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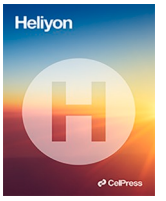

Research article

Genetic consequences of chronic gamma irradiation on agro morphological traits in chili under hydrogel enhance media

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ABSTRACT

Induced mutation for the creation of desirable traits through chronic gamma irradiation provides an opportunity for the selection and development of new chili varieties. This study was conducted to assess the effects of different doses of chronic gamma irradiation on morpho-physiological traits in chili. Ten plants from each variety were exposed to different doses of chronic gamma irradiation for 277.02 h at three weeks after germination under gamma greenhouse facilities, with accumulative dose; 185.61Gy, 83.11Gy, 47.096Gy, 30.474Gy, 19.4Gy, 13.9Gy, 11.1Gy, 8.31Gy, 5.54Gy) and 2.77Gy respectively. Highly significant differences were observed among doses (Rings) of chronic gamma irradiation expressed in mean values for all investigated traits. Relatively moderate doses of chronic gamma irradiation represented by doses 47.096 Gy (Ring 4) and 19.40 Gy (Ring 6) resulted in significant stimulation for most of the studied characters. The highest heritability was recorded in days to flowering at 99.88 while the lowest was observed in fruit dry weight at 34.66 %. High genetic advance were recorded for most of the quantitative traits studied. In addition, a highly significant positive correlation was observed between total fruit per plant, total number of fruit per plant, plant height, fruit fresh weight, number of secondary branches, chlorophyll a, fruit dry weight, total chlorophyll content, stem diameter, fruit length and fruit girth. With increasing chronic gamma dose, mutagenic efficiency and efficacy generally increased. Induced variety of desirable features will considerably increase the chilli's amelioration through mutation breeding, leading to the development of improved varieties. The results of this research offer valuable information for the use of chronic gamma radiation in the mutations breeding of Capsicum annuum L., which will be advantageous for future breeding programs.

1. Introduction

Chili (*Capsicum annuum* L.), a member of the Solanaceae family, is one of the most cultivated vegetable crops in tropical and subtropical climates [1]. It is widely consumed in its fresh, dried, powdered, and paste form as a major ingredient in several dishes.

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Since the advancement of civilization, chili has found important uses in human diets where it is consumed as condiments and spice due to its appealing flavour, pungency and colour. The abundance of medicinal and nutritional quality has made it almost indispensable in daily nutrition [2]. Chili fruit is rich in vitamins A, B (B1, B2, and B3), B-complex, ascorbic acid, antioxidants (carotenoids), and flavonoids. It is considered a good source of minerals such as potassium, manganese, magnesium and iron. Higher capsaicin levels in chilli correspond with higher level of pungency and higher antioxidant activities [2]. Capsaicinoids have been reported to have several pharmacological and physiological effects on the digestive and cardiovascular systems [3]. In Malaysia, annual chili production was estimated at 40,520 Mt covering over 3,581ha in land area [1]. This is extremely low compared to production in countries like India and China who are the highest producers of the crop globally. The huge deficit between production and demand requires that Malaysia import chili to meet up with ever-increasing local demand for chili. The major cause of low productivity of the crop in Malaysia can be attributed to the lack of high-yielding hybrids or varieties. In addition, local varieties are genetically heterogeneous, highly susceptible to pests and diseases and greatly affected by high temperature due to climate change [1].

The development of high-yielding variety having desirable qualities that are highly heritable is one of the existing trends to combat the low productivity of chili. However, selection of desirable traits is a prerequisite for crop improvement and this is achieved through the exploitation of efficient breeding methods. In the absence of genetic variability, induced variations are often used to develop new varieties that can be obtained through mutation breeding techniques. A mutation is any alteration in DNA sequence either in the genes or at the chromosomal level which could be passed down to the offspring. At present, the improvement of major food crops in the world rests on mutation, particularly in cases where variation does not occur naturally. This comes either naturally or through irradiation. Crops with restricted genetic variation require mutagenesis or induced mutation to create desirable and heritable variations in them. By inducing mutation, scientists have been able to increase genetic variation, which breeders depend upon to produce new varieties with desirable traits [4]. Mutation induction is carried out by exposing plant materials such as seeds, seedlings or any propagated plant parts to suitable physical or chemical mutagens [4]. Gamma-ray, applied using two techniques i.e acute and chronic irradiation, is the most commonly used physical mutagen in mutation breeding. Generally, ^{60}Co and ^{137}Cs are two gamma irradiation sources mostly used for irradiation treatments due to their relatively high energy. Gamma ray ionizing radiation reacts with atoms and molecules in the cells of plants to produce free radicals. Production of free radicals depends on the irradiation level which causes modification or damage of components in the plants. Consequently, metabolism and physiology are affected, for example, changes in the rate of photosynthesis, accumulation of phenolic compounds, and variations in the anti-oxidative system [5,6]. This ultimately causes a change in morphology, anatomy, and biochemistry of the plant. Hence, the selection and development of new or improved varieties can be obtained by inducing desirable variations using gamma radiation [7].

Chronic irradiation technique involves the exposure to ionizing radiation at low dose rate over a long period of time (ranging from weeks to months) [8]. This procedure may enhance plants development of recovery mechanisms during long period of irradiation in the gamma greenhouse. This explains why chronic irradiation results in less damage compared to acute irradiation [9]. Changes induced by chronic irradiation procedures are generally significant, manifested as changes in physical appearances, alteration in molecular structures and metabolism modifications. These alterations are random occurrences, inheritable, and the stability is dependent on cell injuries after irradiation at molecular levels. Chronic gamma irradiation has been reported to result in wide range of mutations with less irradiation damage in the production of new mutant cultivars with superior genotypes [10]. The objective of the present study was to investigate the effects of different doses of chronic gamma irradiation on morphological, physiological and

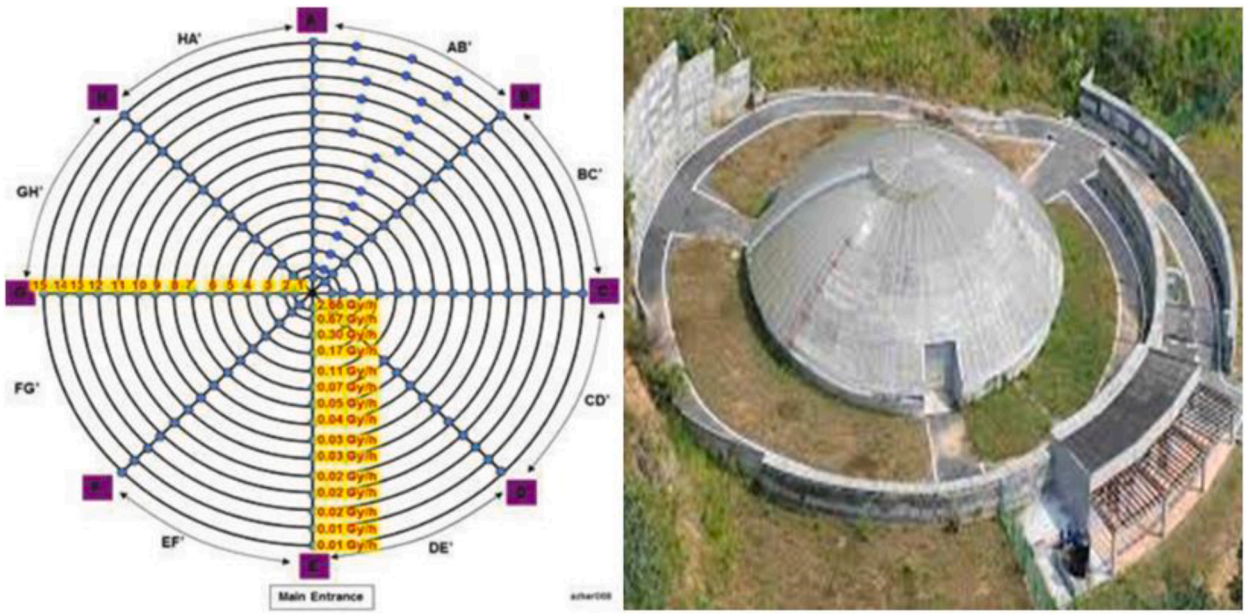

Fig. 1. Gamma Greenhouse dose mapping with coded regions of precise dosage exposures for chronic gamma irradiation.

biochemical variations in two chili varieties.

