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RESEARCH ARTICLE

Optimization of gamma irradiation doses (^{60}Co) for mutagenesis in strawberry (*Fragaria* x *annanasa* Duch) cv. Winter Dawn

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Abstract

Strawberry, a vegetatively propagated crop, exhibits limited genetic variation, which constrains its adaptability and improvement potential. Gamma-ray radiation is a promising approach for enhancing genetic diversity and improving strawberries. Strawberry runners of the Winter Dawn cultivar were irradiated using ^{60}Co, with doses ranging from 20 Gy to 40 Gy administered at a consistent rate of 1.52 Gy per min to evaluate mutagenic effects. The survival effects in gamma irradiation-treated runners were recorded at 30 days after transplantation. An LD_{50} analysis was conducted to assess physiological impacts and the lethal dose value was found to be 40 Gy, resulting in 50% mortality. Distinct differences in survival rates were observed between treated and untreated runners, with the highest survival rate (88%) at 20 Gy. The 30 Gy dose-treated runners exhibited the highest growth parameters, including crown diameter (14.32 mm), primary root length (29.50 cm), secondary root length (5.5 cm) and number of roots (39.6). Among fruit quality traits, maximum fruit length (39.84 mm) and fruit diameter (27.80 mm) were observed in runners treated with a 30 Gy gamma irradiation dose. Significant variations in survival, growth and fruit quality were observed across different gamma irradiation doses. These findings provide a foundation for future efforts to develop potential strawberry mutants with improved traits through gamma irradiation. These findings provide a foundation for future efforts to develop potential strawberry mutants with improved traits through gamma irradiation.

Keywords

^{60}Co; *Fragaria* × *annanasa*; gamma irradiation; mutagenesis; winter dawn

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.), a member of the Rosaceae and Fragaria family, is a perennial plant that produces red fruits. Known as the "Fruit Queen," it boasts exceptionally high nutritional content (1, 2). Efforts to address challenges in cultivation include introducing new, superior varieties with desired traits. Strawberry, being an octoploid plant that reproduces vegetatively, faces challenges due to its relatively low genetic diversity, making conventional breeding difficult (3). However, gamma rays are widely used as a physical mutagen globally. Induced mutation breeding using seeds, pollen and cuttings is a viable approach (4). This method is considered preferable to genetically modified organisms and traditional breeding due to its safety and affordability. Gamma rays pose no harm to humans or the environment, making their application in plant breeding a recommended practice (5).

Mutation improvement is a highly effective strategy for developing plant varieties with enhanced traits. Genetic enhancement techniques can also result in the development of cultivars that are more resistant to biotic and abiotic stressors (6). Although there are many techniques used in mutation breeding, one well-known physical mutagen involves the use of gamma rays (7, 8). Unlike chemical mutagens, physical mutagens like gamma rays are safer for health as they do not necessitate the application of mutagens removal from the material and do not require detoxification post-application (9, 10). According to this method, the number of cultivars resulting from the induction of mutations has been steadily rising (11, 12). Notably, several new cultivars in various plants, such as coriander (13), tomatoes (14), anthurium and mungbean (15), have been developed using gamma rays. By producing free radicals, gamma rays affect the morphological, physiological, biochemical and cytological changes that occur in cells and tissues throughout plant growth and development (16).

Physical mutagens are favored over chemical mutagens due to several advantages. Physical mutagens do not require removal after application, in contrast to chemical mutagens (9) and they do not leave behind hazardous waste that requires special handling. The use of physical mutagens for mutation induction is widespread, with approximately 90% of existing mutants attributed to physical mutations. Of them, X-rays make up 22% and gamma rays 64% (17, 18). Based on information from the IAEA Mutant Database, gamma-ray-induced mutations are responsible for approximately 1665 of the 3362 mutant varieties that have been released to date (19).

The choice of mutagen and the correct dosage is crucial for unlocking opportunities in plant growth by using mutation breeding (20). The dosage of the mutation is emphasized as more critical than the type of mutagen used (5). It is essential to accurately determine the mutation dose before scaling up its application (21). While higher doses of mutations increase the likelihood of obtaining mutants, they can also lead to infertility and individual mortality (22). Numerous studies have shown that mutations can effectively generate genetic diversity with desired traits, making them valuable for plant development programs. One key advantage of mutant breeding is the ability to enhance specific features without altering the overall genotype (23). Therefore, the current radiation study aims to Evaluate how the growth and variability features of the Winter Dawn Strawberry cultivar are affected by different gamma radiation exposure levels.

