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INDUCTION OF GENETIC VARIABILITY ON THE BIOACTIVE CONTENT OF YELLOW PEPPER USING GAMMA IRRADIATION

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ABSTRACT

Hunger affects more than 2 billion people globally. Therefore, there is the need to improve plant species in order to increase yield and mineral contents especially β-carotene. This study was aimed at sourcing for new favourable genetic variations in the M_1 generation that could be exploited in biochemical traits. Mutation was induced in Nsukka Yellow Pepper using varied doses of gamma irradiation (50, 100, 150 and 200 Gy) from ^{60}Co source in the Gamma Irradiation Facility, Nuclear Technology Centre, Sheda Science and Technology Complex, Abuja. The seeds were evaluated in the field during the 2016 planting season using the Completely Randomised Design in the Botanical Garden of the University of Nigeria, Nsukka. Increase in vitamin C, total carotenoid and capsaicin content were observed in fruits of the treated plants when compared with the control. The β-carotene (6.24 ± 0.06 mg/L) and iron content (5.08 ± 0.0 7 mg/L) were higher in fruits of the genotype exposed to 50 Gy. Flavonoid content was observed to decrease in fruits exposed to 50 and 150 Gy. There was reduction in the phytate content across most of the treatments when compared to the control. Gamma irradiation dosage of 50 – 200 Gy could be exploited in the induction of variability relevant for the improvement of biochemical traits in the pepper.

Key words: Hidden hunger; micronutrients; proximate composition; undernourished population; variability
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INTRODUCTION

The undernourished population of Nigeria during 2007-2009 was 11 million people or 7.0% of the total population, which increased to 14 million or nearly 9.0% of the population in 2010-2012 and then 14.6 % in 2020 (FAO, 2012a; FAO, 2012b; Owoo, 2021; Varrella, 2021). Hidden hunger (micronutrient deficiencies), afflicts more than 2 billion individuals, or one in three people, globally (FAO, 2013). It is a major cause of malnutrition among the population especially in pregnant women and children (Ceccarelli, 2012). The human body needs iron from food sources to build blood cells; vitamin A to support immune system and vision; iodine for cognitive development and thyroid function. These essential micronutrients are important because they cannot be synthesised in the body system.

Pepper (*Capsicum annuum*) is a good source of vitamin and β-carotene. β-carotene is known for its nutritional role in provitamin A activity (Zeb and Mehmood, 2004; Jaswir *et al*., 2011). The importance of pepper in the tropics cannot be over-emphasised as it is the third in importance among cultivated vegetables. It is consumed by most households. The highly cherished flavour and the desirability of the carotenoid pigments in food-colouring place pepper on high demand by consumers in rural and urban markets (Abu *et al.*, 2011).

Nsukka yellow pepper, a cultivar of *Capsium. annuum*, is a well-cherished crop, and is regarded as part of the major vegetable crops grown in the derived savannah agro-ecology for its fruits which are distinguished by their unique aroma, hotness due to the high capsaicin content, nutritional values, adaptability to the existing cropping systems and potentials for wealth creation (Uguru, 2000; Abu and Uguru, 2006). Asogwa (2006) noted that the distinctive aroma of Nsukka yellow pepper enhances its acceptability in the market. Consequently, it attracts higher price than other pepper types in the local and urban markets in Nigeria. Similarly, Ajayi and Eneje (1998) reported that the distinctive aroma of the cultivar makes it very much cherished in the diets of several homes and eateries. Nsukka yellow pepper is not widely cultivated in most states in the country but its cultivation is limited to Nsukka agricultural zone and some parts of Kogi State. This may be because of its tendency to lose its pungency, aroma and colouring in other areas, hence the name 'Nsukka Yellow Pepper' (Uguru, 1999). Nsukka agricultural zone is generally considered to be the home of Nsukka yellow pepper (Maga *et al.*, 2012).

Success in plant breeding is directly dependent on genetic variability in the population. The use of genetic engineering to produce such genetic variability has posed quite a number of challenges in Nigeria mostly due to lack of relevant infrastructures. This has made mutation breeding an invaluable tool for crop improvement, especially in such a highly valuable vegetable crop. Wide variations in yield and yield components have been observed in aromatic *C. annuum* genotypes grown in derived Savanna belt of Nigeria (Abu and Uguru, 2006). But there is still the need to source for new genetic combinations that may favour increased carotenoid content especially β-carotene without jeopardising other desirable agronomic attributes. There is the need to scale up production in order to meet demand of the household eateries and to provide raw materials for most pharmaceutical industries. This study was aimed at inducing genetic variability in *C. annuum* (Nsukka Yellow Pepper) in order to source for new favourable genetic variations that could enhance its nutritional value, particularly the β – carotene content.

