Research Article

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Determination of morphological changes using gamma irradiation technology on capsicum specie varieties

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Abstract: The recent increase in the demand of hot and chili pepper at local, national, and international market has drawn the interest of breeders' crop improvement researchers globally. Thus, there is a need to enhance its breeding and development to improve the production of hot and chilies pepper in the quest for food quality and security. This study focused on the germination ability and evaluation of chlorophyll mutations in M_2 generation of gamma irradiates on pepper cultivars. The materials used in this experiment were obtained from the M_1 generation of gamma-irradiated seeds of pepper cultivars (*Capsicum frutescens*, *Capsicum chinense*, and *Capsicum annuum* (hot pepper, bonnet pepper, and bell pepper, respectively)). Seeds of the pepper varieties were subjected to doses of gamma ray (i.e., 0, 100, 150, 200, 250, 300, 350, and 400 Gy) after which they were planted, raised, and harvested in the M_2 generation. The parameters evaluated were germination percentage, survival percentage, epicotyl height, and chlorophyll mutations. However, the highest chlorophyll mutation frequency was observed in *C. frutescens*. This showed that it was more sensitive to gamma irradiation relative to *C. chinense* and *C. annuum*. virescenes were the highest chlorophyll mutant observed in all the three cultivars, while the least was Xanthan. The effective dose ranges between 50 and 250 Gy for germination and survival rate for the three cultivars of pepper give higher desirable mutations in the pepper cultivars. While 400 Gy recorded most efficient for chlorophyll mutation.

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Keywords: capsicum varieties, irradiation technique, chlorophyll mutation, crop germination, survival percentage, food quality

Abbreviations

C	capsicum
DAP	day after planting

1 Introduction

Pepper (*Capsicum frutescens*, *Capsicum chinense*, and *Capsicum annuum*) is an important crop in Nigeria and Sub-Saharan Africa (Adekola and Oluleye 2012). Pepper plays an important role in flavoring of food and nations' economy at large. The researchers all over the world have now focused their works on the importance and development of pepper varieties due to its contribution to food security (Kang et al. 1997; Marín et al. 2004; Matsufuji et al. 2007; Aguilar-Meléndez et al. 2009; Bosland 2012; Plant Mutation Breeding Report 2010; Adekola and Oluleye 2012). It is rich in vitamin C and contains some quantities of vitamins B1, B2, and P. The fruit contains 3–7% sugar, 0.7–0.8% protein, 0.35% carotene, 0.03% capsorubin, 0.07% zeaxanthin, and 0.025% carotenoid. The dry matter content of the sweet fruit is 5–12% and that of hot is 9–20%. The hot fruits contain up to 0.1% capsaicin. Pepper fruits are used as fresh, cooked, pickled, canned, smoked, or grounded as sweet or hot spice (Kume and Todoriki 2013). Pepper is used for flavoring for many savor dishes, preserving, and pickling and in manufacturing of sauces and ketchups. Moreover, pepper is used as an ornamental plant (Bosland 2012; Siddique et al. 2019). Modern scientific innovations are important for developing and providing the quality food for food security (Aremu et al. 2018). The quality food is vital to health and vitality, thereby eliminating hunger and malnutrition associated with Africa and the Sub-Saharan (Halgamuge et al.

2015; Ingram et al. 2008; Obaniyi et al. 2020). The attainable yield potential can be enhanced through innovative research. The previous studies (Bae et al. 2012; Toenniessen 2003; Aremu et al. 2020; Okunola et al. 2020) reported that mutation breeding can be induced through several techniques, but gamma ray is the most effective mutagen. Research has shown that the use of mutation breeding techniques for improvement of the pepper plant has brought remarkable progress to agriculture and food security across the globe (FAO/IAEA 1977, Kang et al. 1997; Daskalov 1986; Adekola and Oluleye 2012; Jo et al. 2016; Siddique et al. 2020).