2. Materials and methods

2.1. Chronic irradiation

Plant rate of mortality indicates the degree of damage that can be induced as a result of exposing seedlings to gamma radiation treatments. Hence, the survival rate was one of the most critical variables that needed to be taken into consideration in any experiment involving the mutagenesis of plants. Therefore, ten healthy plant of three week old seedlings were selected for each variety and exposed to different doses of chronic gamma irradiation represented in rings (2, 3, 4, 5, 6, 7, 8, 9, 11 and 14) for 277.2 h in a gamma greenhouse (GGH) facility at the Malaysian Nuclear Agency Bangi, Selangor, Malaysia (Fig. 1). The gamma greenhouse used Caesium-137 (^{137}C) as the source of chronic gamma irradiation. Dose rates and accumulative doses of gamma-ray for each ring are shown in Table 1. The study was carried out using two inbred chili varieties namely; Chili Bangi 3 and Chili Bangi 5. The seeds of the two varieties were obtained from Chili Bangi Seeds Company, Bangi, Selangor, Malaysia.

2.2. Planting materials

The experimental materials used in this experiment were obtained from the M4 generation of gamma irradiated plant of *Capsicum annuum*. Because Chili Bangi is the most cultivated variety in Malaysia, efforts are constantly being made to increase its yield potentials. Therefore, its plant seedlings were irradiated chronic gamma irradiation to determine radio-sensitivity. After several series of selection and fixation, ten potential lines from each ring with the required adaptive traits such as stable yield were recovered at M4 generation during the 2018–2022 seasons (M0–M4).

2.3. Experimental design and field layout

Irradiated seedlings (after their removal from GGH) were transferred to 16 × 16 cm polybags already filled with coco peat. The experiment was laid down in a Randomized Complete Block Design (RCBD) with three replications and 11 treatments under greenhouse condition at Field 15, Faculty of Agriculture, Universiti Putra Malaysia. The research location lies between 2°59′ north latitudes and 101°43′ east longitudes, with an altitude of 55 m. The temperature in the area ranges from 24 to 31 °C. Hydrostock® fine granule, a commercial biodegradable superabsorbent product, was used in this study. In order to create saturated wet form hydrogel, 20 g of Hydrostock® was dissolved in 1.5 L of water. This hydrogel was then combined with fresh cocopeat in a weight-based ratio of 1:1 for each plant's potting mixture. The polybags were arranged in 75 cm × 100 cm (within and between the line) to avoid competition for light and space. A fertigation system was set up at the greenhouse following Fertigation System Manual, 1st Edition MARDI. The plants were provided daily with stander copper formulations after transplanting. The formulation contains; N 200, P 60, K 300, Ca 170, Mg 50, Fe 12, Mn 2, B 1.5, Zn 0.1, Cu 0.1 and Mo 0.2 (mg L^{-1}), with EC (electronic conductivity) of 0.6. and EC increased slightly weekly until 2.5 depending on plant growth stage [11]. Pesticides such as Confidor, Amiko, Decis and Mitac (Bayer, UK) were applied two to three times a week and fungicide Kencozeb (Kenso, Australia) was sprayed once a week following manufacturer's recommendations.

2.4. Data collection

2.4.1. Morphological, physiological and yield traits

In each variety, three plants were randomly sampled from each block for data collection. Data were collected on height, stem diameter, days to first flowering, days to 50 % flowering, number of primary branches per plant, number of secondary branches per plant, total chlorophyll content, fruit length, fruit girth, fruit fresh weight, fruit dry weight, total number of fruit per plant, total fruit yield per plant, photosynthetic rate, and stomatal conductance as presented in Table 2.

Table 1
Accumulative dose of chronic gamma-rays per ring.

Ring number	Dose rate (Gy/h)	Accumulative dose of gamma-rays (Gy) after 277.02 h
Control	0	0.00
R 2	0.67	185.61
R 3	0.30	83.11
R 4	0.17	47.09
R 5	0.11	30.47
R 6	0.07	19.40
R 7	0.05	13.90
R 8	0.04	11.10
R 9	0.03	8.31
R 11	0.02	5.54
R14	0.01	2.77

2.4.2. Effect of chronic gamma irradiation on biochemical and physiological traits

Capsaicin and dihydrocapsaicin, total phenolic contents were estimated using specific methods in the laboratory with three replications.

2.4.3. Capsaicin and dihydrocapsaicin content

Concentrations of capsaicin and dihydrocapsaicine were analyzed using High Performance Liquid Chromatography (HPLC) (Waters, 2998) Photodiode Array Detector, Model E2695. Fully matured chili fruits (10 fruits) from each treatment (doses) were collected and samples were oven-dried at 60 °C for 3–5 days and grounded using a laboratory mill. The grounded samples were stored in 20 °C until further use. Capsaicinoids extraction from chili fruit was carried out following the method described by Usman et al. [2]. Quantification and extraction were done in duplicates for each variety under study. The conversion of capsaicinoids concentration to Scoville Heat Unit (SHU) was done by increasing chili fresh and dry weight capsaicinoids amount (μg/ml) by a coefficient of heat 16.1 for both capsaicin and dihydrocapsaicin [2].

$$\text{Total SHU} = \{\text{capsaicin} + \text{dihydrocapsaicin}\} \times 16.1$$

It is categorized as.

(i) 0–700 SHU nonpungent
(ii) 700–3000 SHU mildly pungent
(iii) 25,000–70,000 SHU highly pungent
(iv) 3000–25,000 SHU moderately pungent
(v) 80,000 SHU very highly pungent.

2.4.4. Total phenolic content (TPC)

Gallic acid stock solution was prepared by dissolving 0.5 g gallic acid in 10 mL ethanol before adding distilled water to make up 100 mL. The prepared stock was stored in amber glass vessels for 2 weeks at 4 °C. The stock preparation was cooled to room temperature (25 °C) before use. To prepare the working standards, 1, 2, 5, and 10 mL were respectively drawn from the stock solution and diluted to 100 mL with distilled water in a volumetric flask to make concentrations of 50, 100, 250 and 500 mg L^{-1}. Sodium carbonate solution was obtained by dissolving 7.5 g anhydrous sodium carbonate in 100 mL distilled water. The preparation was stirred homogeneously on a mechanical stirrer for 24 h at room temperature before use. Total phenolic content (TPC) microscale was assayed by mixing 40 μL of extract with 3.16 mL distilled water and 0.20 mL Folin–Ciocalteu reagent. The mixture was allowed to stand at 25 °C for 6 min before adding 0.60 mL of 7.5 % sodium bicarbonate (Na_2CO_3) aqueous solution. The mixture was shaken vigorously and incubated for 2h at 25 °C. The color of Folin–Ciocalteu reagent changed from yellow to blue. Absorbance against a blank was measured at 765 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The same procedure was applied to the Gallic acid of standard solutions. The TPC was expressed as GAE mg per gram of extract from the calibration curve of Gallic acid standard solution, ($y = 0.0016x + 0.0837$), where y is absorbance and x is concentration (mg L^{-1}).

2.4.5. Estimation of chlorophyll a and b

Exactly 0.5 g of fresh leaves from each treatment was weighed and grounded using a pestle and mortar with 20 ml of 80 % acetone. Homogenized sample of the mixture was centrifuged at 10000 rpm at 4 °C for 5 min. The supernatant was relocated and the process was repeated until the residue became colourless. Absorbance of the solution was measured at 645 and 663 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of Chlorophyll *a* and Chlorophyll *b* was calculate using the

Table 2
List of quantitative parameters.

Quantitative traits	Abbreviation (Unit)	Description
Plant height	PH (cm)	Height of each plant from the coco peat level to the tip of the plant was measured using measuring tape.
Stem diameter	SD (cm)	Stem diameter measured 5 cm from the base of the plant stalk using an Absolute Digimatic caliper.
Days to first flowering	DTF (days)	Counted from the number of days after transplanting to first flowering.
Days to 50 % flowering	DT50 % (Days)	Counted from the number of days after transplanting to 50 % flowering.
Number of primary branches per plant	PB (no.)	Measured from the number of branches produced from the main stem of plant.
Number of secondary branches per plant	SB (no.)	Measured from the number of branches produced from the primary branches.
Total chlorophyll content	TCH(μg/ml)	Total chlorophyll was measured on the third to fifth leaf from the tip using SPAD-502 Plus.
Fruit length	FL (cm)	Length of one matured fruit per plant from calyx to the tip of the fruit was measured.
Fruit girth	FG (cm)	Girth of one matured fruit (0.3 cm below the calyx) was measured.
Fruit fresh weight	FFW (g)	Weight of one matured fruit per plant was measured
Fruit dry weight	FDW (g)	Weight of one matured fruit per plant was measured.
Total number of fruit per plant	TNF (no.)	Counted from the total number of fruits per plant harvest during harvesting period.
Total fruit yield per plant	TYP (kg)	Total weight of fruits per plant was determined at harvest period.
Photosynthetic rate and Stomata conductance	PHO(*μ*mol/m2/s) & SC	Photosynthesis rate, stomata conductance were measured on the third or fourth leaf from the tip) using a photosynthesis portable system (LI-6400xt, LI-COR, Lincoln, NE, USA)

following formula:

$$\text{Chlorophyll } a : 12.7\ (A_{633}) - 2.69\ (A_{645})$$

$$\text{Chlorophyll } b : 22.9\ (A_{645}) - 4.68\ (A_{663})$$

2.4.6. Variance components, broad sense heritability and genetics advance

Phenotypic and genotypic coefficients of variation were estimated as described by Singh and Chaudhary [12].

$$\text{PCV}\ (\%) = \frac{\sqrt{\sigma_P^2}}{\overline{X}} \times 100 \quad (1)$$

$$\text{GCV}\ (\%) = \frac{\sqrt{\sigma_g^2}}{\overline{X}} \times 100 \quad (2)$$

Where, σ_p^2 is the phenotypic variance, σ_g^2 is the genotypic variance and $\overline{X}$ is the mean of the trait. Genotypic coefficient variation (GCV) and phenotypic coefficient variation (PCV) values were categorized as low (0–10 %), moderate (10–20 %) and high (20 % and above) values, following Oladosu et al. [13].

