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Effect of Gamma Irradiation on Fruits of Three Pepper Varieties

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Abstract:
Breeders have centered efforts on productivity and paid little attention to fruit quality, including traits that can increase consumers appeal. Induced mutation is a methods used in creating genetic variations resulting to new varieties with different features. The aim of this study was to qualitatively evaluate the effect of gamma irradiation on fruit colour and shape of three pepper cultivars. Mutation was induced using different doses of gamma irradiation (50, 100, 150 and 200 Gy from ^{60}Co source) in the Gamma Irradiation Facility (GIF), at Nuclear Technology Centre (NTC) of the Sheda Science and Technology Complex, Abuja. The fruit colour at the immature phase exhibited two phenotypic classes of green and dark green. In Tatase 0 Gy and 50 Gy showed 100 % dark green fruits, while 100, 150 and 200 Gy had 100 % green fruits. In Nsukka yellow pepper, there was an increase in the frequency of green colour with 100 Gy, 150 Gy and 200 Gy dosage. At full maturity, the frequency distribution for Tatase showed one phenotypic class of deep red colour while Nsukka yellow pepper showed two phenotypic classes of yellow and orange colour. The results recorded increased frequency of fruits with pointed blossom end in Shombo fruits exposed to the mutagen. The variation obtained in the percentage frequency of the various treatments could then suggest an effect of gamma irradiation on pepper fruit characteristics. Therefore gamma irradiation dosage of 50 Gy – 200 Gy could be exploited in the creation of variability relevant for the diversification of pepper fruit colour and shape.

Keywords: *Capsicum annuum, Variation, Fruit, Colour, Shape, Phenotypic ratio*

1. Introduction

The production of pepper (chile, bell and specialty-type) worldwide was nearly 200 million tonnes of green pepper and 33 million tonnes of dry peppers in 2013(Naegele et al., 2016).However, the global production of peppers was recently estimated at 14.4 billion dollars (FAO, 2016), approximately forty times bigger than what was obtained in the 80's. Clearly, the market and consumption of peppers is still growing mainly owing to its fruits nutritional value, however, consumers choice could be based on the phenotype attributes (Kim et al., 2014).

Selection in fruit crops was probably founded on nutritious, non-toxic, and palatable features. Pleasurable and culinary qualities, including flavor, succulence, juiciness, and other consumer-desirable characteristics were added later (Gascuel et al., 2017).Due to wide genetic variability and diversity of peppers, alternatives to several new gene rearrangements are possible. Pepper breeding are mainly aimed at features such as productivity, disease and pest resistance, fruit characteristics (bioactive compounds, pungency and flavor), and abiotic stresses (drought, salinity) (Kuhn et al., 2016; Manzur et al., 2014). However, since the 30's breeders, have centered their efforts on productivity and have basically neglected traits that increases consumers appeal (e.g., fruit colour, size and shape).

According to Huh et al.(2011), pepper fruit colour is regulated by three genetic loci – y, c1, c2. The unique red colour of pepper fruit is due to capsanthin, capsorubin and capsanthin-5,6-epoxyde (Tomlekova et al., 2016) while accumulation of α- and β- carotene, zeaxanthin, lutein and β-cryptoxanthin leads to yellow-orange fruit colour (G´omez-Garc´ıa and Ochoa-Alejo, 2013). It was confirmed that changes in the expression levels of the genes for capsanthin/capsorubin synthase (Ccs), phytoene synthase (Psy), lycopene- β -cyclase (LcyB) and β –carotene hydroxylase (CrtZ) result in different color combinations (Tomlekova et al., 2016; Thian et al., 2014). Among them, the most noticeable effect was confirmed for beta-carotene hydroxylase (Tomlekova et al., 2016). EMS induced mutation of a red-fruit variety resulting in orange fruit colour and high beta-carotene levels, was identified as another known mutation in the CrtZ-2gene (Borovsky et al., 2013; Paran et al., 2007). The aim of this study was to qualitatively evaluate the effect of gamma irradiation on the colour and shape of three pepper variety fruits.