Gamma irradiation (^{60}Co) is one of the ways with which food security can be enhanced through effective mutation breeding processes. In common bean (*Phaseolus vulgaris*), gamma irradiation has been found to improve the crop survival rate (Ulukapi and Ozmen 2018). The effects of gamma ray on Lima bean and Cowpea crop types had been reported (Borkar and More 2010; Awoleye 1995; Ojomo and Ched 1971). The economic importance of pepper is increasing and its demands are expanding; therefore, the need for its improvement becomes imperative. Hitherto, improvement programs were based mainly on using natural source of germplasm, cross breeding, and heterosis. However, the amount of variability in the Capsicum is tremendous and has largely been untapped. This is the knowledge gap that this research intends to cover. More importantly, this research will further help to ascertain some mutants for ornamentals purposes. Furthermore, it will add to the existing knowledge on how gamma irradiation can be useful in crop improvement and productivity. The finding of this study will be disseminated by extension personnel to stakeholders and practitioners in the agricultural production and food industry, as extension providers perform the roles of disseminating new technologies to the necessary users (Owolabi et al. 2019) as traditional ways of development has some shortcomings (Ajala et al. 2019). Moreover, ornamental pepper can be developed and cultivated; therefore, the main objective of this study is to identify the early morphological changes and the amount of variability in the Capsicum, which is tremendous in nature, and yet not untapped, this finding will help us to create ornamental pepper cultivars and to understand the mechanisms of chlorophyll accumulation in certain tissues in a tropical and subtropical regions of the world.

2 Material and methods

The experimental materials used in this experiment were obtained from the M_1 generation of gamma irradiated seeds of *Capsicum frutescens*, *Capsicum chinense*, and *Capsicum annuum* (hot pepper, bonnet pepper, and bell pepper, respectively). Culturally, the pepper names tested are called Sombo, Rodo, and Tatashe in the South–West, Nigeria. Seeds of these pepper varieties were subjected to doses of gamma ray (100, 150, 200, 250, 300, 350 and 400 Gy). Duration of exposure was between 5 and 20 min. The irradiation equipment used is CGR/MP50 X-ray machine at the radiology unit of Landmark University Health Service Centre. Each of the three varieties was subjected to these treatments after which the seeds were each raised in a nursery bed of 4 × 3 cm and depth of 1 cm using sterilized soil medium and mulched for 10 days with 3 replicated. This was repeated two times, and the average mean of germination, survival percentages, epicotyl height (cm), and leaf mutant (chlorophyll mutation) were evaluated by counting the number of seedlings that emerged at 16 days after planting (16DAP). This was expressed as a percentage of the seeds planted. The survival percentages of the three cultivars were also evaluated by counting the number of seedlings that survived at 35 days after planting (35DAP). This was expressed as a percentage of the seeds planted. The epicotyl height was measured at 30 days after planting (30DAP) with measuring ruler calibrated in centimeters. This was measured from cotyledon levels to the main stems.

2.1 Procedure for measuring chlorophyll mutations

(i) The procedure includes daily observation of the traits for change in color and the observed 35 mutants were recorded.
(ii) Chlorophyll mutations were observed on the leaves between 14 and 21 days after planting.
(iii) The observations were made per treatment, and there were 24 treatments altogether.
(iv) This was carried out on row basis, and the number of seedlings affected by chlorophyll mutation was recorded.
(v) Type of mutations was also identified and classified using Gustafson and the frequency of chlorophyll mutations was calculated.
(vi) The data collection for chlorophyll mutations was transformed using chlorophyll transformation package as used in the studies by Brun and Rademakers (1997), Mensah et al. (1990), and Subramaniand (1981).

2.2 Statistical analysis of data

Data were analyzed using analysis of variance (ANOVA). The treatment means were separated and ranked by Duncan Multiple Range Test. Data obtained were subjected to ANOVA using Genstat 5 (Release 3.2), and significant treatment means were separated by the New Duncan's Multiple Range Test (DMRT). Correlation analysis was also carried out to determine relationships (B9) between the variables evaluated. The main software used for the statistical analysis was Statistical Package for Social Science (SPSS) version 23. It is one of the current software for estimation of mean, standard deviation, correlations, and other desired analysis (Table 1).

Ethical approval: This project was approved by the University Ethical committee.

3 Results and discussion

3.1 Physiological injuries

Physiological injuries are cytological and are determined or measured on organ or the whole organism (Tables 2 and 3). The mutagenic treatment of plant materials causes physiological damage or injury often resulting in significant reduction in early morphological trait performance (germination, seedling growth, seedling survival, etc.). The degree of physiological injury caused by the mutagen is usually correlated with the frequency of induced mutations especially with regards to radiation (Kume and Todoriki 2013). Therefore, a quantitative determination of the injury is commonly used as a check on the effectiveness of the mutagen (Sadecka 2007). In this study, the indicators of physiological injuries used were germination percentage, survived percentage, and epicotyl height reduction.

3.2 Germination percentage

Germination of gamma-irradiated seeds of M_2 of the three cultivars ranged between 41 and 62%. The nonirradiated control had 68% germination (Table 2) at the lower doses of 100, 150, 200, and 250 Gy with germination percentage of 62, 59, 53, and 51, respectively. Differences between 200 and 250 Gy were not significant at 5% level of probability. However, at 300 Gy, germination decreased 48%, representing a significant percent reduction over lower irradiated doses.