Heritability estimate: Broad sense heritability (h_B^2) is the ratio of the genetic variance (σ_g^2) to the phenotypic variance (σ_p^2).

$$h_B^2\ (\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100 \quad (3)$$

Where, σ_g^2 is the genotypic variance and σ_p^2 is the phenotypic variance. The heritability percentage is categorized as low (0–30 %), moderate (30–60 %) and high (>60 %), following Usman et al. [2].

Genetic advance: The genetic advance (GA) (as percentages of the mean) was calculated with selection intensity (K) assumed to be 5 %. The genetic advance was categorized as low (0–10 %), moderate (10–20 %) and high (>20 %), following Oladosu et al. [13].

$$\text{GA}\% = K \times \frac{\sqrt{\sigma_P^2}}{\overline{X}} \times h_B^2 \times 100 \quad (4)$$

Where, K is a constant that represents the selection intensity. When K is 5 %, the value is 2.06, $\frac{\sqrt{\sigma_P^2}}{\overline{X}}$ is the phenotypic standard deviation, h_B^2 is the heritability and $\overline{X}$ is the mean of the traits.

Table 3
Mean squares of analysis of variance for effects of chronic gamma irradiation on quantitative traits.

S.O.V	DF	PH	NL	SD	DTF	DT50 %	PB	SB
Rep	2	141.22	25.62	0.065	1.56	1.01	0.073	0.665
Genotypes (G)	1	602.55**	2.37ns	0.329ns	6.68*	0.062ns	0.002	0.288
Rings (R)	10	582.47**	3744.33**	2.37**	699.44**	35.19**	0.539**	5.90**
R × G	10	55.01**	291.21*	0.254ns	1.98*	0.061ns	0.082	0.251
Error	42	9.32	117.38	0.254	0.71	0.65	0.09	0.326
S.O.V	DF	PHO	SC	TCH	CHA	CHB	CAP	DIH
Rep	2	0.069	0.015	4.16	25.69	17.76	2341.37	651.86
Genotypes (G)	1	2.52**	0.269**	1.81ns	20.11*	2.00ns	12272.72	977.51
Rings (R)	10	61.31**	0.506**	24.63*	12.93**	16.52**	18747.01**	3904.62**
R × G	10	3.81**	0.114**	26.84*	15.76**	24.94**	8707.16	2160.41
Error	42	0.0275	0.004	9.28	4.26	5.603	5969.36	1216.08
S.O.V	DF	TPC	FL	FG	FFW	FDW	TNF	TFY
Rep	2	10482.04	0.009	0.002	0.317	0.049	51.1	0.0013
Genotypes (G)	1	387741.69**	0.15	0.023**	2.07**	0.016*	18.11	0.049**
Rings (R)	10	106851.09**	0.615**	0.048**	1.41**	0.037**	258.00**	0.062**
R × G	10	6221.02	0.065	0.002**	0.312**	0.013**	39.16*	0.014**
Error	42	11773.92	0.072	0.0009	0.061	0.002	18.08	0.0017

Note: S.O.V: Source of variation; DF: Degree of freedom; **highly significant at $P \leq 0.01$ level; *significant at $P \leq 0.05$ level; ns non-significant; PH: Plant height; NL: Number of leaves; SD: Stem diameter; DTF: Days to first flowering; PB: Number of primary branches; SB: Number of secondary branches; PHO: Photosynthesis rate; SC: Stomatal conductance; TCH: Total chlorophyll content; CHA: Chlorophyll *a* content; CHB: Chlorophyll *b* content; CAP: Capsaicin content; DIH: Dihydrocapsaicin content; TPC: Total phenolic content; FL: Fruit length; FG: Fruit girth; FFW: Fruit fresh weight; FDW: Fruit dry weight; TNF: Total number of fruit per plant; TYP: Total fruit yield per plant.

2.4.7. Statistical analyses

Analyses of variance (ANOVA) was carried out on all the traits measured using SAS program Version 9.4 to determine significance in variations among gamma irradiation treatments and the two chili varieties. Mean, range, coefficient of variation (CV) and standard deviation were calculated for each trait. Mean comparisons were performed using Least the Significant Difference (LSD) test. The phenotypic correlation coefficient estimates were also carried out using proc corr in SAS, Version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Effect of chronic gamma irradiation on morphological characters

In this study, the effect of chronic gamma irradiation on the performance of two chili varieties was observed by measuring various parameters. The results showed highly significant differences among doses (Rings) of chronic gamma irradiation expressed in mean values for all investigated traits (Table 3). Generally, the relatively moderate doses of chronic gamma irradiation represented by doses 47.096 Gy (Ring 4) and 19.40 Gy (Ring 6) resulted in significant stimulation for most of the studied characters. For plant height, the tallest plants (109.70 cm) in Chili Bangi 3 was recorded in chili irradiated with dose 19.40 Gy (ring 6), while in Chili Bangi 5, the tallest plant recorded heights of 112.17 at dose 30.94 Gy and 117.30 cm at dose 19.40 (Ring 6) whereas height in control was 87.30 cm and 80.50 cm in Chili Bangi 3 and Chili Bangi 5, respectively (Table 4). In mutation breeding, treatment of plant materials (seeds, seedlings, scion, and cutting rhizomes) with gamma irradiation can induce micro-mutations affecting quantitative traits and through phenotypic selection, beneficial mutant lines can be identified to develop better and improve varieties [13]. Pitrimovae [14] reported that increase in height of plants irradiated by chronic gamma irradiation was due to synthesis of nucleic acid which stimulate cell division resulting in an increase in plant height. Plants irradiated with 19.40 Gy were observed to have the highest means in terms of stem diameter, having 14.32 mm and 14.24 mm, followed by mutants irradiated with 47.09 Gy of 13.91 and 13.91 mm for Chili Bangi 3 and Chili Bangi 5 respectively. The lowest values for stem diameter was observed in plants treated with 5.54 Gy (12.38 mm) for the Chili Bangi 3, and 5.54 Gy (12.17 mm) for Chili Bangi 5 (Table 4).

Agrobiotechnology and crop improvement are critical in improving harvests for future generations. Although conventional plant breeding is already accessible for selecting superior genotypes from genetically variable populations, however, induced mutation provides a more rapid option [4]. In the 1980s, Malaysia plant mutagenesis activities were centred on acute gamma radiation. Chronic gamma radiation facility, known as gamma greenhouse (GGH), was established in 2005 to support plant improvement by mutagenesis through chronic radiation. Mutagenesis is the process through which an organism's genetic information is modified in a stable manner, resulting in mutation at the molecular level [4]. Gamma radiation is a physical mutagen for plant mutagenesis through which DNA changes might result in novel features for crop development. Genetic diversity through induced mutagenesis is essential because it has proven to be a reliable source of genetic variability. By changing the dose of the mutagen, it is possible to control the frequency and saturation of mutations [13], and mutagenic agents can cause different lengths of genomic damage, from base mutations to larger fragment insertions or deletions [4].

Among the doses used in this study, the highest number of primary branches per plant in Chili Bangi 3 was recorded in plant irradiated with 47.096 Gy (3.21 branches per plant) while the lowest value for this trait (2.40 branches per plant) was recorded in plant exposed to 5.54 Gy. In control, the mean value for number of primary branches per plant was 2.51 for Chili Bangi 3. In Chili Bangi 5, higher mean values for this trait were recorded in plants irradiated with doses 186.61, 83.11, 47.096, 30.90 and 19.40 Gy with mean values ranging from 3.00 to 3.11 branches per plant whereas in untreated plants, the mean value recorded was 2.50 branches per plant. For number of secondary branches per plant, Chili Bangi 3 recorded higher mean in plants irradiated with 47.096 Gy (6.55 branches

Table 4
Effects of chronic gamma irradiation on morphological quantitative traits.