MATERIALS AND METHODS

The seeds of Nsukka yellow pepper used for the study were divided into five sets each and exposed to varied gamma irradiation concentrations (0, 50, 100, 150 and 200 Gy). Irradiation of accessions of Nsukka Yellow Pepper was carried out in the Gamma Irradiation Facility (GIF) at the 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 mixed with poultry manure (Ojua and Abu, 2018; Ojua *et al.*, 2019) and watered daily. Seedlings were then transplanted 6 weeks after sowing into individual polyethylene bags filled with well filtered top soil mixed with poultry manure and laid out in a Completely Randomised Design (CRD). This was carried out at the Botanical Garden of the University of Nigeria, Nsukka. The M_1 fruits were collected for the biochemical analysis.

The biochemical studies were carried out at the Department of Crop Science Teaching and Research Laboratory, University of Nigeria, Nsukka. The fruits of each treatment were collected from all the plants into a bag, mixed together and selected at random. The parameters evaluated were β – carotene, total carotenoids, capsaicin and ascorbic acid. Proximate analysis was carried out to determine crude proteins, crude fat, fibre, moisture and ash, as well as phytochemicals (flavonoids, alkaloids, tannin and phytate) and some mineral elements (iron, zinc and calcium).

β – carotene, total carotenoids, ascorbic acid, proximate composition, flavonoids, phytate, iron, zinc and calcium were determined using the official methods described by the Association of Official Analytical Chemists (AOAC, 2005). Protein was determined by the Kjeldahl method as outlined by Pearson (1976). Kjeldahl method involved digestion of the sample in Tetraoxosulphate (VI) acid, which was neutralised with sodium hydroxide. This freed the ammonia, which was removed from the mixture by steam distillation. The distillate from the Kjeldahl mixture was received in boric acid solution and titrated with standard hydrochloric acid (HCl). For each ammonia molecule found in the distillate, one nitrogen atom was present in the sample. The crude protein in the sample was calculated by multiplying the total nitrogen by an empirical factor ($N \times 6.25$).

The percentage fat in the sample was determined as outlined by Pearson (1976). This was by extraction from dried, ground sample using light petroleum in Soxhlet extraction apparatus under controlled conditions. Crude fibre content was determined by Weende method as described by Peatson (1976). The crude fibre of a sample is the residue remaining after protein, starch, fat and digestible carbohydrate have been hydrolysed out of the sample. The ash of a sample is the inorganic residue remaining after the organic matter has been burnt off.

The percent ash of each sample was determined using the procedures described by Pearson (1976), while moisture content was determined by indirect distillation method which involved the measurement of weight loss due to evaporation of water (AOAC, 2005). Tannin was determined according to Folin Denis Spectrophotometric method (Pearson, 1976) while alkaloid was determined using the modified method described by Harbone (1973).

Data collected were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 4 software and means were separated using the least significance difference test at 5 and 1% levels of probability.

RESULTS

The analysis of variance (ANOVA) showed significant differences in the proximate, vitamin/carotene, phytochemicals and mineral content across the dosages used (Table 1). Figure 1 shows that fruits from plants irradiated with 50 Gy recorded the highest β –carotene content with a mean value of 6.24 ± 0.06 mg/100 g which was significantly higher ($p < 0.05$) than those of the other dosages except the control. Fruits from the genotype irradiated with 200 Gy recorded the lowest β –carotene content of 2.75 ± 0.27 mg/100g (Figure 1).