2. Materials and Methods

Fruits of the three pepper cultivars (Tatase, Shombo and Nsukka yellow pepper) were divided into five sets each and exposed to varied gamma irradiation concentrations of 0 Gy (Gray), 50 Gy, 100 Gy, 150 Gy and 200 Gy. Irradiation of Capsicum peppers was carried out in the Gamma Irradiation Facility (GIF), at Nuclear Technology Centre (NTC) of the Sheda Science and Technology Complex, Abuja. The Bruker e-scan alanine dosimetry reader system, model SCO205, manufactured by Bruker Biospin Corporation, U.S.A, was used to measure the absorbed dose.

The seeds were nursed according to the separate treatments in nursery baskets filled with well filtered top soil mix with poultry manure and watered daily. Seedlings were then transplanted at 6 weeks when the seedlings were 5 to 10 cm tall to individual polybags filled with mixed soil and manure according to Uguru (1999) and laid out in a complete randomized design (CRD). This was carried out at the Botanical Garden of the University of Nigeria, Nsukka and phenotypic data on fruit colour and shape were then collected.

Fruit colour at immature and maturity stage were determined by comparing all the fruits from each treatment and graded. Fruit shape and the shape at the tip of the fruit were examined by observing all the fruit shapes and graded, andtheir respective frequency recorded as a percentage of the plant population.

3. Results and Discussion

The frequency distributions of the immature fruit colour are presented on Table 1. The variation observed inthe fruits colour at the immature phase exhibited two phenotypic classes of green and dark green (Figure 1). Tatase control and plants raised from seeds irradiated with 50 Gy showed 100 % dark green fruits, while plants raised from seeds irradiated with 100, 150 and 200 Gy had 100 % green fruits (Table 1; Figure 1a and d). This result revealed a complete dominance of one trait (green) over the other (dark green) in the control. The immature fruits of Shombo were similarly grouped into two colour groups (green and dark green), where seeds irradiated with 50, 100 and 200 Gy showed almost equal percentage frequencies between the two groups as compared to the untreated seeds (Table 1, Figure 1b and e). Nsukka yellow pepper showed variation in immature fruit colour (Figure 1c and f) in both the control and plants raised from seeds irradiated (Table 1). The Nsukka yellow pepper control plant fruits had a green and dark green colour ratio of 1:1.11which is equivalent to 1:1 ratio. This results obtained in control group was in agreement with what was reported by Maga (2012). Therefore, it is reasonable to say that there was a codominance effect between the traits. Fruits from plants raised with seeds exposed to 50 Gy induced an increased effect of the dark green traits giving rise to a phenotypic ratio of 1: 2, while 100, 150 and 200 Gy increased the green colour trait with a phenotypic ratio of 4:1. However, the mutagenic treatments must have induced an increased ability of the green genotype to fully express itself with higher dosage in the generation.

Figure 1 :(A B C) Tatase, Shombo and Nsukka Yellow Pepper with Green fruit Colour,(D-F): Tatase, Shombo and Nsukka Yellow Pepper with Dark with Green fruits Colour at Immature Stage

	Tatase		Shombo		Nsukka Yellow Pepper	
Dosage	Green	Dark green	Green	Dark green	Green	Dark green
0 Gy	0	100	60.2	39.8	47.5	52.5
50 Gy	0	100	45.3	54.7	37	63
100 Gy	100	0	57.4	42.6	82.9	17.1
150 Gy	100	0	85.3	14.7	100	0
200 Gy	100	0	50	50	80	20

Table 1: Effect of Gamma Irradiation on Percentage Frequency Distribution of Immature M_1 Fruit Colour Ofc Annuum

At full maturity, the frequency distribution for Tatase showed one phenotypic class of deep red (Figure 2c) while Shombo and Nsukka yellow pepper showed two phenotypic classes; yellow and orange (Figure 2a, b and d, e). As presented in Table 2, a phenotypic ratio of 2 (67.5): 1 (32.5) was recorded for the controlled plants. This is in agreement with similar reports of Maga (2010) for two seasons. Irradiation dosage of 50 Gy had a change from the norm (i.e. higher yellow colour fruits) to induction of higher orange colour fruits with a phenotypic ratio of 1(22.2): 3 (77.8). Tomlekova et al., 2016 observed lack of amplification of CrtZ-2 gene in mutant variety, suggesting that the gamma irradiation had led to significant genome changes on chromosome 3 in the vicinity of CrtZ-2gene, most likely induction of deletion of the CrtZ-2 gene. As a result, carotene biosynthesis pathway was disrupted thus leading to accumulation of beta-carotene at high levels.