Ring	PH (cm)		SD (mm)		DTF (days)		PB		SB	
	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5
R0	87.30^{d}	80.50^{d}	12.93^{cde}	12.52^{def}	20.5^{f}	21.0^{g}	21.51^{bc}	2.50^{bc}	5.20^{cd}	5.00^{cd}
R2	96.92^{c}	102.17^{cd}	13.76^{abc}	13.70^{ab}	49.3^{a}	50.7^{a}	3.11^{a}	3.11^{a}	6.44^{a}	6.40^{a}
R3	101.90^{bc}	103.40^{cd}	13.92^{ab}	13.30^{bcd}	48.3^{a}	49.0^{b}	3.0^{ab}	3.00^{a}	6.50^{ab}	6.10^{b}
R4	102.80^{b}	110.27^{b}	13.91^{ab}	13.91^{ab}	30.7^{b}	29.7^{c}	3.21^{a}	3.10^{a}	6.55^{a}	6.41^{a}
R5	100.80^{bc}	112.10^{ab}	13.60^{abc}	113.04^{cd}	30.7^{b}	29.0^{c}	2.90^{ab}	3.00^{a}	6.08^{bc}	6.20^{b}
R6	109.70^{a}	117.30^{a}	14.32^{a}	14.24^{a}	21.30^{de}	22.70e	3.00^{ab}	3.00^{a}	6.20^{b}	6.30^{b}
R7	87.50^{d}	96.75^{e}	12.56^{ef}	12.11^{f}	20.70^{e}	21.30^{fg}	2.60^{b}	2.73^{ab}	5.33^{cd}	5.37^{c}
R8	88.73^{d}	97.30^{e}	12.78^{def}	12.96^{cde}	21^{e}	21.7^{ef}	2.80^{ab}	2.71^{ab}	5.80^{bc}	5.36^{c}
R9	91.47^{d}	101.7^{de}	12.67^{ef}	13.15^{bcd}	22.7^{cd}	22.7^{e}	2.50^{bc}	2.21^{c}	5.00^{d}	4.33^{e}
R11	87.70^{d}	87.84^{f}	12.38^{f}	12.17^{ef}	23.3^{cd}	25.3^{d}	2.40^{c}	2.11^{c}	4.75^{e}	4.22^{e}
R14	88.09^{d}	86.37^{f}	12.92^{cdef}	12.54^{def}	23.7^{c}	24.7^{d}	2.50^{bc}	2.33^{bc}	4.50^{e}	4.66^{de}
Mean	92.15	93.38	13.52	13.33	28.22	29.13	2.81	2.72	5.64	5.6
LSD	5.01	5.607	0.907	0.805	1.59	1.288	0.599	0.407	0.663	0.576
SE	1.575	2.048	0.131	0.134	1.835	1.818	0.068	0.071	0.135	0.138

Note: Mean values with different letters within same columns are significantly different by LSD test at 5 % significant level; SE: Standard Error. PH: Plant height; SD: Stem diameter; DTF: Days to first flowering; PB: Number of primary branches; SB: Number of secondary branches.

per plant) and 83.11 Gy (6.50 branches per plant), followed by plants administered with 185.61 Gy (6.44 branches per plant) and 19.40 Gy (6.20 branches per plant). Meanwhile, in control treatment of Chili Bangi 3, the mean value recorded was 5.20 branches per plant. In Chili Bangi 5, higher mean values for number of secondary branches per plant were respectively observed in plants irradiated with 185.61 Gy (6.40 branches per plant), 47.096 Gy (6.41 branches per plant) and 19.40 Gy (6.30 branches per plant) compared to control (5.00 branches per plant) (Table 4). Accordingly, the high and moderate doses of chronic gamma irradiation induced stimulatory effects as shown in increased numbers of primary and secondary branches in irradiated chili plants. Gamma irradiation induced substantial improvement in plant height, number of branches per plant, grain yield, and oil content in Mutant plant. The previous study conducted by Shamsiah et al. [5], found that the effects of mutagens on the physiological system were responsible for either an increase or a decrease in the length of the shoots. According to Aisha et al. [16], some plant traits that are associated with increasing trend in the dosage of gamma radiation may be linked to the loss of water and nutrient absorption caused by an intense influence of irradiation on the growth and development of the root system. Additionally, it is possible that the activation of auxin and the in vivo division rate contributed to the outcomes that were produced as a result of the irradiation treatments. It is possible to draw a provisional conclusion that the stimulating effects of low doses of gamma-ray on plant development may be attributed to the stimulation of cell division or cell elongation, as well as the enhancement of metabolic processes that affect the production of phytohormones or nucleic acids. On the other hand, it was found that exposure to large amounts of gamma irradiation could be hazardous [15].

Okamura [17] reported a beneficial effect of chronic irradiation where few traits were improved due to reduced occurrence of radiation damage. Compared to control, day to first flowering was delayed in all plants treated with chronic gamma doses (Rings) for both Chili Bangi 3 and Chili Bangi 5. However, this was not the case for mutants derived from 13.90 Gy (Ring 7) which showed first flowering at the same time as the control (21 days after transplanting). Gamma irradiation severely delayed first flowering among the chili plants studied. Plants irradiated with higher gamma irradiation doses at 185.61 Gy and 85.11 Gy showed first flowering at 50 and 49 days after transplanting for the two varieties, respectively. In days to 50 % flowering, non-irradiated (control) plants reached 50 % flowering earlier (24.70 days after transplanting) compared to mutants at all doses (rings) except for plants treated with 13.90 Gy and 11.1 Gy where days to 50 % flowering was attained at 25.0 and 25.70 days, respectively for Chili Bangi 3 and plants irradiated with 13.90 Gy (25.3 days after transplanting) for Chili Bangi 5. As presented in Table 4, plants exposed to higher doses at 185.61 Gy and 85.11 Gy took longer time (56.70 and 54.70 days after transplanting, respectively) to attain 50 % flowering compared to other doses for Chili Bangi 3 and Chili Bangi 5 at 57.00 and 54.60 days after transplanting, respectively. High doses of irradiation appeared to increase number of days to 50 % flowering. As reported by Pushparajan et al. [18], higher doses of gamma-rays delayed days to flowering in okra plants. Similarly, inhibitory effects of high doses of chronic gamma irradiation on flower development characteristics of *Curcuma alismatifolia* was reported whereas desirable variations were stimulated by low gamma irradiation doses [19]. These reports, corroborate results from our study that days to flowering may be significantly delayed by high doses of chronic gamma irradiation. According to Shah et al. [20], different flowering days may be related to the seed metabolism and the initial stage of the process of DNA synthesis as a consequence of the effects of gamma rays. This may be largely owing to the fact that cells that sustain relatively more chromosomal damage as a result of high exposures to gamma radiation are at a high risk due to the diplontic segment, which is unable to integrate effectively with normal cells and ultimately causes a minor delay in flowering.

3.2. Effects of chronic gamma irradiation on physiological and biochemical characters

Gamma irradiation can induce desirable alterations of physiological parameters [21]. Biological effects of gamma-rays depend on interaction with cells particles or molecules; especially water to generate free radicals, which can cause damage to different vital compounds of plant cell leading to physiological and biochemical changes [21]. In the present study, relatively moderate and low doses of chronic gamma irradiations result in higher mean value of chlorophyll content in the two varieties. Higher chlorophyll content

Table 5
Effects of chronic gamma irradiation on physiological traits.