Germination of the three varieties of pepper decreased with an increase in gamma irradiation and was drastically affected at 300 Gy dose. This confirmed the fact that higher doses had the severe effect on cultivar germination (Sağel et al. 2009; Arunal et al. 2010). Therefore, this explains the extent of physiological injury caused by the application of higher doses on germination trait of the three pepper varieties. The germination response of the three varieties showed that *C. annum* had the highest followed by *C. frutescens* and *C. chinense*, even as there were no statistical differences from one another. This finding is in agreement with the previous studies on the effects of physical and chemical mutagen on seed germination of hot pepper (Iqbal et al. 2016; Mba 2013).

3.3 Survival percentage

The survival of the seedling decreased with an increase in gamma irradiation with the highest percent mortality observed at 35 days after planting (35DAP) using 400 Gy. The survival rate of nonirradiated control was 68%, whereas the survival rate of gamma-irradiated seeds of the three cultivars ranged between 28.5 and 56.3% (Table 2). At the lower doses of 100, 150, and 200 Gy, the survival rate was 56.3, 53.2, and 50.2%, respectively. However, at 250 Gy, the survival rate fell to 47.4%, representing a significant percent reduction over those irradiated at 100 and 150 Gy,

Table 1: Mean square of traits and irradiation doses evaluated

Treatment	df	Germination percentage	Survival percentage	Epicotyl height	Chlorophyll mutation
Variety	2	79.4	92.1	2.4	310.9*
Dose	7	756.9**	1379.4**	4.18**	310.9*
Variety × dose	14	129.3	153.6	0.51	84.2
Error	46	80.9	94.1	0.9	67.7

*,**Significant at 5 and 1% level of probability.

Table 2: Effect of different gamma irradiation doses on the four traits of three pepper cultivars

Treatments	Germination (%), ±SD	Survived (%), ±SD	Epicotyl height (cm), ±SD	Chlorophyll mutation frequency (±SD)
Gamma dose (Gy)				
0	$68.4^{a} \pm 0.77$	$68.4^{a} \pm 0.77$	$3.2^{b} \pm 0.17$	—
100	$62.3^{ab} \pm 1.04$	$56.3^{b} \pm 0.84$	$3.5^{ab} \pm 0.22$	$25.5^{c} \pm 0.50$
150	$58.9^{bc} \pm 0.84$	$53.2^{b} \pm 0.72$	$4.5^{a} \pm 0.43$	$30.8^{bc} \pm 0.42$
200	$53.0^{cd} \pm 1.41$	$50.2^{b} \pm 0.24$	$4.5^{a} \pm 0.39$	$30.9^{b} \pm 0.41$
250	$50.8^{cd} \pm 1.30$	$47.4^{bc} \pm 0.61$	$2.9^{b} \pm 0.47$	$32.5^{b} \pm 0.62$
300	$47.6^{d} \pm 0.70$	$42.2^{cd} \pm 0.48$	$4.5^{a} \pm 0.39$	$32.7^{b} \pm 0.57$
350	$46.0^{de} \pm 0.64$	$36.3^{de} \pm 0.35$	$4.2^{a} \pm 0.17$	$36.5^{b} \pm 0.81$
400	$41.0^{e} \pm 0.99$	$28.5^{e} \pm 0.50$	$3.1^{b} \pm 0.35$	$44.9^{a} \pm 0.51$
Mean	53.6	41.16	3.8	29.23

Values in the same column followed by a different small letter are significantly different ($P < 0.05$).

respectively. This showed that the survival rate of the three cultivars of pepper decreased with an increase in gamma irradiation and was drastically affected at 250 Gy upward. This finding is similar to the study by Vitányi et al. (2013) on chlorophyll and protochlorophyllide forms in *Pisum sativum*. This situation was severe at 400 Gy, where only 28.5% survival rate was recorded. This confirmed the fact that high doses had severe effects on the survival of the seedlings of the three varieties, while the effect was mild at lower doses. The maximum physiological injuries were recorded at 400 Gy, followed by 350, 300, and 250 Gy, respectively.

The survival responses of these three varieties (Table 3) showed that *C. frutescens* had the higher rate of survival (49.8%) followed by *C. chinense* (47.8%) and *C. annuum* the lowest (45.9%). This showed that *C. frutescens* was more resistant to gamma rays with regards to proportion of survival than the other two pepper varieties.

