute for Food and Agriculture (NIFA) Peshawar, Pakistan.

Results and discussion

Gamma irradiation, being known for its mutagenic effect also showed pronounced effect on M2 generation of Pea. Pea variety responded to gamma rays in M2 generation. Following results explore the findings of present investigation.

Days to germination

Table 1 represent the effect of gamma irradiation on days to germination of pea plant in M2 generation. The results show that days to germination were significantly affected by gamma irradiation. Days to germination decreased significantly at 7Krad (13 days) followed by 6 Krad, 8Krad, 9Krad, and 10Krad (14 days in each) as compared to control (15 days). Some researchers found that gamma irradiation causes faster seed germination. This is probably due to the fact that shortwave photons (i.e. gamma rays) are more energetic than visible light photons (> 400 nm) and, therefore, have a stronger effect on the surface of the plant cells. This causes the final breakdown of the seed coat allowing germination to occur [23]. Similar results were found by [24].

Germination percentage

In (Table 1) the results revealed that the germination percentage was non-significantly affected by gamma irradiation. Germination percentage was kept maximum by all the doses as compared to control (100 %).

The stimulating effect of gamma rays on germination may be due to the activation of RNA or protein synthesis, which occurs during the initial stage of germination after irradiation of the seed [25]. Similar results were found by [25].

Seedling survival percentage

Table 1 represent the data which show the effect of gamma irradiation on seedling survival percentage of pea in M2 generation. Gamma irradiation kept the seedling survival percentage unchanged as compared to control (100 %). It can be concluded that gamma irradiation showed no effect on seedling survival percentage. [26] also observed similar results. The results of [27] have shown that survival of plants to their maturity depends on the nature and extent of chromosomal damage.

Flower initiation

Table 1 shows the effect of gamma irradiation on days to flower initiation of pea in M2 generation. The result show that gamma irradiation non-significantly affected days to initiation. Statistical results revealed that all the doses kept their rate parallel to control. The Days obtained are at control (92 days), 6Krad (93 days), 7Krad (90 day), 8 Krad (91 days), 9Krad (92 days) and 10Krad (90 days).

Flower maturation

Table 1 represents the effect of gamma irradiation on days to flower maturation of

pea in M2 generation. Non-significant effect of gamma irradiation was obtained in M2 generation on this temporal trait. Statistical results revealed that the results were non-significant. At control (95 days), 6Krad (96 days), 7Krad (94 days), 8 Krad (97 days), 9Krad and 10Krad (98 days each) were observed. Mutants with changes in flowering and maturity time have been reported by many workers because, generally due to radiation, flowering and maturity is late [28].

Fruit initiation and maturation

Data in (Table 1) represents the effect of gamma irradiation on days to fruit maturation of pea in M2 generation. At control (110 days), 6Krad (111 days), 7Krad (107 days), 8Krad (114 days), 9Krad (115 days) and 10Krad (118 days) result was found. Its proving that gamma irradiation delayed fruit initiation. Table 1 represent the effect of gamma irradiation on days to fruit maturation of pea plants in M2 generation. Fruit maturation was non-significantly affected. Increasing radiation delayed fruit maturation. Highest mean was obtained at 10Krad (146 days) followed by 9Krad and 8Krad (144 days and 142 days respectively) as compared to control (137 days), while lowest mean value was obtained at 7Krad (133 days), it can be concluded that gamma irradiation delayed fruit initiation and fruit maturation.

Table 1. Effect of gamma irradiation on flowers and fruits

Radiation doses	Flower initiation	Flower maturation	Fruit initiation	Fruit maturation	Days to germination	Germination percentage	Seedling survival percentage
Control	92 a	95 a	110 a	137 a	15 a	100% a	100% a
6Krad	93 a	96 a	111 a	141 a	14 ab	100% a	100% a
7Krad	90 a	94 a	107 a	133 a	13 b	100% a	100% a
8Krad	91 a	97 a	114 a	142 a	14 ab	100% a	100% a
9Krad	92 a	98 a	115 a	144 a	14 ab	100% a	100% a
10Krad	90 a	98 a	118 a	146 a	14 ab	100% a	100% a
LSD value at $\alpha=0.05$	10.551	4.7064	32.572	41.894	1.1315	0	0

Data are represented as Mean (n = 05). Means followed by different letter within the column are significantly different (P < 0.05). (ANOVA followed by Tukey LSD test).

Plant height (cm)

Table 2 shows the effect of gamma irradiation on plant height (cm) of pea

plants in M2 generation. Plant height was maximum at 10Krad (22.4 cm) followed by 8Krad (22 cm) as compared to control (21.4

cm), while the height was decreased significantly at 6Krad (19.6 cm) followed by 9Krad (20.8 cm) as compared to control. Non-significant effect was observed at 7Krad. Gamma rays induced reduction in plant height may be due to destruction or damage to apical meristem [29], hampered respiratory enzyme synthesis and reduction in the level of amylase activity [30] and to temporary suspension of cell division or delay in mitosis [31]. They mentioned that ionizing radiation causes inactivation of growth regulators that lead to delayed growth of plants. [32]. believe that the delay in height of the plant may be due to an increase in the production of active radicals that are responsible for lethality or due to the increase in gross structural chromosomal changes induced by radiation.

Number of pods/pot

Table 2 represents the data which show the effect of gamma irradiation on number of pods/pot of pea in M2 generation. Number of pods/pot decreased significantly at 6Krad (11.4 pods/pot) followed, by 10Krad (11.8pods/pot), and 8Krad (14.6pods/pot) while increased significantly at 7Krad (17.4pods/pot) as compared to control (15.2pods/pot). 9Krad showed non-significant effect. [33] screened out the high yielding mutants in chemical mutagens induced progeny of *Vigna radiata* and reported the increase in number of pods produced per plant and total seed yield at lower doses of chemical mutagens in *Vigna radiata*.