Materials and Methods

This study selected the commercially farmed first-generation Winter Dawn strawberry cultivar for its economic importance. In October 2022, runner plants were collected from the ICAR Regional Station Shimla, Himachal Pradesh and immediately transferred to Punjab Agricultural University in Ludhiana, Punjab, for gamma irradiation using 60Co.

Based on several reviews of literature, it has been found that doses between 10 Gy and 50 Gy were found to be beneficial in fruit crops. Hence, Doses were given at a rate of 1.52 Gy per minute to four groups of plants: 0 Gy (Control), 20 Gy, 30 Gy and 40 Gy (Fig. 1). Each treatment for the Winter Dawn cultivar comprised 25 runner plants and was administered once. Following irradiation, the runners were transplanted into 5 kg plastic pots with a substrate mixture of vermicompost, cocopeat and sand in equal proportions 1:1:1 ratio was used to fill each pot (Fig. 2). The transplanted runners were placed in a greenhouse at the agriculture farm of Lovely Professional University, Phagwara, Punjab. The experiment was set up in a completely randomized design (CRD) with 5 replications, each consisting of 5 Winter Dawn variety plants.

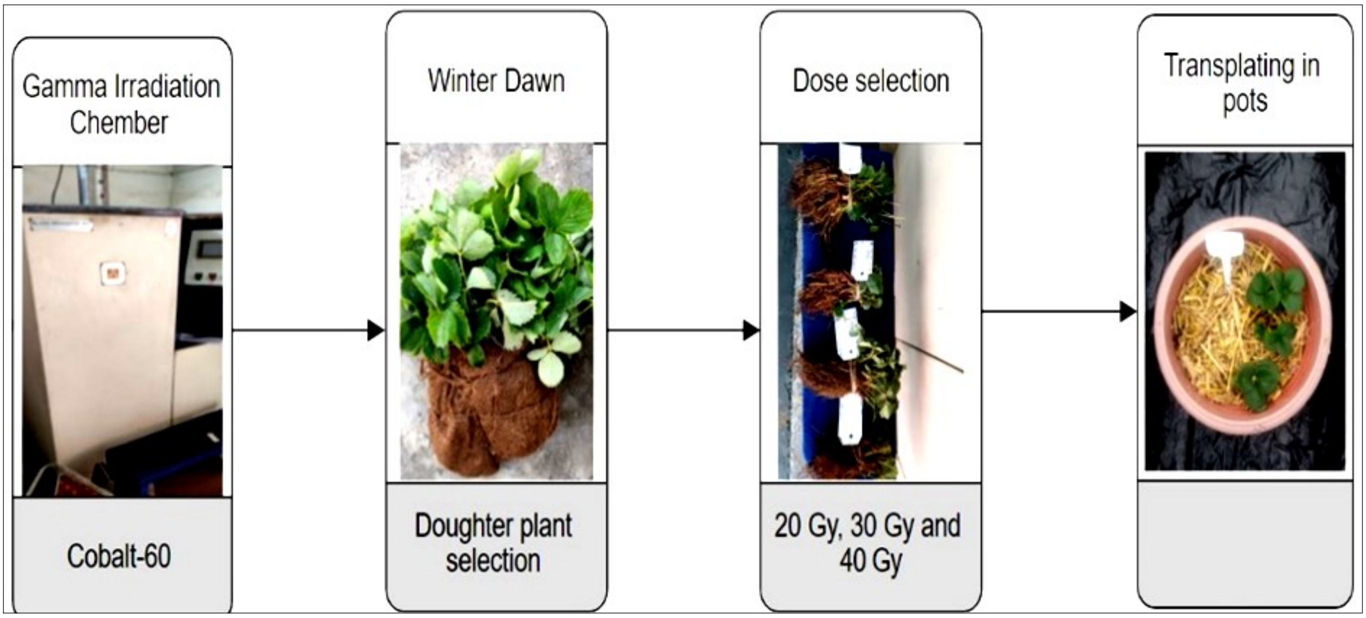

Fig. 1. Sample preparation for gamma irradiation treatment

Fig. 2. Different doses of gamma irradiation and their effects

The plants were clipped to eliminate dead leaves, runners and flowers prior to undergoing irradiation treatment. Then, after being split up into 3 groups and given varying doses of gamma irradiation, they were carefully placed in irradiation containers. The treatment duration for each radioactive dose decay was documented during the runners' treatment and the plants were allocated into 3 groups for 3 doses (Table 1). There were 4 groups of runners: 25 untreated (control) runners, 25 runners of 20 Gy, 25 runners of 30 Gy and 40 Gy-treated runners.