Figure 2: (A): Shombo with Light Red Fruits at Maturity,
(B): "Nsukka Yellow Pepper" with Yellow Fruits Colour at Maturity,
(C-D):Tatase And Shombo with Deep Red Fruits at Maturity,
Nsukka Yellow Pepper with Orange Fruit Colour at Maturity

	Tatase		Shombo		Nsukka Yellow Pepper	
Dosage	Light red	Deep red	Light red	Deep red	Yellow	Orange
0 Gy	0	100	37.5	62.5	67.5	32.5
50 Gy	0	100	16.8	83.2	22.2	77.8
100 Gy	0	100	25.7	74.3	90.2	9.8
150 Gy	0	100	41.2	58.8	40.9	59.1
200 Gy	0	100	44.7	55.3	67.5	32.5

Table 2: Effect of Gamma Irradiation Onpercentage Frequency Distribution of Ripped M_1 Fruit Colour Ofc Annuum

There was also a considerable range of variation in the M_1 fruit shape which was divided into elongated and conical fruits (Table 3) and (Figure 3a to 3d). Tatase variety showed a higher frequency of conical fruit shape than elongated fruit shape in all treatment which was almost the same trend with the control dosage. But in Nsukka yellow pepper, 150 Gy irradiation dosage induced more elongated fruits in a ratio of 2.66: 1 which is approximately 3: 1 ratio as compared to the unirradiated which had more conical fruits. Maga (2010) had earlier reported Nsukka yellow pepper having higher oblong and conical fruit shape as compared the elongated fruit shapes in two planting seasons which the unirradiated fruit corroborates with. This could probably suggest a significant effect of gamma irradiation in fruit elongation.

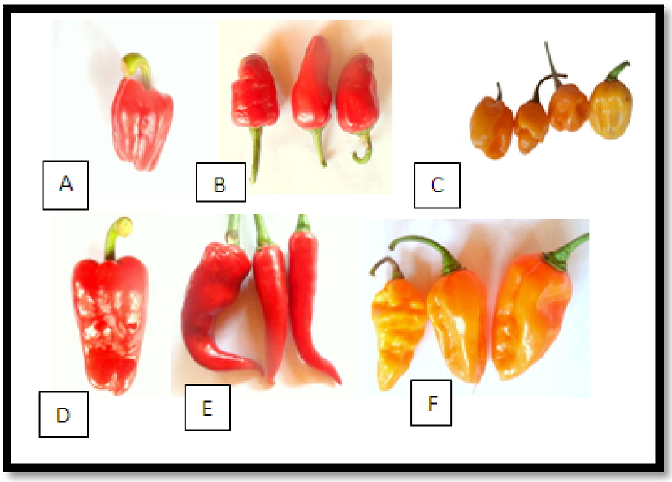

Figure 3: (A-C): Conical Fruits of Tatase, Shombo and Nsukka Yellow Pepper, (D-F): Elongated Fruits of Tatase, Shombo and Nsukka Yellow Pepper

Dosage	Tatase		Shombo		Nsukka Yellow Pepper	
	Elongated	Conical	Elongated	Conical	Elongated	Conical
0 Gy	38.5	61.5	63.6	36.4	10	90
50 Gy	0	100	73.7	26.3	59.3	40.7
100 Gy	4.5	95.5	76.5	23.5	51.2	48.8
150 Gy	40	60	58.8	41.2	72.7	27.3
200 Gy	0	100	63.2	36.8	45	55

Table 3: Effect of Gamma Irradiation on percentage Frequency Distribution of M_1 Fruit Shape Ofc Annuum

The frequency distribution with respect to fruit shape at blossom end (fruit tip) revealed that most of the fruits had pointed tips, some had blunts tips while others had shrunken tips as shown in Table 4 and Figure 4 a - i.