Ring	PHO		SC		TCH		CHA		CHB	
	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi 5	Bangi3	Bangi5
R0	15.23^{h}	15.35^{h}	0.379^{d}	0.388^{e}	38.80^{cd}	38.20^{ab}	22.01^{b}	23.17^{b}	12.16^{cd}	11.86^{bc}
R2	16.78^{e}	18.85^{d}	1.22^{b}	0.566^{cd}	42.83^{abc}	41.80^{a}	16.95^{d}	23.68^{abcd}	15.35^{bc}	13.1^{bc}
R3	18.85^{d}	18.58^{e}	1.38^{a}	0.745^{b}	41.17^{abc}	42.30^{a}	22.48^{bc}	24.56^{abcd}	9.78^{d}	13.00^{bc}
R4	24.55^{a}	24.21^{a}	0.668^{c}	0.635^{c}	41.30^{abc}	42.35^{a}	19.11^{cd}	20.15^{d}	15.62^{bc}	17.45^{ab}
R5	20.35^{c}	21.75^{b}	0.758^{c}	0.557^{d}	42.50^{abc}	43.32^{a}	17.10^{d}	27.27^{ab}	20.87^{a}	19.86^{a}
R6	20.75^{b}	20.75^{c}	0.748^{c}	0.931^{a}	44.64^{a}	43.10^{a}	27.17^{ab}	27.88^{a}	13.27^{cd}	11.65^{bc}
R7	15.73^{h}	15.29^{i}	0.336^{d}	0.313^{f}	35.96^{de}	41.02^{a}	24.09^{bc}	28.08^{a}	12.61^{cd}	12.71^{bc}
R8	15.35^{i}	16.35^{g}	0.269^{d}	0.195^{g}	43.49^{ab}	34.80^{b}	24.02^{bc}	24.18^{abcd}	19.97^{ab}	10.35^{c}
R9	16.25^{f}	17.65^{f}	0.317^{d}	0.347^{ef}	$37.5d^{e}$	39.16^{ab}	25.83^{b}	22.8^{bcd}	11.95^{cd}	17.45^{ab}
R11	15.24^{j}	15.25	0.363^{d}	0.335^{ef}	42.13^{abc}	40.02^{ab}	26.48^{b}	21.53^{cd}	16.23^{ab}	17.86^{ab}
R14	16.57^{g}	14.02^{k}	0.365^{d}	0.367^{ef}	40.07^{bcd}	40.20^{a}	23.92^{bc}	24.05^{abcd}	16.18^{abc}	17.23^{b}
Mean	17.25	18.17	0.6	0.49	52.48	51.94	23.55	24.3	15.28	14.45
LSD	0.37	0.02	0.15	0.01	4.115	5.307	5.34	4.6	5.16	2.95
SE	0.57	0.53	0.066	0.038	0.687	0.627	1.02	0.575	0.867	0.694

Note: Mean values with different letters within same columns are significantly different by LSD test at 5 % significant level; SE: Standard Error. PHO: Photosynthesis rate; SC: Stomata conductance; TCH: Total chlorophyll content; CHA: Chlorophyll *a* content; CHB: Chlorophyll *b* content.

of mutants (44.64 μg/ml) was recorded at dose 19.40 Gy in Chili Bangi 3 whereas, in Chili Bangi 5, higher mean for this trait (42.35 μg/ml) was recorded in plant irradiated with 47.096 Gy (Table 5). Chlorophyll content for untreated plants was 38.80 μg/ml in both chili varieties (Table 5). In terms of chlorophyll *a*, the highest mean value was recorded in mutants of Chili Bangi 3 at 19.40 Gy (27.17 μg/ml) while chlorophyll *a* in control was 22.01 μg/ml. In Chili Bangi 5, the highest chlorophyll *a* was recorded in plants irradiated with 13.90 Gy (28.80 μg/ml) followed by 19.40 Gy (27.88 μg/ml) while chlorophyll *a* in the untreated plant was 23.17 μg/ml (Table 5). The highest means in term of chlorophyll *b* were observed at dose 30.47 Gy (20.87 and 19.86 μg/ml) for Chili Bangi 3 and Chili Bangi 5 respectively, while chlorophyll *b* was 12.16 and 11.86 μg/ml in control for both varieties (Table 5). Alikamanoğlu et al. [22] reported that the levels of total chlorophyll, chlorophyll *a* and *b* in *Paulownia tomentosa* plants increased upon treatment with gamma-ray. Contrarily, Kiong et al. [21] reported a 55.9 % reduction in total chlorophyll content of *Orthosiphon stamineus* plants treated with 70 Gy compared to non-treated plants. The detrimental effects of gamma irradiation on growth limiting factors were primarily related to the changes that occur during the process of photosynthesis and chlorophyll concentration. However, different results were observed where chlorophyll had no notable response to low doses of gamma-ray. Furthermore, a study reported significant increase in chlorophyll levels in red pepper plants irradiated at 16 Gy [23].

For photosynthesis rate and stomatal conductance, the result showed significant variation among the gamma-irradiated doses (Rings). Mutant plants derived from 47.096 Gy of both chili varieties showed higher photosynthesis rate (24.55 and 24.21 μmol CO_2 m^{-2} s^{-1}) compared to control (15.23 and 15.35 μmol CO_2 m^{-2} s^{-1}). Stomatal conductance was higher in mutants produced by irradiating Chili Bangi 3 with 85.61 Gy (1.38 mol H_2O m^{-2} s^{-1}) and 19.40 Gy (0.931 mol H_2O m^{-2} s^{-1}), respectively compared to untreated plants where stomatal conductance was 0.379 and 0.388 mol H_2O m^{-2} s^{-1}in both Chili Bangi 3 and Chili Bangi 5, respectively (Table 5). This result is in agreement with the findings of Wani et al. [24] who reported the stimulatory effects of gamma-ray doses on photosynthesis rate and stomatal conductance in Pongamia pinnata plants. In contrast, the findings of Moghaddam et al. [25] reported that there was no notable difference in terms of photosynthesis rate and stomata conductance among irradiated plants of *Centella asiatica*.

Chili Bangi 3 showed higher capsaicin content at dose 19.40 Gy (373.00 μg/mL) and 13.90 Gy (332.00 μg/mL) compared to control (117.80 μg/mL). The lower capsaicin content among chronic doses was observed at 2.77 Gy (154.00 μg/mL) in this variety. Chili Bangi 5 recorded higher capsaicin content at dose 11.10 Gy (406.30 μg/mL) followed by 30.47 Gy (364.00 μg/mL) and 19.40 Gy (319.60 μg/mL) while, lower values for this trait was recorded in control (108.60 μg/mL) (Table 6). In the case of dihydrocapsaicin content, Chili Bangi 3 recorded higher mean (191.3 μg/mL) at an irradiation dose of 19.40 Gy followed by 13.90 Gy (149.70 μg/mL), whereas control recorded lower mean values (99.00 μg/mL) of dihydrocapsaicin content. Chili Bangi 5 recorded higher dihydrocapsaicin content at an irradiation dose of 11.1 Gy (222.30 μg/mL) followed by 19.90 Gy (154.00 μg/mL) and 30.47 Gy (150.00 μg/mL) while, lower values for dihydrocapsaicin content in Chili Bangi 5 was recorded in control (88.00 μg/mL) (Table 6). The main reaction responsible for this is ionization, which generates ionized water particles (H_2O) as well as H- and OH-. In organic tissue, these ionizations are stimulated along the entire path of radiation causing series of reactions, which generate secondary reactive oxygen species (ROS). Aruldoss et al. [26] studied the effect of gamma-ray and EMS on phytochemical components such as capsaicin content in chili (*Capsicum annuum* L.). The researcher reported a variation in capsaicin contents induced at 40 kR (0.41 %) of gamma-ray and 30 mM EMS (0.43 %) compared to non-treated plants (0.36 %). However, there was no effect of gamma irradiation (3 and 7 kGy) on colour and capsaicinoids in red peppers powder [26]. The higher value in degree of pungency represented by Scoville Heat Unit (SHU) values for Chili Bangi 3 was recorded in plant irradiated with 19.40 Gy (9085.23 SHU) followed by 13.90 Gy (7755.37 SHU), whereas lower values of SHU was recorded in Chili Bangi 3 irradiated with 5.54 Gy (3965.43 SHU) and control where the SHU value of 3490.48 was recorded. In Chili Bangi 5, the highest SHU was recorded in plant irradiated with 13.90 Gy (10120.46 SHU) followed by 19.40 Gy (7624.96 SHU) and 30.47 Gy (8275.40 SHU). Lower values of SHU for Chili Bangi 5 were recorded by control (3165.26 SHU). According to the classification of SHU, all chronic doses and control as observed in their values within the range of 3000–25,000 SHU as categorized as

Table 6
Effects of chronic gamma irradiation on biochemical traits.

