

Assessing the mutagenic effects of gamma irradiation on *Cajanus cajan* (L.) Huth and *Vigna unguiculata* (L.) Walp landraces using morphological markers

Ogbuagu Udensi Ugorji^{1*}, Ekei Victor Ikpeme²,
Joseph Abebe Obu³, Ekpe Daniel Ekpenyong⁴

¹Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria

²University of Calabar, Calabar, Nigeria

³Department of Physics, University of Calabar, Calabar, Nigeria

⁴Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria

*Corresponding author, e-mail: princeuou4u@yahoo.com

Abstract

Giving the high adaptability, heritability, genetic variability and nutritive values reported in the indigenous landraces of legumes, combined efforts should be geared towards their improvement. The present study aimed at evaluating the sensitivity of "Fiofio" *Cajanus cajan* (L.) Huth and "Olaudi" *Vigna unguiculata* (L.) Walp to gamma irradiation using morphological markers. Seeds of these legumes were exposed to gamma irradiation at 0, 200, 400, 600, 800 Gy from ⁶⁰Co source. There was significant effect of gamma irradiation on days to 50% seedling emergence in a dose-dependent fashion for "Fiofio" but it did not significantly affect the seedling emergence of "Olaudi". Germination percentage was not affected concerning the two crops but the survival percentage was significantly reduced as the dose of exposure increased, especially for "Fiofio" variety. Growth and yield traits were increased in plants raised from seeds exposed to 200 Gy of gamma irradiation for both legumes. "Fiofio" plants raised from seeds treated with 400, 600 and 800 Gy have died after two months, while those of "Olaudi" have presented a retarded growth. Explicitly, exposing the seeds of these legumes to 200 Gy of gamma irradiation could serve as a good springboard for their improvement.

Key words: Mutation breeding, gamma irradiation, Fiofio, Olaudi, improvement

Efeitos mutagênicos de irradiação gamma em variedades nativas de *Cajanus cajan* (L.) Huth e *Vigna unguiculata* (L.) usando marcadores morfomoleculares

Resumo

Dadas as elevadas adaptabilidade, hereditariedade, variabilidade genética e valores nutritivos relatados nas raças autóctones de leguminosas, os esforços combinados devem ser orientadas para a sua melhoria. O presente estudo teve como objetivo avaliar a sensibilidade de feijão guandu *Cajanus cajan* (L.) Huth e "Olaudi" *Vigna unguiculata* (L.) Walp à irradiação gama, utilizando marcadores morfológicos. As sementes destas vagens foram expostas a irradiação gama de 0, 200, 400, 600, 800 Gy a partir de fonte de ⁶⁰Co. Houve efeito significativo da irradiação gama em dias com a emergência de 50% das sementes em uma dose-dependente para "Fiofio", mas isto não afetou significativamente a emergência de plântulas de "Olaudi". A percentagem de germinação não foi afetada naquilo que se refere às duas culturas, mas a percentagem de sobrevivência foi significativamente reduzida à medida que houve aumento da dose de exposição, especialmente para a variedade "Fiofio". Características de crescimento e rendimento foram aumentadas em plantas cultivadas a partir de sementes expostas a 200 Gy de radiação gama para ambos os legumes. Plantas "Fiofio" oriundas de sementes tratadas com 400, 600 e 800 Gy morreram depois de dois meses, enquanto as de "Olaudi" apresentaram um crescimento retardado. Explicitamente, expor as sementes dessas leguminosas a 200 Gy de radiação gama poderia servir como um bom trampolim para a sua melhoria.

Palavras-chave: reprodução de mutação, irradiação gama, Fiofio, Olaudi, melhoria

Received: 21 July 2012
Accepted: 22 September 2012

Introduction

Legumes are valuable sources of carbohydrates, dietary fibers, vitamins, minerals and proteins (17-40%) higher than cereals (7-13%) but coincidentally equals to the protein in meat (18-25%) (Tharanathan & Mahadevamma, 2003; Costa et al., 2006; Udensi et al., 2011a). The high adaptability, heritability, genetic variability and nutritive values reported of locally grown pulses (landraces) (Udensi et al., 2011a, b) call for combined efforts towards their improvement because of the importance attached to food security, especially in Sub-Saharan Africa.