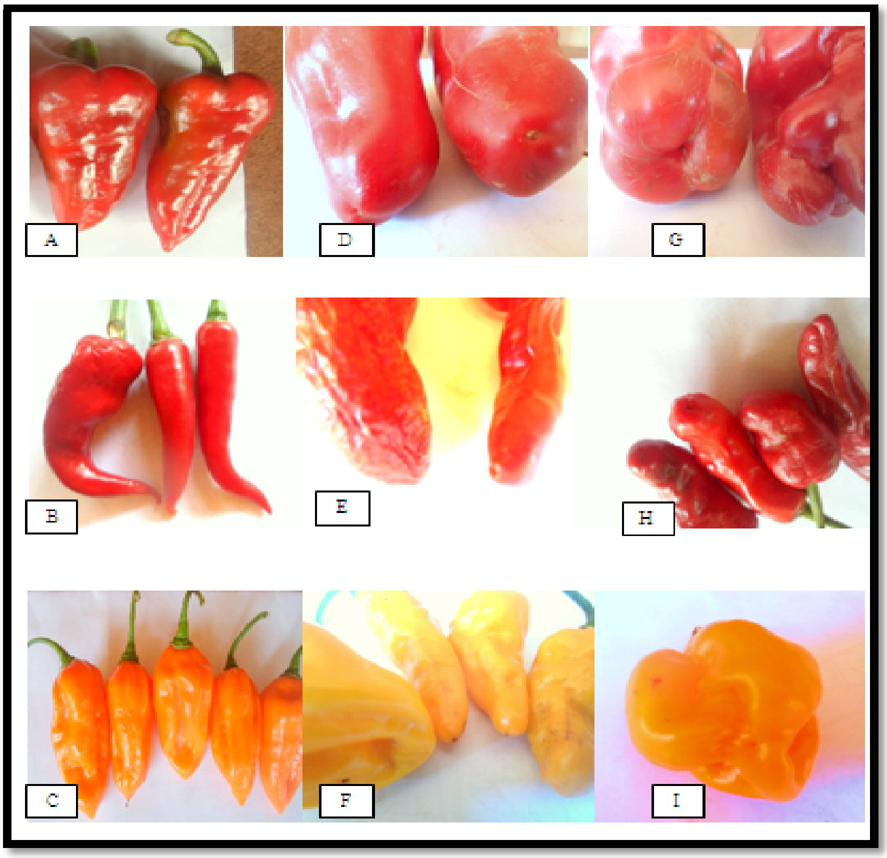

Figure 4: (A-C) Tatase, Shomboand Nsukka Yellow Pepper Fruits with Pointed Tips, (D-F) Tatase, Shomboand Nsukka Yellow Pepper Fruits with Blunt Tips (G-I) Tatase, Shomboand Nsukka Yellow Pepper Fruits with Shrunken Tips

Dosage	Tatase			Shombo			Nsukka Yellow Pepper		
	Pointed	Blunt	Shrunken	Pointed	Blunt	Shrunken	Pointed	Blunt	Shrunken
0 Gy	28.6	42.9	28.6	28.4	51.1	20.5	67.5	22.5	10
50 Gy	45	15	40	54.7	24.8	20.4	51.9	44.4	3.7
100 Gy	54.5	18.2	27.3	52.9	32.4	14.7	31.7	63.4	4.9
150 Gy	20	20	60	61.8	33.8	4.4	59.1	27.3	13.6
200 Gy	0	37.5	62.5	44.3	44.3	11.3	30	52.5	17.5

Table 4: Effect of Gamma Irradiation on Percentage Frequency Distribution of Fruit Shape Ofc Annuum

The variation obtained in the percentage frequency of the various treatment dosage as compared to the control, could then suggest a significant effect of gamma irradiation on pepper fruit characteristics. Even though qualitative traits have little relevance in crop improvement programmes nowadays (Lotti et al., 2007), their description could proffer a more detailed understanding of the genotype morphology. Therefore, within the scope of this study, gamma irradiation dosage of 50 Gy – 200 Gy could be exploited in the creation of variability relevant for the improvement pepper fruit colour and shape. This study will form a base for pepper fruit shape and colour breeding via gamma irradiation in the future.