Table 1. Treatment dose rates and timing required for doses

Treatments	Dose rate	Time of radioactive decay
T_1	20 Gy	10 min 14 sec
T_2	30 Gy	15 min 22 sec
T_3	40 Gy	20 min 29 sec
T_0	0 Gy	Control

After treatment, these runners were planted in multiple plastic pots on the same day. A light irrigation was applied right after planting and more irrigations were applied as needed in accordance with the moisture content of the soil. The plants were grown separately in individual pots, with daily temperatures maintained at 28 °C and nighttime temperatures at 18 °C. The photoperiod was calculated at 14 hr of light and 10 hr of darkness, with the relative humidity maintained between 60% and 75%. During cultivation, 3 rounds of hand weeding were done: the first round occurred 20 days after transplanting and the second round followed 25 days later. After recovering from the initial shock of transplanting, 5 plants, selected at random, were observed at different intervals in each replication of the 20 treatments.

Growth related observations

The Probit analysis method was employed to ascertain the radiation doses at which lethal effects manifest 15 and 30 days post-treatment. The LD_{50} dose was determined by Probit analysis utilizing data on sprouting percentage and survival rate.

The count of shoots displaying non-browning growth was recorded at intervals of 15, 20 and 30 days post-treatment. The percent survival was calculated using the following formula.

$$\% \text{ of Survival} = \frac{\text{Number of explants that survived}}{\text{Number of runners treated}} \times 100$$

....(Eqn.1)

The height of each plant was measured in centimeters using a meter scale and the average height was calculated for all plants in each pot. The leaf count for each plant in every pot was tallied, leading to the determination of the average leaf count. The Leaf Area , measured in cm^2, was acquired using the CL-202 Leaf Area Meter (USA), employing a destructive method that focused on mature leaves using a SPAD meter on mature leaves from each plant in each pot, the chlorophyll content of each plant was determined non-destructively. The duration from transplantation to flower bud initiation, flowering and fruiting was documented and the mean value was then computed.

Yield related observation

The total number of flower buds, flowers and fruits per plant was recorded, along with the number of completely bloomed flowers and ripe fruits in each pots. Fruit quality was assessed by measuring the length and diameter of fruits in millimeters (mm) using the Digital Caliper DC-515. Average values were calculated for each treatment. A digital refractometer (ERMA, Tokyo, Japan) was used to measure brix percentages.

Statistical analysis

Data analysis was performed using SPSS-21 software one-way ANOVA was used to assess the significance of treatment differences for various observable parameters. A significance threshold of 5% and treatment differences were analyzed using the least significant difference (LSD) test.

Results

LD_{50} lethal dose

In the Winter Dawn variety, LD_{50} analysis indicates that gamma radiation significantly affects plant survival rates. At lower doses, survival rates are higher, with the highest survival observed at 20 Gy. As radiation doses increase, a decreasing trend in survival rates is evident, with significant effects at higher doses, where the 50% survival threshold is achieved at 40 Gy. The log dose values represent the 50% mortality against survival rates showing a dose-dependent plant response to gamma radiation. Higher radiation doses significantly affect plant survival, with the LD_{50} determined at 40 Gy. The dose-dependent plant response is evident from the log values correlating with gamma radiation, as shown in Table 2. This dose selection is critical because it allows researchers to apply a level of gamma radiation that can effectively induce genetic changes while maintaining a viable population for further study.

Table 2. Dosages and survival percentage of plant and LD_{50}

Concentration	Total no. of plant	No. of killed plant	No of survived	% of survival	Log dose	Exp.Prop	Emp probit	Exp probit	Work. probit
20 Gy	25	3	22	88%	0.113	0.016	-1.212	-1.431	-1.786
30 Gy	25	6	19	76%	0.403	0.192	-1.096	-1.634	-1.783
40 Gy	25	11	14	50%	0.679	0.330	-0.786	-0.646	-0.733
0 Gy	25	7	18	72%	0.545	0.167	0.272	-0.532	-2.543