Table 2. Effect of gamma radiation on yield and yield components.

Radiation doses	Pod length (cm)	Number of pods/pot	Number of seeds per pod	1000 seeds weight	Plant height (cm)
Control	6.058a	15.2ab	4.2565a	180.04b	21.4ab
6Krad	5.38b	11.4b	4.235ab	200b	19.6b
7Krad	5.36b	17.4a	3.602b	169.155b	21.4ab
8Krad	5.242b	14.6ab	3.62b	179.028b	22ab
9Krad	5.266b	15.2ab	3.768ab	173.041b	20.8ab
10Krad	5.409b	11.8ab	4.081ab	169.13b	22.4a
LSD value at $\alpha=0.05$	0.6097	5.7887	0.7053	121.12	2.4652

Data are represented as Mean (n = 05). Means followed by different letter within the column are significantly different (P < 0.05). (ANOVA followed by Tukey LSD test

Pod length (cm)

Pod length showed a decreasing tendency with increasing radiation doses. Maximum mean value for this trait was observed at control (6.058 cm), the rest of the doses decreased pod length significantly showing that gamma irradiation decreased pod length at 8Krad (5.242 cm) followed by 9Krad (5.266cm), 7Krad (5.36cm), 6Krad (5.38cm) and 10Krad (5.409cm) as compared to control (6.058cm).

Number of seeds/pod

Table 2 represents the data which shows the effect of gamma irradiation on number of seeds /pod in M2 generation. Gamma irradiation showed inhibitory effect on number of seeds/pod. Highest mean value was obtained for control (4.2565 seeds/pod) and the rest of the doses decreased number of seeds/pod significantly i.e. at 7Krad (3.602 seeds/pod), followed by 8 Krad (3.62 seeds/pod), 9Krad (3.768 seeds/pod), 10Krad (4.081 seeds/pod) and 6Krad (4.235 seeds/pod) as compared to control (4.2565 seeds/pod).

1000 seed weight (gram)

Table 2 represents the effect of gamma irradiation on 1000 grains weight of M2 generation's pea seeds. 1000 grain seeds weight were maximum at 6Krad (200 gm) but decreased non-significantly i.e. at 7Krad (169.155 gm), 8Krad (179.028 gm), 9Krad (173.041 gm), and 10 Krad (169.13 gm) as compared to control (180.04 gm). [34] observed similar results in *Pisum sativum*.

Ash (%)

Table 3 represents the effect of gamma irradiation on ash content (%) on pea in M2 generation. Ash contents (%) at control were (2.25131 %), and increased at 9Krad (3.15442%), followed by 6Krad (2.94296%), 7Krad (2.969247%), 10Krad (2.878497%), and 8Krad (2.70219%).

Moisture (%)

Table 3 represents the data which show the effect of gamma irradiation on moisture content (%) of Pea in M2 generation. Moisture contents (%) decreased significantly at 8Krad (7.6790596 %) followed by 7Krad (8.9090546 %) as compared to control (9.45085972 %). Moisture content (%) showed non-significant change at 6Krad (9.66054 %), 9Krad (9.819458 %), and 10Krad (8.948604 %) as compared to control (9.45085972 %).

Protein contents (%)

Data in (Table 3) represent the effect of gamma irradiation on proteins content (%) of Pea in M2 generation. Statistical analysis show that proteins content (%) were increased non-significantly at 7Krad (34.895764%), followed by 8Krad (34.83348%), 10Krad (33.4612%), 6Krad (33.4257756%) and 9Krad (33.2878%) as compared to control (31.7690156%).

Fats (%)

Table 3 represents the data which show the effect of gamma irradiation on fats content (%) of Pea in M2 generation. Fats content (%) were increased significantly at 8Krad (7.48914 %), followed by 9Krad (6.28994 %), 10Krad (5.72059 %), 6Krad (5.17432 %) and 7Krad (5.10677 %) as compared to control (4.0029 %).

Table 3. Effect of gamma radiation on proximate analysis of *Pisum sativum*

Radiation doses	Protein %	Fats %	Ash %	Moisture %
Control	31.7690156 a	4.0029 d	2.25131 a	9.45085972 a
6Krad	33.4257756 a	5.17432 c	2.94296 a	9.66054 a
7Krad	34.895764 a	5.10677 c	2.969247 a	8.9090546 a
8Krad	34.83348 a	7.48914 a	2.70219 a	7.6790596 b
9Krad	33.2878 a	6.28994 b	3.15442 a	9.819458 a
10Krad	33.4612 a	5.72059 bc	2.878497 a	8.948604 a
LSD value at $\alpha=0.05$	5.1752	0.8949	22	0.9638

Data are represented as Mean (n = 05). Means followed by different letter within the column are significantly different ($P < 0.05$). (ANOVA followed by Tukey LSD test)

Conclusion

Gamma irradiation significantly affected morphological and proximate parameters in M2 generation of *Pisum sativum*. The most significant dose was 7Krad. Proximate parameters were significantly increased in higher doses of gamma irradiation as compared to control. It is concluded that lower dose (7Krad) can be used for agricultural purposes to improve yield attributes of Pea.

Authors' contributions

Conceived and designed the experiments: WM Khan, Performed the experiments: A Younas, Analyzed the data: N Akhtar, Contributed Seeds material: Z Muhammad, Wrote the paper: T Burni & N Umar.

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