3.4 Epicotyl height reduction

The mean epicotyl height of nonirradiated control was 3.2 cm. In this experiment, the reduction in the epicotyl height was observed at 250 and 400 Gy. Dosage of 250 Gy caused 9% height reduction, while 400 Gy caused 2% reduction in seedling height (Table 2). Seedling height is the most commonly used indicator for seed irradiation to study the mutagen effect. This is possible because it portrays the level of physiological injury or damage caused to the plant. It is established in many crops particularly the cereals that the optimum dose of gamma rays for maximum desirable mutations should cause 30–50% reduction in the seedling height. However, the seedling height reduction is not the same for the different mutagens and crop families (Vitányi et al. 2013; Böddi et al. 2005; Daskalov 1981). However, there were stimulatory effects at 100, 150, 200, 300, and 350 Gy, which caused 10, 40, 39, 40, and 31% increase over the control. The stimulatory effect may be as a result of mutagenic ineffectiveness in M_2 generation relatives to M_1 generation. Likewise, stimulatory effects may be as a result of effects of gamma irradiation on the gene responsible for height since the epicotyl height of nonirradiated seedling was reduced relative to the irradiated one. The result is similar to that of Mensah et al. (1990) where the low dosage of 100 and 150 Gy increased the seedling heights of *Vigna*

Table 3: Pepper varietal mean trait performance across gamma irradiation doses

Variety	Germination (%), ±SD	Survival (%), ±SD	Epicotyl height (cm), ±SD	Chlorophyll mutation frequency (±SD)
C. chinense	$53.5^{a} \pm 2.25$	$47.8^{a} \pm 2.2$	$3.8^{a} \pm 0.44$	$32.3^{b} \pm 1.37$
C. frutescens	$54.2^{a} \pm 1.03$	$49.8^{a} \pm 1.8$	$4.2^{a} \pm 0.31$	$37.9^{a} \pm 1.8$
C. annuum	$51.4^{a} \pm 1.5$	$45.9^{a} \pm 1.1$	$3.5^{a} \pm 0.25$	$30.6^{b} \pm 1.2$
S.E. (dose)	3.0	3.2	0.3	2.7
S.E. (Cultivars)	1.8	2.0	0.3	3.9

% = percentage; cm = centimeter.
Values in the same column followed by a different small letter are significantly different ($P < 0.05$).

Table 4: Effect of different gamma doses on chlorophyll mutations of the three pepper cultivars

		Chlorophyll mutation										
***Capscicum* spp.**	**Dose**	**Vir.**	**Chl.**	**Mac.**	**Albov.**	**mag.**	**Tig.**	**Xan.**	**Vir. X.**	**No of chlorophyll mutation**	**No of seedlings**	**Freq/100 M_2 seedlings**
1	100	3	1	1	0	0	0	0	0	5	26	19.2
	150	6	1	4	0	0	0	0	0	11	50	22
C. chinenses	200	3	0	4	1	0	2	0	0	10	42	24
	250	2	3	1	4	1	0	0	0	11	45	24
	300	6	0	3	0	0	0	0	0	9	34	26
	350	7	4	0	0	0	0	0	0	11	37	30
	400	5	1	4	1	0	0	1	0	9	16	56
Total		**32**	**10**	**17**	**6**	**1**	**2**	**1**	**0**	**66**	**250**	**201.2**
2	100	3	0	3	3	0	0	0	0	9	51	1
	150	5	2	10	3	7	2	0	0	29	92	32
C. frutescens	200	4	2	5	1	2	0	0	0	14	43	33
	250	4	1	0	5	0	1	0	0	11	31	35
	300	3	0	2	4	2	1	0	1	13	32	41
	350	3	0	2	2	0	6	0	0	12	24	50
	400	12	6	1	1	1	6	0	5	32	52	62
Total		**34**	**11**	**23**	**19**	**12**	**16**	**0**	**6**	**120**	**325**	**254**
3	100	2	0	1	1	0	3	0	0	7	66	11
	150	2	0	1	1	0	0	0	0	4	24	17
	200	1	1	3	2	0	0	0	0	7	31	23
C. annuum	250	3	1	0	4	0	4	0	0	12	39	31
	300	4	2	4	2	0	0	0	0	12	37	32
	350	4	2	3	0	2	2	0	3	16	47	34
	400	5	0	3	0	0	0	1	0	9	22	41
Total		**21**	**6**	**15**	**10**	**2**	**9**	**1**	**3**	**67**	**266**	**189**

Code: Vir. = Virescenes, Chl. = Chlorine, Mac. = Maculate, Albov. = Alboviridis, mag. = Magnate, Tig. = Tigrigna, Xan. = Xanthan, Vir. X. = Virido Xanthan.

unguiculata and soybean seedling, respectively. The stimulatory effects of mutagens on plant height have been reported.