Ring	CAP		DIH		SHU		TPC	
	Bangi 3	Bangi 5	Bangi 3	Bangi 5	Bangi 3	Bangi5	Bangi3	Bangi 5
R0	117.8 ^{cd}	108.60^{dc}	99.00^{bc}	88.00^{c}	3490.48^{c}	3165.26^{c}	509.45^{d}	497.27^{d}
R2	251.00^{bcd}	215.00^{bc}	128.00^{bc}	125.00^{bc}	6101.00^{bc}	5474.00^{bc}	787.53^{a}	734.98^{b}
R3	239.00^{bcd}	169.30^{c}	118.00^{bc}	104.00^{bc}	5747.70^{bc}	4400.13^{c}	771.54^{a}	763.23^{a}
R4	223.00^{bcd}	261.30^{abc}	136.00^{abc}	104.00^{bc}	5779.90^{bc}	5881.33^{bc}	776.72^{a}	732.48^{a}
R5	265.00^{abc}	364.00^{ab}	136.30^{abc}	150.00^{bc}	6460.93^{bc}	8275.40^{ab}	762.46^{a}	753.94^{a}
R6	373.00^{a}	319.60^{abc}	191.30^{a}	154.00^{b}	9085.23^{a}	7624.96^{abc}	785.40^{a}	762.90^{a}
R7	332.00^{ab}	298.00^{abc}	149.70^{ab}	149.00b^{c}	7755.37^{ab}	7196.70^{abc}	721.85^{ab}	715.6^{bc}
R8	227.00^{bcd}	406.30^{a}	125.00^{bc}	222.30^{a}	5667.20^{bc}	10120.46^{a}	727.27^{bcd}	740.6^{c}
R9	231.00^{bcd}	273.00^{abc}	129.00^{bc}	149.30^{bc}	5796.00^{bc}	6799.03^{abc}	682.9^{b}	615.6^{cd}
R11	209.00^{cd}	237.30^{bc}	113.30^{bc}	118.00^{bc}	3965.43^{c}	5720.33^{bc}	633.31^{bc}	635.6^{cd}
R14	154.00^{d}	225.30^{abc}	92.30^{c}	139.00^{bc}	5139.03^{c}	5865.23^{bc}	578.1^{d}	620.6^{cd}
Mean	243.88	271.15	128.79	136.48	5999.93	6562.95	752.91	698.61
LSD	110.17	154.83	55.933	64.657	2542	3429.6	109.48	104.28
SE	14.01	17.479	6.444	17.478	320.017	399.67	29.81	25.88

Note: Mean values with different letters within same columns are significantly different by LSD test at 5 % significant level; SE: Standard Error; CAP: Capsaicin content; DIH: Dihydrocapsaicin content; SHU: Soville Heat Unit; TPC: Total phenolic content.

moderately pungent (Table 6).

In terms of total phenolic content, higher mean values were observed in plants irradiated with 185.61Gy (787.53 mg/g) and 19.40 Gy (785.40 mg/g) followed by 83.11 Gy (771.54 mg/g), 47.096 Gy (776.72 mg/g) and 30.47 Gy (762.46 mg/g) for Chili Bangi 3. In Chili Bangi 5, total phenolic content doses was 85.61Gy (763.23 mg/g), 19.40 Gy (762.90 mg/g) and 30.47 Gy (753.94 mg/g). Lower total phenolic content (509.45 mg/g and 497.27 mg/g) was recorded in untreated plants of the two varieties (Table 6). These data were consistent with the findings of Akshatha and Chandrashekar [27], in their study on *Pterocarpus santalinus*, where they observed that treatment with gamma irradiation considerably enhanced total phenolic content of irradiated samples compared to untreated samples. Stimulating effects of gamma irradiation on total phenolic content could be due to the damaging effects of irradiation and oxidation, which break chemical bonds of polyphenols into smaller molecular weights such as soluble phenols [28].

3.3. Effect of chronic gamma irradiation on yield and yield characteristics

Improved yield is a major objective in plant breeding program. Improved yield and yield characteristics such as fruit length, fruit girth, number of fruits per plant, fruit fresh weight and fruit dry weight are priorities for chili breeders because all of these traits directly affect total yield per plant. In the present study, doses of chronic gamma irradiation induce desirable variations in fruit length as plants irradiated with doses 30.47 and 19.40 Gy recorded higher means for fruit length in the two varieties. The mean fruit length of plant irradiated with 30.47 and 19.40 Gy was 10.30 cm and 10.32 cm for Chili Bangi 3 while mean value for this trait at the two irradiation doses were 10.31 cm and 10.20 cm, respectively for Chili Bangi 5 (Table 7). The rest of the doses (rings) showed similar performance with control. Significant variation was observed in fruit girth (cm) among doses of chronic irradiation (Rings). In both varieties, high to moderate means were recorded in plants irradiated with dose 185.61, 85.11, 47.096, 30.90 and 19.40 Gy with means ranging from 1.59 to 1.61 cm. Lower irradiation doses such as 5.54 Gy and 2.77 Gy results in decrease fruit girth for Chili Bangi 3 (1.43 cm and 1.44 cm, respectively) and Chili Bangi 5 (1.39 cm and 1.41 cm, respectively). The mean fruit girth for untreated plants were 1.50 cm for Chili Bangi 3 and 1.38 cm for Chili Bangi 5 (Table 7). Increased fruit fresh weight was recorded in plants irradiated with moderate gamma irradiation for the two chili varieties under study.

In terms of fresh fruit weight among mutants of Chili Bangi 3, the highest fresh weight was recorded in plants exposed to 30.47 Gy (9.71 g) and 19.40 Gy (9.86 g) whereas control plants had a mean value of 9.10 g. Mutants of Chili Bangi 5 (185.61, 85.11, 47.096, 30.90 and 19.40 Gy) had higher fruit fresh weight with values ranging from 10.01 g (185.61 Gy) to 10.43 g (19.40 Gy) while lower mean value (8.63 g) was recorded for this trait in unirradiated plant (Table 7). Higher fruit dry weight was recorded in Chili Bangi 3 irradiated with19.40 Gy (1.33 g) and 30.47 Gy (1.31 g) while mean fruit dry weight in the rest of the chronic doses was similar to control (1.12 g).

In Chili Bangi 5, higher fruit dry weight was observed in mutants exposed to 83.11 Gy (1.38 g), 47.096 Gy (1.39) and 19.40 Gy (1.37 g) compared to control (1.13 g). Significant differences was recorded in total number of fruits per plant due to different doses of chronic gamma irradiation (Rings) and interaction between varieties and doses (Table 3). Higher mean value for total number of fruits per plant in Chili Bangi 3 was recorded in plants irradiated with 83.11 Gy (123.96), 47.096 Gy (119.90), 30.47 Gy (121.43) and 19.40 Gy (120.80), whereas mean value in control was 108.50. In Chili Bangi 5, the mutants irradiated with 47.096 Gy (118.40) and 19.40 Gy (120.60) scored higher means for total number of fruits per plant compared to untreated plants (110.00) (Table 7). The analyses of variance revealed highly significant differences in total fruit yield per plant among the different doses (Rings), while, significant interaction was also observed between varieties and doses.

For Chili Bangi 3, higher total fruit yield per plant was obtained by mutants irradiated with 185.61, 85.11, 47.096, 30.90 and 19.40 Gy with mean value ranging from 1.06 kg (185.61 Gy) to 1.09 kg (47.096 Gy) compared to 0.989 kg in untreated plants. The highest total fruit yield per plant in Chili Bangi 5 was recorded at 19.40 Gy (1.31 kg) while mean value for total fruit yield in control was 1.06

Table 7
Effects of chronic gamma irradiation on yield and yield-related traits.