Growth attributes

The survival rates of runners improved when transferred to plastic pots following 20 Gy gamma irradiation treatments, compared to those subjected to a 40 Gy dosage or left untreated. The result itself dictates better results than control runners. Consequently, it can be inferred that survival improved as a result of radiation treatment. Plant height, number of leaves, leaf area index (LAI) and chlorophyll content were affected significantly (Fig. 1). In growth-related observations, plant height, number of leaves, leaf area index (LAI) and chlorophyll content were recorded and illustrated (Fig. 1). The highest plant height (24.6 cm) was noted with a 30 Gy gamma irradiation treatment. In contrast, the lowest height was documented at a 40 Gy exposure. Similarly, the number of leaves, a key factor in photosynthesis, was found to be highest (13.3) in runners treated with the 30 Gy dose, with the minimum number of leaves recorded at the 40 Gy dose. The largest leaf area (23.1 cm^2) was recorded at the 30 Gy treatment, whereas the smallest was observed in untreated runners. Chlorophyll content was highest (52.51) in plants treated with the 30 Gy dose. Overall, the findings indicated that runners treated with 20 Gy and 30 Gy showed maximum growth instead of 40 Gy dose (Table 3).

Table 3. Effect on growth-related observation in Winter Dawn

Dosage	Plant height	Leaf number	LAI (cm^2)	Chlorophyll content
20 Gy	22.8^{b} (4.88)	12.8^{ab} (3.71)	21.4^{b} (4.74)	50.71^{b} (7.19)
30 Gy	24.6^{a} (5.6)	13.3^{a} (3.78)	23.1^{a} (4.91)	52.51^{a} (7.31)
40 Gy	21.8^{b} (4.77)	11.8^{bc} (3.58)	21.2^{b} (4.71)	50.23^{bc}(7.15)
0 Gy	21.4^{b} (4.73)	10.7^{c} (3.32)	21.1^{b} (4.70)	49.39^{c} (7)
C.D.	1.359 (0.138)	1.309 (0.179)	1.438(0.152)	1.13 (0.078)
SE (m)	0.436 (0.044)	0.42 (0.057)	0.462(0.049)	0.363 (0.025)
SE (d)	0.617 (0.063)	0.594 (0.081)	0.653(0.069)	0.513 (0.035)
C.V.	3.844 (1.826)	7 (3.189)	4.244(2.043)	1.431 (0.698)

The values in the table represent the average of 5 replications. According to the Ducan Multiple Range Test, distinct letters within the same column indicate significant variations at a significance level of P < 0.05. The values enclosed in brackets are transformed

Yielding attributes

The time required for the first bud initiation, measured from the day of transplanting, varied with the radiation dose. The 20 Gy dose resulted in the earliest bud initiation. On the other hand, the 40 Gy dose was found to have the longest duration for the first bud initiation (Table 4). The interval between bud emergence and flowering was also recorded, showing that plants receiving no radiation (0 Gy) flowered earlier instead of 40 Gy dose, while the 40 Gy dose delayed flowering. The time to fruit formation after transplantation was calculated across different treatment doses. Plants treated with 20 Gy exhibited the earliest fruit setting, while the untreated runners showed the longest time to fruit formation (Table 5).

The number of buds was recorded in both mutagen-treated and untreated runners. The maximum number of buds was observed in runners treated with a 20 Gy dose, while the minimum number of buds was recorded in runners treated with a 40 Gy dose. Similarly, the maximum number of flowers was recorded at the 20 Gy dose, whereas the minimum number of flowers was observed at the 40 Gy dose (Table 4). In the case of fruits, the maximum number was recorded at the 30 Gy dose, while the minimum number of fruits was observed at the 40 Gy dose (Table 5). Plants treated with 20 Gy had the highest counts of flower buds, flowers and fruits, while untreated plants had the lowest counts for these parameters.

Quality attributes

The 30 Gy exposure resulted in the thickest fruit among all gamma radiation doses, while the untreated runners produced the thinnest fruit (Table 5). Fruit length and diameter were significantly smaller in the untreated runners compared to the treated plants. The 30 Gy dose produced the largest fruit length and diameter among all treat-