The epicotyl height response of the three varieties showed that *C. annuum* was more resistant to the gamma irradiation, followed by *C. chinense* and *C. frutescens* (Table 3). The result showed that less physiological injury was recorded in *C. frutescens*, relative to *C. chinense* and *C. annuum* with regards to height expression.

3.5 Chlorophyll mutations

Performance of the three varieties of pepper for the four traits *C. frutescens* recorded highest chlorophyll mutation frequency, and this increased with an increase in the gamma level (Table 3). The lowest dosage (100 Gy) recorded the lowest chlorophyll mutation frequency in all, while the highest dosage (400 Gy) recorded highest chlorophyll mutations in the three cultivars. On chlorophyll mutation, 60 mutants were observed in *C. chinense* over a range of 100–400 Gy. Of these mutants, 32 were virescence and manifested as light-green uniform colors, which gradually changed to dark green and finally to normal. There were 14 maculates, which depicted chlorophyll destruction in the form of dots distributed over the leaf.

Generally, yellowish-green colors were prevalent (Table 4). A somewhat darkening, which showed great number of chlorine, and some alboviridis, which is green base with white tips, were also recorded. Likewise, transverse destruction of pigments were recorded as Tirana, which were two in number with only one magnate (dark green leaf with white edges) and xanthan (yellow). Chlorophyll mutation frequency per 100 M_2 seedlings for dosage of 100, 150, 200, 250, 300, 350, and 400 Gy were 19, 22, 24, 26, 30, and 56%, respectively. *C. chinense* showed seven types of chlorophyll mutations while no virido xanthan was observed.

C. frutescens also showed increase in chlorophyll mutation frequency with the increased dosage level. Chlorophyll mutation frequency per 100 M_2 seedlings at the dosage level of 100, 150, 200, 250, and 400 Gy for

C. frutescens were 18, 32, 35, 41, 50, and 62%, respectively. *C. frutescens* showed seven types of chlorophyll mutations excluding xanthan, which was present in *C. chinense*. Thus, *C. frutescens* showed highest sensitivity to gamma irradiation with respect to chlorophyll mutation. The numbers of mutants in the range of 100 Gy were 120. Of these mutants, 34 were virescence, 22 were maculate, 19 were alboviridis, 16 were Tigrinya, 12 were magnates, 11 were chlorine, and the least virido xanthan, which were six in number. *C. annuum* with an increase in the dosage level showed lowest chlorophyll mutation frequency, indicating less sensitivity to gamma irradiation. This finding corroborate the findings of Wang and Bosland (2006), who discovered many morphological traits from *C. annum*. Sadecka (2007) reported pepper sensitivity to irradiation doses in a review of species. According to the study by Iqbal et al. (2016), gamma irradiation up to 600 Gy supports carotenoid concentration in hot pepper.

An increase in the gamma level for the three pepper varieties increased chlorophyll mutations even as the highest dosage of 400 Gy recorded the largest chlorophyll mutation. This result is similar to that of the studies by Böddi et al. (2005) and Tekniklerin (2001) who reported that the percentage of chlorophyll mutation was low under lower dosages of 100 kV at 140 rpm for soybean and Mungbean, respectively. While Iqbal et al. (2016) reported that total carotenoids and ascorbic acid substantially increased with the increased doses of gamma rays on *C. annuum*. Therefore, this research affirms that mean chlorophyll mutation frequency increased with an increase in dosage of gamma irradiation. From this study, 400 Gy dosage recorded the highest chlorophyll mutation and hence confirmed mutagenic effectiveness of the dose. However, only 20% survivals were observed at 400 Gy of gamma rays, while above average survival rate was recorded at 200 Gy dosages. This was shown in the generation parameter also, which was in agreement with the findings of Borkar and More (2010) and Khan et al. (2000), and it is therefore affirmed that possible effective dose for the mutation breeding in the pepper cultivars may be between 150 and 250 Gy gamma irradiation levels, which cause less physiological injury and survival rate of over 50%.

4 Conclusion

This article concludes that the highest chlorophyll mutation frequency was observed in *C. frutescens*. This showed that it was more sensitive to gamma irradiation relative to *C. chinense and C. annuum*. The effective dose for highest chlorophyll mutation was 400 Gy. This result further adds to the early morphological changes and the amount of variability in Capsicum, which is tremendous in nature and could be used as ornamental plants.

Moreover, this study revealed that possible effective dose for mutation breeding in pepper cultivars could be between 150 and 250 Gy gamma irradiation dose levels, which cause less physiological injury and survival rate of over 50%. These dosage levels would give higher desirable mutations in pepper varieties.

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