Table 1: Mean Squares from the ANOVA Table

Proximate	Mean square	Vitamin/carotene	Mean square	Phytochemical	Mean square	Minerals	Mean square
Moisture	76.97^{***}	Vitamin C	18.30^{***}	Flavonoids	2.11^{***}	Fe	2.86^{***}
Ash	14.31^{***}	β – carotene	6.32^{***}	Alkaloid	21.94^{***}	Ca	4.99^{***}
Fat	0.01^{NS}	Total carotene	15.83^{*}	Tannin	0.63^{***}	Zn	0.81^{***}
Fibre	5.64^{***}	Capsaicin	0.11^{***}	Phytate	0.19^{***}		
Protein	1.22^{***}						

***-significant at $p< 0.001$; *-significant at $p < 0.05$; NS- not significant

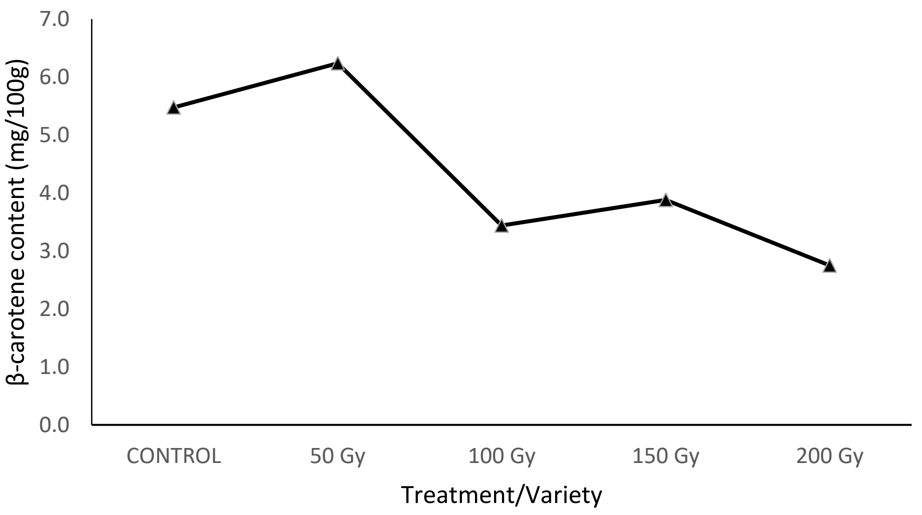

Figure 1: Effect of gamma irradiation on the β – carotene content

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Table 2 shows significant differences ($p < 0.05$) among the various treatments for vitamin C, total carotenoid and capsaicin. Fruits harvested from plants irradiated with 50 Gy had the highest vitamin C content with a mean of 11.28 ± 0.32 mg/ 100 g. Exposure of seeds to 150 Gy and 200 Gy resulted in increased Vitamin C content when compared to the control. There was a significant increase in alkaloid content in seeds exposed to varied dosage of irradiation when compared with the control. There was a reduction in the tannin and phytate content across all the treatments when compared with the control, which had 1.28 ± 0.03% and 1.2 ± 0.01 %, respectively (Table 2).

Table 2: Effect of gamma irradiation on vitamins and phytochemicals (mg / 100 g) in the fruits

Treatment	Gamma Irradiation					LSD(0.05)
	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy	
Vitamin C	5.24 ± 0.47[c]	11.28 ± 0.32[a]	5.28 ± 0.24[c]	7.72 ± 0.01[b]	7.03 ± 0.06[b]	0.88
Total carotene	14.35 ± 0.58[bc]	12.02 ± 0.07[c]	12.66 ± 0.02[bc]	15.48 ± 0.57[ab]	17.76 ± 2.07[a]	3.14
Capsaicin	0.82 ± 0.01[c]	0.75 ± 0.00[c]	1.07 ± 0.05[a]	0.58 ± 0.00[d]	0.97 ± 0.00[b]	0.07
Flavonoids	2.27 ± 0.03[a]	0.84 ± 0.01[b]	0.65 ± 0.00[c]	2.23 ± 0.12[a]	0.68 ± 0.01[bc]	0.18
Alkaloid	12.83 ± 0.23[c]	18.60 ± 0.21[a]	18.73 ± 0.03[a]	14.80 ± 0.26[b]	14.07 ± 0.12[b]	0.60
Tannin	1.28 ± 0.03[a]	0.45 ± 0.01[c]	0.36 ± 0.01[cd]	1.08 ± 0.04[b]	0.27 ± 0.06[d]	0.12
Phytate	1.22 ± 0.01[a]	0.56 ± 0.01[d]	0.70 ± 0.02[c]	0.71 ± 0.01[c]	0.86 ± 0.01[b]	0.04

Means followed by the same letter (s) within the same column are not significantly different using the LSD at 5% level of probability.