Ring	FL (cm)		FG (cm)		FFW (g)		FDW (g)		TNF		TYP (kg)	
	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5	Bangi3	Bangi5
R0	9.50^{d}	9.20^{f}	1.51^{b}	1.38^{b}	9.10^{bcd}	8.63^{d}	1.12^{c}	1.13^{cd}	108.50^{cd}	110.00^{abc}	0989^{bc}	1.06^{c}
R2	10.10^{ab}	10.20^{abc}	1.61^{a}	1.61^{a}	9.13^{bcd}	10.01^{a}	1.21^{c}	1.34^{ab}	113.80^{bc}	115.43^{ab}	1.06^{a}	1.21^{b}
R3	10.02^{ab}	10.04^{abc}	1.59^{a}	1.59^{a}	9.26^{bc}	10.40^{a}	1.22^{bc}	1.38^{a}	123.96^{a}	114.12^{abc}	1.07^{a}	1.61^{b}
R4	9.69^{abc}	10.10^{abc}	1.59^{a}	1.60^{a}	9.46^{ab}	10.20^{a}	1.19^{c}	1.39^{a}	119.90^{ab}	118.40^{a}	1.09^{a}	1.20^{b}
R5	10.30^{a}	10.31^{a}	1.59^{a}	1.59^{a}	9.71^{a}	10.20^{a}	1.31^{ab}	1.26^{bc}	121.43^{ab}	115.20^{ab}	1.07^{a}	1.17^{b}
R6	10.32^{a}	10.20^{ab}	1.61^{a}	1.61^{a}	9.86^{a}	10.43^{a}	1.33^{a}	1.37^{a}	120.80^{ab}	120.60^{a}	1.09^{a}	1.31^{a}
R7	9.66^{bcd}	9.97^{bcde}	1.45^{bc}	1.38^{b}	8.81^{d}	8.80^{cd}	1.18^{c}	1.10^{f}	104.90^{d}	111.20^{bcd}	0.919^{d}	0.95^{de}
R8	9.40^{d}	9.70^{de}	1.48^{bc}	1.41^{b}	9.10^{bcd}	8.96^{bcd}	1.19^{c}	1.11^{ef}	106.70^{cd}	100.03^{f}	0.96^{cd}	0.846^{f}
R9	9.40^{d}	9.83^{bcde}	1.46^{bc}	1.41^{b}	8.91^{cd}	9.33^{b}	1.21^{c}	1.2^{cde}	106.80^{cd}	103.43^{ef}	1.03^{ab}	0.98^{de}
R11	9.50^{cd}	9.60^{ef}	1.43^{c}	1.39^{b}	8.80^{d}	9.10^{bc}	1.13^{c}	1.13^{def}	105.80^{d}	106.13^{def}	0.99^{bc}	1.00^{cd}
R14	9.70^{bc}	9.75^{cde}	1.44^{c}	1.41^{b}	9.12^{bcd}	9.20^{bc}	1.14^{c}	1.16^{def}	104.90^{d}	107.63^{cde}	0.94^{cd}	0.91^{ef}
Mean	9.79	9.83	1.53	1.54	9.01	9.1	1.17	1.19	114.1	113.91	972.6	965.6
LSD	0.496	0.415	0.065	0.0382	0.407	0.449	0.092	0.077	7.679	7.124	0.061	0.078
SE	0.0723	0.0642	0.0139	0.0182	0.0699	0.1204	0.015	0.022	1.443	1.245	0.012	0.026

Note: Mean values with different letters within same columns are significantly different by LSD test at 5 % significant level; SE: Standard Error; FL: Fruit length; FG: Fruit girth; FFW: Fruit fresh weight; FDW: Fruit dry weight; TNF: Total number of fruit per plant; TYP: Total fruit yield per plant.

kg (Table 7). These results suggest that stimulatory effects were induced by relatively high doses; 185.61 Gy and 83.11 Gy and moderate doses 47.096 Gy, 30.474 Gy and 19.40 Gy of chronic gamma-ray on yield and yield characteristics of chili varieties. Treatment with gamma irradiation have effects on morphological characters such as plant height, tillers, number of filled grains number of fruits per plant, total yield per plant and plant maturity of mutant plants in chili and rice [13,16].

3.4. Estimation of genetic parameters (coefficient of variation, heritability and genetic advance)

In crop improvement, selection based on phenotypic expressions can be effective when high phenotypic variation is detected and accompanied with high genotypic variation. This reflects the existence of high genetic variability for various traits and less impact of environment [2]. The magnitude of variability in respect to phenotypic ($\sigma 2p$) and genotypic ($\sigma 2g$) variance and phenotypic (PCV) and genotypic coefficient of variation (GCV) for morphological, physiological, biochemical, yield and yield characters of this study are presented in Table 8. The estimation of PCV and GCV in investigated characters ranged from 6.29 % (fruit length) to 75.64 % (days to first flowering) for the former, and from 5.79 % (fruit length) to 75.59 % (days to first flowering) for the latter. High PCV (>20 %) was observed in days to first flowering (75.64 %), primary branches per plant (23.40 %), secondary branches per plants (36.37 %), chlorophyll *b* (28.48 %), chlorophyll *a* (19.53 %), capsaicin content (48.49 %), dihydrocapsaicin content (42.87 %) and total phenolic content (22.01 %). For GCV, higher percentages were recorded in days to first flowering (75.59 %), primary branches per plant (21.03 %), and secondary branches per plants (35.05 %), chlorophyll *b* (24.67 %), capsaicin content (40.64 %) and dihydrocapsaicin content (36.06 %). This implies that these characters are dominated by additive genes. Consequently, phenotypic selection is sufficient to bring about desirable improvement for these traits. The findings of this research are in agreement with those reported by Usman et al. [2]. Phenotypic variation is a result of the interaction between genetic and environmental factors.

Heritability is a measure of the degree of phenotypic variation caused by genetic effects. Successful selection can only be achieved if the additive effects are sufficiently higher than the effect of environment. Heritability percentage is considered low at range between 0 and 30 %, while 30–60 % is classified as moderate and values above 60 % is regarded as high. Broad sense heritability values of the studied characters of chili are presented in Table 8. All the studied characters were highly heritable, these include plant height (97.55 %), days to first flowering (99.88 %), stem diameter (84.41 %), number of primary branches (80.82 %), number of secondary branches (92.92 %), chlorophyll *a* (83.14 %), chlorophyll *b* (75.05 %), total chlorophyll content (72.34 %), capsaicin content (70.23 %), dihydrocapsaicin content (70.25 %), total phenolic content (70.55), fruit length (84.91 %), fruit girth (96.16 %), fruit fresh weight (94.88 %), total number of fruit per plant (92.27 %) and total fruit yield per plant (96.37 %), however, fruit dry weight recorded moderate value among the traits studied (34.66 %).

Genetic advance (GA) is the direct relationship between heritability and selection responses. Information on the extent and nature of variation among mutants for specific traits has been considered important for making effective selection for many traits in chili pepper improvement. Genetic advance measures how the selection of population can be successful and effective. Johnson et al. [29] categorized genetic advance as low when the value ranges from 0 to 10 %, moderate at values between 10 and 20 % and high at values of ≥20 %. In this study, traits such as plant height (37.56 %), days to first flowering (55.53 %), number of primary branches (35.03 %), number of secondary branches (67.09 %), chlorophyll *a* (30.51 %), chlorophyll *b* (38.14 %), capsaicin content (58.79 %), dihydrocapsaicin content (52.56 %), total phenolic content (22.45 %), fruit girth (22.23 %), fruit fresh weight (19.68 %), total number of

Table 8
Estimates of broad sense heritability, genotypic, and phenotypic coefficient of variance and genetic advance for 17 traits.

Trait	$\sigma^2 p$	$\sigma^2 g$	PCV (%)	GCV (%)	h^2_B (%)	GA (%)
PH	328.69	336.94	18.69	18.92	97.55	37.56
SD	1.037	1.228	7.81	8.49	84.41	13.57
DTF	466.11	466.66	75.59	75.64	99.88	55.53
TCH	21.83	30.17	11.87	13.96	72.34	17.69
PB	0.322	0.397	21.03	23.4	80.82	35.03
SB	3.88	4.176	35.05	36.36	92.92	67.09
CHA	17.63	21.21	17.81	19.53	83.14	30.51
CHB	14.76	19.67	24.67	28.48	75.05	38.14
CAP	10950.38	15593.23	40.64	48.49	70.23	58.79
DIH	2287.8	3233.65	36.06	42.87	70.75	52.56
TPC	34121.6	49345.66	42.24	35.12	69.15	60.17
FL	0.323	0.3799	6.29	5.79	84.91	10.13
FG	0.028	0.0295	11.44	11.22	96.16	22.23
FFW	0.89	0.938	10.07	10.34	94.88	19.68
FDW	114.98	331.75	327.84	556.87	34.66	34.07
NF	147.46	159.82	10.85	11.29	92.27	20.62
TYP	0.0377	0.0391	18.56	18.91	96.37	36.85

Note: $\sigma 2g$: genotypic variation; $\sigma 2p$: phenotypic variation; GCV: genotypic coefficient variation; PCV: phenotypic coefficient variation, h^2_B : broad–sense heritability; GA: genetic advance; PHAH: plant height at harvest; SD: stem diameter; DTF: days to flowering; CAP: capsaicin content (μg/mL); DIH: dihydrocapsaicin content (μg/mL); TCH: chlorophyll content, CHA: chlorophyll content CHB: chlorophyll content; TPC: Total phenolic content; FL: fruit length; FG: fruit girth; FFW: fruit fresh weight; FDW: fruit dry weight; NF: number of fruit per plant; TYP: Total yield per plant.

Table 9
Combined analysis for phenotypic correlations coefficient among the investigated traits of chronic gamma radiation.