Table 4. Effect on fruit-related observation in Winter Dawn

Dosage	Days to bud	Number of Bud	Days to flowering	Number of flowers
20 Gy	55.66^{b} (7.22)	31.60^{a} (5.70)	59^{b} (7.74)	32.2^{a} (5.76)
30 Gy	55.66^{b} (7.54)	26.57^{b} (5.25)	62.25^{a} (7.95)	28.5^{bc} (5.43)
40 Gy	58^{a} (7.66)	26.35^{b} (5.22)	63.25^{a} (8)	27.6^{c} (5.35)
0 Gy	57.66^{a} (7.68)	27.12^{b} (5.30)	64.5^{a} (8.09)	28.9^{ab} (5.47)
C.D.	N/A (0.214)	1.254 (0.117)	2.564 (0.160)	1.121 (0.100)
SE (m)	1.323 (0.069)	0.402 (0.037)	0.823 (0.051)	0.36 (0.032)
SE (d)	1.871 (0.097)	0.569 (0.053)	1.164 (0.073)	0.509 (0.046)
C.V.	4.002 (1.821)	2.884 (1.395)	2.644 (1.293)	2.45 (1.169)

The values in the table represent the average of 5 replications. According to the Ducan Multiple Range Test, distinct letters within the same column indicate significant variations at a significance level of P < 0.05. The values enclosed in brackets are transformed

Table 5. Effect on yield-related observations in Winter Dawn

Dosage	Days to fruit set	Number of fruits	Fruit Length	Fruit Diameter	Fruit weight
20 Gy	81.7^{b} (9)	30.13^{b} (5.57)	38.37^{b} (6.27)	26.91^{ab} (5.40)	10.40^{b} (3.37)
30 Gy	82.5^{b} (9.13)	32.79^{a} (5.81)	39.84^{a} (6.39)	27.80^{a} (5.59)	12.1^{a} (3.62)
40 Gy	85^{b} (9.27)	27.99^{c}(5.38)	37.51^{b} (6.20)	25.94^{bc} (5.32)	9.985^{b}(3.31)
0 Gy	88^{a} (9.43)	29.88^{b}(5.55)	33.53^{c} (5.87)	25.22^{c} (5.29)	10.27^{b} (3.35)
C.D.	3.107 (0.169)	1.118(0.099)	1.382 (0.111)	0.928 (0.117)	0.768 (0.113)
SE (m)	0.997 (0.054)	0.359 (0.032)	0.444 (0.036)	0.298 (0.037)	0.246 (0.036)
SE (d)	1.41 (0.077)	0.508 (0.045)	0.627 (0.051)	0.421 (0.053)	0348 (0.051)
C.V.	2.36 (1.175)	2.37 (1.137)	2.377(1.157)	2.25 (1.1387)	4.609 (2.119)

The values in the table represent the average of 5 replications. According to the Ducan Multiple Range Test, distinct letters within the same column indicate significant variations at a significance level of P < 0.05. The values enclosed in brackets are transformed ments. While observing the treated strawberry runners, it was noted that the fruit quality was affected, with a significant reduction in fruit length and diameter at a 40 Gy dose of gamma irradiation. In contrast, the maximum fruit length and diameter were observed at a 20 Gy dose. In terms of fruit weight, the 40 Gy dose-treated runners had the lowest weight whereas the 30 Gy gamma irradiation dose treatment had the maximum fruit weight.

Root length, number of root and crown diameter

The root acts as the foundation for plant growth and an increase in root length is anticipated to improve the plants' strength and Vigor. In a study conducted on strawberry runners subjected to varying doses of gamma irradiation (20 Gy, 30 Gy, 40 Gy and 0 Gy), the longest primary root length was observed at 30 Gy, followed by 20 Gy. In comparison, the shortest was recorded at 40 Gy and the untreated runners. Similarly, secondary root length was highest at 30 Gy and 20 Gy and lowest at 40 Gy and in the untreated runners. The number of primary and secondary roots was highest in plants treated with 30 Gy and lowest in untreated plants (Table 6).

The strawberry crown diameter, a critical parameter for plant vigor and fruit yield, was assessed after gamma irradiation treatments. The largest crown diameter was observed at 30 Gy, followed by 20 Gy (Table 6). Conversely, the smallest diameter was recorded with a 40 Gy dose, followed by control plants.