4. References

i. Borovsky Y., Tadmor, Y., Bar, E., Meir, A., Lewinsohn, E., Paran, I. (2013). Induced mutation in b-CAROTENE HYDROXYLASE results in accumulation of b-carotene and conversion of red to orange color in pepper fruit.Theoretical and Applied Genetics, 126 (3): 557–565.

ii. G´omez-Garc´ıa M., Ochoa-Alejo, N. (2013). Biochemistry and Molecular Biology of Carotenoid Biosynthesis in Chilli Peppers (Capsiumspp), International Journal of Molecular Science, 14, 19025-19053.

iii. Gascuel, Q., Diretto, G., Monforte, A. J., Fortes, A and Granell, A (2017). Use of natural diversity and biotechnology to increase the quality and nutritional content of tomato and grape. Frontiers in Plant Science, 8: 625

iv. Huh J. H., Kang, B. C., Nahm, S. H., Kim, S.K. S. Ha, M. H. Lee, B. D. Kim (2001). A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (Capsicum spp.).Theor. Appl. Genet., 102, No 4, 524–530.

v. Kim, S., Park, M., Yeom, S. I., Kim, Y. M., Lee, J. M., Lee, H. A., Seo, E., Choi, J., Cheong, K., Kim, K. T., Jung, K., Lee, G. W., Oh, S. K., Bae, C., Kim, S. B., Lee, H. Y., Kim, S. Y., Kim, M. S., Kang, B. C., Jo, Y. D., Yang, H. B., Jeong, H. J., Kang, W. H., Kwon, J. K., Shin, C., Lim, J. Y., Park, J. H., Huh, J. H., Kim, J. S., Kim, B. D., Cohen, O., Paran, I., Suh, M. C., Lee, S. B., Kim, Y. K., Shin, Y., Noh, S. J., Park, J., Seo, Y. S., Kwon, S. Y., Kim, H. A., Park, J. M., Kim, H. J., Choi, S. B., Bosland, P. W., Reeves, G., Jo, S. H., Lee, B. W., Cho, H. T., Choi, H. S., Lee, M. S., Yu, Y., Do Choi, Y., Park, B. S., van Deynze, A., Ashrafi, H., Hill, T., Kim, W. T., Pai, H. S., Ahn, H. K., Yeam, I., Giovannoni, J. J., Rose, J. K.,Sørensen, I., Lee, S. J., Kim, R. W., Choi, I. Y., Choi, B. S., Lim, J. S., Lee,Y. H. and Choi, D. (2014). Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nature Genetics, 46(3): 271-278.

vi. Kuhn, H., Padilha, M and Barbieri, R. L. (2016). Plant breeding of chili peppers (Capsicum, Solanaceae) – A review. Australian Journal of Basic and Applied Sciences,10(15): 148-154

vii. Maga, T. J. (2012). Pattern of genetic variability in Nsukkayellow pepper (Capsicumannuum L.) genotypes. A dissertation submitted to the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka.

viii. Manzur, J. P., Oliva-Alarcón, M. and Rodríguez-Burruezo, A. (2014). In vitro germination of immature embryos for accelerating generation advancement in peppers (Capsicum annuum L.). Scientia Horti culturae, 170: 203-210.

ix. Naegele, R. P, Mitchell, J. and Hausbeck, M. K. (2016). Genetic Diversity, Population Structure, and Heritability of Fruit Traits in Capsicum annuum, PLoS ONE 11(7): e0156969.

x. Padilha, H. K. M., Pereira, E. S., Munhoz, P. C., Vizzotto, M., Valgas, R. A. and Barbieri, R. L. (2015). Genetic variability for synthesis of bioactive compounds in peppers (Capsicumannuum) from Brazil. Food Science and Technology, 35(3): 516-523.

xi. Paran I., Y. Borovsky, S. Nahon, O. Cohen (2007). The use of induced mutations to study shoot architecture in Capsicum,Israel Journal of Plant Sciences, 55 (2): 125–131.

xii. ThianS., Li. L., Chai, W., Shah, S. and Gong, Z. (2014). Effects of silencing key genes in the capsan thin biosynthetic pathway on fruit color of detached pepper fruits, BMC Plant Biology., 14: 314

xiii. Tomlekova, N., Spasova-Apostolova, V. and Panchev, I. (2016). Characterization of mutation affecting a region of the chromosome 3 in orange-coloured and beta-carotene-rich mutant registered as the variety Oranzhevakapiya. Biologiemol´eculaire 69: 2

xiv. Uguru, M. I. (1999). Location effects on the growth yield and flavour Expression of Nsukka aromatic yellow Pepper. J. Appl. Chem. Agric. Res. 6:84-87.