Significant differences were observed for moisture, ash, fat, fiber and protein content as shown in Table 3. The moisture content ranged from 73.43% to 85.83%. It was observed that fruits from plants grown from seeds exposed to 50 Gy had the lowest moisture content which was significantly ($p < 0.05$) lower than the other treatments except 100 Gy. Similarly, the ash content ranged from 2.17% to 8.13%, being significantly ($p < 0.05$) lower in fruits harvested from plants grown from seeds exposed to 50 Gy. Fat was not significantly ($p > 0.05$) different across the treatments, ranging from 0.47 to 0.63% while fiber ranged from 16.00% to 18.97%; crude protein content ranged from 3.60% to 5.05%. Fibre was significantly ($p < 0.05$) highest at 100 Gy and 0 Gy while protein was significantly ($p < 0.05$) highest at 100 Gy treatment.

Table 3: Effect of gamma irradiation on percentage proximate composition in Nsukka yellow pepper fruits

Treatment	Gamma irradiation					LSD
	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy	
Moisture	81.23 ± 1.14[b]	73.43 ± 0.26[d]	74.97 ± 1.01[cd]	85.83 ± 0.56[a]	76.70 ± 0.25[c]	2.34
Ash	5.37 ± 0.09[b]	2.17 ± 0.12[d]	4.97 ± 0.29[b]	3.93 ± 0.32[c]	8.13 ± 0.09[a]	0.65
Fat	0.63 ± 0.07	0.47 ± 0.03	0.57 ± 0.09	0.50 ± 0.00	0.57 ± 0.03	NS
Fibre	18.80 ± 0.45[a]	16.00 ± 0.35[b]	18.97 ± 0.35[a]	16.53 ± 0.07[b]	16.77 ± 0.13[b]	0.96
Protein	4.94 ± 0.01[b]	3.60 ± 0.03[c]	5.05 ± 0.09[a]	4.41 ± 0.32[b]	3.88 ± 0.08[c]	0.49

Means followed by the same letter (s) within the same row are not significantly different using the LSD at 5% level of probability

The values for Fe, Ca and Zn contents in fruits of different treatment dosages are shown in Table 4. The Fe, Ca and Zn contents ranged from 2.34 - 5.08 mg/ 100 g, 1.22 – 4.65 mg/100 g and 12.08 – 13.18 mg/100 g, respectively. There was a significant increase ($p< 0.05$) in Fe and Ca contents of seeds exposed to 50 Gy as compared to the control (Table 4).

Table 4: Effect of gamma irradiation on mineral content (mg/ 100 g) in Nsukka yellow pepper fruits

Treatment	Gamma irradiation					LSD
	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy	
Fe	3.76 ± 0.06[b]	5.08 ± 0.07[a]	3.56 ± 0.03[b]	2.34 ± 0.23[c]	3.44 ± 0.00[b]	0.35
Ca	3.58 ± 0.41[b]	4.65 ± 0.13[a]	3.92 ± 0.01[b]	1.22 ± 0.12[c]	3.62 ± 0.07[b]	0.63
Zn	12.92 ± 0.12[a]	12.88 ± 0.02[a]	12.08 ± 0.04[b]	12.05 ± 0.01[b]	13.18 ± 0.21[a]	0.35

Means followed by the same letter (s) within the same row are not significantly different using the LSD at 5% level of probability

DISCUSSION

The results of biochemical screening of Nsukka yellow pepper in this study revealed that pepper fruits contain health- supporting compounds. The existence of nutritional or anti-nutritional factors in pepper has been reported by numerous researchers (Uguru, 2000; Bosland and Votava, 2000; Ogunlade *et al.*, 2012, Wahua *et al.*, 2013, 2014; De-Figueiredo *et al.*, 2014, Abu *et al*., 2019; Abu *et al*., 2020). Palma *et al.* (2015) noted that pepper fruits are low in calorie content with high levels of antioxidant especially ascorbic acid (Vitamin C) and β-carotene (provitamin A).