Trait	NL	SD	DTF	TCH	PB	SB	CHA	CHB	FL	FG	FFW	FDW	TYP
PH	0.68**	0.51**	0.48**	0.03^{ns}	0.40**	0.43**	0.11^{ns}	0.02ns	0.62**	0.65**	0.75**	0.14^{ns}	0.60**
NL		0.58**	0.53**	0.05^{ns}	0.59**	0.65**	−0.18*	0.09ns	0.56**	0.76**	0.68**	0.23**	0.66**
SD			0.35**	0.04^{ns}	0.48**	0.54**	0.02^{ns}	−0.08	0.40**	0.68**	0.53**	0.01^{ns}	0.48**
DTF				0.38**	0.31**	0.51**	0.36**	0.08ns	0.29**	0.59**	0.36**	0.01^{ns}	0.35**
TCH					−0.1^{ns}	−0.25*	0.59**	0.62**	0.12^{ns}	−0.04^{ns}	0.16*	0.19*	0.22*
PB						0.84**	−0.1^{ns}	0.08^{ns}	0.38**	0.57**	0.48**	0.19*	0.40**
SB							−0.2^{ns}	0.09^{ns}	0.33**	0.64**	0.41**	0.16*	0.41**
CHA								−0.2*	0.06ns	−0.13^{ns}	0.001^{ns}	0.02^{ns}	0.22*
CHB									0.11ns	0.002^{ns}	0.14*	0.23**	0.07^{ns}
FL										0.58**	0.62**	0.21*	0.42**
FG											0.63**	0.13^{ns}	0.62**
FFW												0.11^{ns}	0.71**
FDW													0.19*

Note: ** Highly significant at P $\leq$ 0.01 level; * Significant at P $\leq$ 0.05 level; ns non-significant; PH: Plant height, NL; Number of leaves; SD: Stem diameter; DTF: Day to first flowering; TCH: Total chlorophyll content; PB: Number of primary branches per plant; SB: Number of secondary branches per plant; CHA: Chlorophyll *a* content; CHB: Chlorophyll *b* content; FL: Fruit length; FG: fruit girth; FFW: Fruit weight; FDW: Fruit dry weight; TNF: Number of fruits; TYP: Total fruit yield per plant.

fruit per plant (20.62 %) and total fruit yield per plant (36.85 %) were observed to have high genetic advances (≥20 %). This suggests that phenotypic expression of mutants is significantly affected by the additive gene with much less influence of environment. Thus, the effective selection for this trait can be expected in the subsequent generations (M2 and M3). Additonally, moderate genetic advances were shown by some traits such as stem diameter (13.57 %), total chlorophyll content (17.69 %) and fruit girth (10.13 %) (Table 8).

The evaluation of a trait's genotypic coefficient of variation (GCV) in relation to its phenotypic coefficient of variation (PCV) is the most accurate method for determining the amount of genetic variation present in a morphophysiological trait. In this study, slight variation between the GCV and the PCV was observed in plant height, days to flowering, fruit girth, fruit fresh weight, and total yield per plant. This suggests that genotype has a highly significant effect on phenotypic expression, whereas environment has a very small effect. On the other hand, when comparing GCV with PCV, significant variations were found in the amounts of chlorophyll *b*, capsaicin, dihydrocapsaicin, total phenolic content, and fruit dry weight which revealed the influence of the environment. In this experiment, a high GCV was observed in several traits such as fruit dry weight and days to flowering. This shows that these traits have a high degree of variability and that further selection might be employed to improve the genotypes for the traits. On the other hand, a low GCV indicates that there has been minimal improvement for the traits through selection.

High heritability along with high genetic advance was recorded for most of the studied characters such as plant height, days to first flowering, stem diameter, number of primary branches, number of secondary branches, chlorophyll *a*, chlorophyll *b*, capsaicin content, dihydrocapsaicin content, fruit length, fruit fresh weight, total number of fruits per plant and total fruit weight per plant. This strongly implies that the expressions of the traits are governed by gene action, thus, such traits can be exploited for developing superior genotype based on phenotypic performance. High heritability associated with moderate genetic advance was shown for stem diameter, total chlorophyll content and fruit girth. This indicates that such characters are less influenced by environmental factors and can, therefore, be inherited in subsequent generations of M2 and M3. According to variability and estimation of heritability, traits such as plant height, days to first flowering, stem diameter, number of primary branches, number of secondary branches, chlorophyll *a*, chlorophyll *b*, capsaicin content, dihydrocapsaicin content, fruit girth, fruit length, fruit fresh weight, total number of fruits per plant and total fruit yield per plant can be utilized in selection for chili varietal improvement. According to Usman et al. [2], the efficacy of selection is dependent not only on heritability but also on genetic progress. The combination of a high GCV with a high heritability and genetic progress provides more information than heritability alone [13]. The high heritability and genetic progress observed in this study indicate that these traits are primarily controlled by additive genes. Therefore, selection may be useful for enhancing these characteristics in chili pepper. Several characteristics of chili have been found to have a high heritability alongside genetic progress, as reported in Ref. [2]. This study corroborates the findings of [32], who observed high genetic progress and high heritability in anthracnose-resistant and heat-tolerant chile inbred lines.

3.5. Phenotypic correlation coefficient of quantitative traits

Improvement of yield is the main aim in most crop breeding program. Yield is a result of complex correlations with other characters that affect yield. It is a quantitative character controlled by many genes. Selection for improved yield cannot be established on yield only because yield is more affected by its related components. Thus, other yield associated traits must be given equal consideration [30]. The correlation coefficient provides an indication of the various relations with yield and yield components, and this correlation may be positive or negative. In the present study, phenotypic correlation coefficient of quantitative traits in worked out to estimate the association among these traits. In this generation, the results showed significant positive, negative and non-significant phenotypic correlation coefficient among quantitative traits. Total fruit yield per plant showed a highly significant positive correlation with fruit fresh weight (0.71) and fruit girth (0.62), total number of fruits per plant (0.60) and plant height (0.60). Moderate significant positive correlations were observed in fruit length (0.42), stem diameter (0.48), number of secondary branches (0.41), number of primary branches (0.40) and days to first flowering (0.35). Therefore, effective selection of high-yielding mutant lines can be achieved using these traits. The results agree with results reported by Usman et al. [31]. Low significant positive correlation coefficients were recorded in total chlorophyll content (0.22), chlorophyll *a* (0.22) and fruit dry weight (0.19). Low whereas non-significant positive correlation was observed only in chlorophyll *b* content (0.07) (Table 9).

Total number of fruits per plant showed moderately significant positive correlation with fruit girth (0.61), plant height (0.50), fruit length (0.44), fruit fresh weight (0.45), stem diameter (0.43), number of secondary branches (0.31) and number of primary branches (0.30), and days to first flowering (0.31). However, a low significant positive relationship was recorded in fruit dry weight (0.16). Low and non-significant positive correlation were observed on total chlorophyll content (0.02), chlorophyll *a* (0.06) and chlorophyll *b* content (0.09). Most of the other quantitative traits were observed to be have either moderate or low significant positive correlation with each other (Table 9).

4. Conclusions

In plant breeding, the major improvement in varietal development depends on existing variability among plant species. Mutagenesis has a greater possibility of bringing about genetic variability among treated populations which can be exploited to achieve desirable enhancement in the selection program. The development of mutant varieties with high-yielding and good quality traits can be achieved by exploring genetic aspects of their quantitative traits. In this study, different doses of chronic gamma irradiation 30.11 Gy (Ring 5), (83.11 Gy) Ring 3 and 185.61Gy (Ring 2) showed stimulatory effects on morphological, physiological and biochemical yield and yield-related traits. The irradiation doses 19.4 Gy (Ring 6) and 47.096 Gy (Ring 4) proved to be appropriate doses for inducing desirable micro mutation on all quantitative traits. High heritability along with high genetic advance was recorded for most

of the quantitative traits. This indicated that their expressions are more influenced by genetic factors rather than environmental factors, thus such traits can be useful for developing superior genotypes based on phenotypic variations. Total fruit yield per plant appeared to have highly significant positive correlation with total number of fruit per plant, plant height, fruit fresh weight, number of secondary branches, chlorophyll *a*, fruit dry weight, total chlorophyll content, stem diameter, and fruit length, fruit girth. This indicates the potential improvement of yield in mutant plants as a result of appropriate association of desirable component genes.

Data availability statement

The data in this study has not been linked or stored in a publicly accessible repository. All the data presented in this study is included and reference in this article and there are no additional data sources available.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Aisha Hashim: Writing – original draft, Methodology, Conceptualization. **M.Y. Rafii:** Supervision, Project administration, Funding acquisition, Conceptualization. **Oladosu Yusuff:** Writing – review & editing, Writing – original draft, Methodology. **Abdul Rahim Harun:** Supervision, Methodology, Conceptualization. **Shukor Juraimi:** Supervision, Conceptualization. **Azizah Misran:** Supervision, Conceptualization. **Samuel Chibuike Chukwu:** Writing – review & editing, Methodology. **Fatai Arolu:** Writing – review & editing, Methodology. **Asma Ilyani Kadar:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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