Table 6. Effect on yield-related observations

Dosage	Primary root length (cm)	Secondary root length (cm)	Number of primary with secondary root	Crown/ Stem Diameter (mm)
20 Gy	25.17	4.82	32	12.28
30 Gy	29.50	5.5	39.6	14.32
40 Gy	19.33	3.66	30.4	11.94
0 Gy	21.9	4.48	29.6	12.10
C.D.	1.216	0.308	1.711	0.973
SE (m)	0.402	0.102	0.566	0.322
SE (d)	0.569	0.144	0.8	0.455
C.V.	3.753	4.929	3.845	4.692

The values in the table represent the average of 5 replications. According to the Ducan Multiple Range Test, distinct letters within the same column indicate significant variations at a significance level of $P < 0.05$. The values enclosed in brackets are transformed

Discussion

Spontaneous and induced mutations have been pivotal in improving traits such as fruit yeild, disease resistanceand fruit quality in fruit crops through targeted breeding efforts. Mutagenesis techniques have been effectively utilized to enhance traits such as plant size, flowering time, fruit ripening, color, self-compatibility, self-thinning and resistance to pathogens. The number of cultivars developed through mutation induction continues to rise (24). The LD_{50} findings for the Winter Dawn variety align with previous research, which shows that increased gamma radiation doses reduce survival rates in a dose-dependent manner. This threshold varies among plant types (25). These effects are due to low to moderate radiation doses stimulating mutation while maintaining cell viability, which is significantly reduced at higher doses (26). The observed trend, where survival rates decrease as radiation levels increase, is consistent with findings on other crops, such as rice and barley, where LD_{50} values are often between 30-50 Gy (26). These effects are due to low to moderate radiation doses stimulating mutation while maintaining cell viability, which is significantly reduced at higher doses (26). This supports the utility of identifying an LD_{50} dose range that maximizes mutagenic potential while maintaining viability for breeding programs (27).

This study aimed to establish standardized gamma irradiation doses for Winter Dawn. Higher doses decreased plant height, whereas lower doses increased it, consistent with findings in other studies on different plant species (28). The reduction in plant height induced by gamma rays may result from damage to the apical meristem. Leaf numbers are influenced by genetic variations and environmental factors such as temperature and light, as observed in studies in Punjab (29). High levels of irradiation doses adversely affected leaf numbers, possibly due to tissue damage (30). However, some reports suggest that irradiated plantlets did not exhibit reduced leaf numbers. This might be due to variations in genetic structure and physiological responses of plants due to gamma radiation, which can also contribute to these inconsistencies. While higher radiation doses are generally associated with reduced growth and leaf numbers due to cellular damage and inhibited cell division, lower or optimal doses can stimulate growth by inducing beneficial mutations or enhancing hormonal activity (31). Earlier studies have shown that lower gamma irradiation doses are favorable for leaf area index (LAI), while higher doses decrease it (32).

The plantlet treated with low levels of gamma radiation increased chlorophyll concentration. This increase was attributed to the activation of an enzyme system, consequently boosting the photosynthetic rate and enhancing yield (33). Conversely, higher doses of gamma radiation caused a gradual decline in chlorophyll content in treated plants. This decline may occur due to the liberation of chlorophyll from its protein complex, followed by degradation or oxidative stress (34).

Application of gamma irradiation dose at 15 Gy increased the chlorophyll content (both a and b) of *Dracaena* plants cultivated *in vitro*. The level of chlorophyll in a plant tends to fluctuate depending on the dose of irradiation and the specific plant species (35). Low levels of gamma radiation can enhance chlorophyll production by activating enzyme systems and improving yield components, whereas higher doses typically have an inhibitory effect (36).

Conclusion

This study suggests that targeted gamma radiation can be effectively integrated into strawberry breeding programs, enhancing both sustainability and resilience by promoting desirable genetic traits and improving stress tolerance. Our findings indicate that irradiation at specific doses, particularly 30 Gy, promotes desirable growth characteristics, such as crown diameter, maximum root length and number of roots, while also enhancing chlorophyll content and the leaf area index (LAI). Conversely, higher doses 40 Gy resulted in increased mortality and reduced growth performance, highlighting the importance of dose optimization in mutation breeding. The LD_{50} found at 40 Gy dose confirms a clear relationship between radiation dose and survival rates, reinforcing the necessity of careful dosage selection to balance mutation induction with plant viability. This study contributes to the broader understanding of how physical mutagens can effectively generate genetic variation in strawberry crops, which is critical for developing new cultivars with improved traits. Overall, gamma radiation presents a promising avenue for advancing strawberry breeding programs, offering a practical approach to improving key traits and enhancing the long-term sustainability of this vital crop.

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Authors' contributions

RR developed the concept, methods, and assumptions. RRR and SS conducted the research and performed the statistical analyses. KP, HK, KJ, MC, and JS collected the necessary information and contributed to the manuscript drafts. All authors reviewed and approved the final version of the manuscript for publication.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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