In this study, there were significant variations in β-carotene, vitamin C, total carotene and capsaicin contents of pepper fruits. Higher β-carotene content was obtained from the accessions raised from seeds exposed to 50 Gy. Jaswir *et al*. (2011) noted that carotenoids function as precursors of vitamin A as well as antioxidant, anticancer, antiobesity and have anabolic effect on bone components. Ashraf (2003) attributed the increase in β –carotene to production of reactive oxygen by gamma irradiation which induces oxidative stress that affects structural and functional molecules, thereby distortion the normal metabolic pathways. The increase in β –carotene content could be due to biological response towards induced oxidative damage in the cell (hormesis) which could be heritable across generations. The success in developing pepper varieties with higher β-carotene content could address micro-nutrient deficiency (hidden hunger) because pepper is consumed in many households as raw in salads or cooked in dishes irrespective of the status or class of the individual.

Chung (2016) noted that several programmes on nutrition which aimed to expand the range of foods available to people in the developing world have encountered challenges. To overcome these challenges, the United Nations International Children's Emergency Fund (UNICEF), World Health Organisation (WHO) and Global Alliance for Improved Nutrition (GAIN) resorted to fortification. According to UNICEF (2021), fortification programmes are effective in preventing micronutrient deficiencies at minimal cost. Unstable food prices, natural resource depletion, globalisation, urbanisation and climate change are just a few examples of major food security challenges today (FOA, 2018). Therefore, exposure of pepper seeds to gamma irradiation before planting could be a good approach in developing breeding lines with higher β-carotene content.

Pungency or the hot taste of pepper fruits is a function of capsaicinoid concentration. These compounds are recognised for their therapeutic effects on gastric ulcers and rheumatoid arthritis (Sathyanarayana, 2006). Capsaicin and alkaloid content were observed to increase in fruits harvested from plants grown from seeds exposed to 100 Gy. These pepper lines could be advanced from the exposure of seeds to 100 Gy for increased capsaicin content. On the other hand, exposing seeds to 150 Gy could result in the production of pepper lines with low capsaicin contents, which can be used as food colourants in order to make the food to look more appetising (Grubben and Denton, 2014). Pepper lines with low capsaicin and high β-carotene as observed with fruits from plants grown from seeds exposed to 50 Gy can be eaten as vegetable in higher amount for increased intake of β-carotene, which is vital for children and pregnant women. Phytochemical properties such as flavonoid and alkaloid have an indirect effect on provender quality. Flavonoids, with properties such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action were observed to be high at 150 Gy exposure. This study confirmed that gamma irradiation can increase phenolic plant metabolites, as has also been reported by Variyar *et al.* (2004) in soybean.

Gamma irradiation reduced tannin and phytate level in pepper fruits mostly at 100 and 200 Gy treatment dosages. Tannins bind dietary protein and digestive enzymes and are known to form complexes that are not readily digestible; they also cause decrease in palatability and reduce growth rate (Soetan and Oyewole, 2009). Even though tannins and phytate are grouped as anti-nutritional, selection with identifiable content can still be in the right direction provided that other important minerals are not jeopardised. This is because the amount of tannin and phytate present in pepper is below the level that can be harmful to the body. Phytic acid is a common storage form of phosphorus in seeds and in a few tubers and fruits (Adedayo *et al.,* 2010). There was also a reduction in phytate concentration across the treatments as compared to the control. Results showed that exposing seeds to gamma irradiation, especially at 50 Gy dosage, could lead to the development of pepper lines with low anti-nutritional factors.

Iron, calcium and zinc are essential minerals for the human body. According to Geissler and Powers (2005), iron has a variety of biochemical functions in the body, including oxygen-binding in haemoglobin and as a catalytic centre in a variety of enzymes, such as cytochrome oxidase. Iron is associated with the production of red blood cells while calcium is one of the most important components in human bone. Zinc is essential for the immune system (Sousa *et al.*, 2014). The treatments were observed to be effective in developing pepper breeding lines with improved Fe, Ca and Zn content in Nsukka yellow pepper. This corroborates the previous reports of Abu *et al*. (2019) that the enhancement or inhibition of biological responses is a frequently observed symptom in gamma irradiated plants. The increase in mineral content could be due to biological response towards induced oxidative damage in the cells (Abu *et al.*, 2019).

CONCLUSION

Results of this study showed that the bioactive composition of Nsukka yellow pepper could be increased via gamma irradiation treatment. The selection of breeding lines that could combine increased β-carotene with iron as observed in plants exposed to 50 Gy could be a step towards combating nutritional insecurity (hidden hunger) among the rural populace. If this mutagenic process is monitored and directed carefully, it could proffer significant alternative for improving β-carotene content in *Capsium annuum*.

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