Interestingly, the role of mutation breeding in increasing food production and sustainable agriculture is well recognized (Ahloowalia & Maluszynski, 2001). For the past few decades, researchers and scientists have made great efforts with little success to develop reliable transformation systems for cowpea and pigeon pea, which are very important as good sources of protein and energy for people in developing countries of Asia and Africa. There have been reports of failures surrounding the regeneration of cowpea and pigeon pea plants via callus (Geetha et al., 1998; Kumar et al., 1985; Somers et al., 2003; Chandra & Pental, 2003; Popelka et al., 2006). Problems ranging from transient gene expression to no evidence of stable integration have been a recurrent decimal in the different trials. Similarly, the constraints underlying recombinant DNA technology are the difficulty of producing reliable and stable transformants (Popelka et al., 2006; Chaudhury et al., 2007; Ivo et al., 2008; Solleti et al., 2008), technical inadequacies in Sub-Saharan regions, infrastructural and instrumentation unavailability. Obviously, relying solely on rDNA technology for cowpea and pigeon pea improvement might spell doom to food security in the region with increasing refugees' episodes (Udensi et al., 2011b). It should be understood that combating food insecurity in Sub-Saharan Africa demands diversification of approaches that could lead to sustainable agriculture through crop improvement.

Ionizing radiation has been routinely employed in the generation of genetic variability for breeding and genetic studies (Boureima et

al., 2009) and this process, according to Udensi et al. (2012c), could be highly instrumental in achieving sustainable agriculture and food security, especially in countries that are bedeviled with poor infrastructure and instrumentations, political instability, fractured policy making and implementation, etc.

In conformity with Mahandjiev et al. (2001), mutation breeding serves as an important complement to genetic crop improvement. By varying the specific mutagens dose, the frequency and saturation of mutations can be regulated (Menda et al., 2004). Wheat, rice, barley, cotton, peanut and cowpea are among crops that have been improved through mutation breeding (Khan & Al-Qurainy, 2009). Genetic variability is very pivotal to successful breeding program (Udensi et al., 2011a). Mutagenic agents such as chemicals, gamma rays, x-rays, electron beam irradiation, etc. are usually employed to induce this variability artificially, leading to the production of mutants (Ciftci et al., 2006; Boureima et al., 2009). These mutants could express economic important traits whose genes could be isolated, identified, cloned and used in designing crops for yield and quality traits (Ahloowalia & Maluszynski, 2001).

Agricultural sustainability and food security are twin cardinal issues in the Millennium Development Goal (MDGs) of Nigerian government. It therefore becomes pertinent to develop techniques that could drive the vision. Interestingly, the high nutritive value, high adaptability, drought tolerance and considerable high yield performances of pigeon pea and cowpea landraces in marginal soils (Udensi et al., 2011a, b) make them suitable crops for further improvement. It is on this premise that this paper seeks to evaluate the efficacy of gamma irradiation in improving yield performances of these landraces.

Material and Methods

Seed collection and gamma irradiation

Seeds of three landraces of pulses viz Brown "Fiofio", white "Fiofio" (*Cajanus cajan* (L) Huth) and "Olaudi" (*Vigna unguiculata* (L.) Walp) were obtained from germplasm collections of Dr. Udensi, O. Ugorji of the Department of Genetics

and Biotechnology, University of Calabar, Nigeria. The seeds were exposed to gamma irradiation at the National Atomic Energy Commission (NAEC), Abuja, Nigeria. Seeds of each variety were divided into five groups of fifty grams weight of fifty seeds each and were exposed to different doses of gamma irradiation – 200, 400, 600, 800Gy from ^{60}Co source while the fifth group served as control. The application was done in accordance with the FAO/IAEA Agricultural and Biotechnology Laboratory in Seibersdorf, Austria, in February 2008 (Ciftci et al., 2006). Irradiated seeds were kept at 4°C in a refrigerator prior to planting.

Planting and cultural practices

A plot of land measuring 12x12 meters was manually cleared in the University of Calabar Experimental Farm. Five beds were made with a spacing of 2 meters between them. Three seeds were sown in a hole of 4cm deep per variety according to the method of the Center for New Crops and Plants Products (2002) as modified by Udensi et al. (2012b) using randomized complete

block design (RCBD) with 6 replications. Spacing of 50 x 75cm (cowpea) and 20 x75cm (pigeon pea) were maintained. Weeding was done as the need arose. There was no fertilizer application.

Data collection and analysis

Data on germination, morphological and yield attributes were recorded and subjected to analysis of variance (ANOVA) using PASW version 18.0 statistical software.

Results

Germination indices

The gamma irradiation significantly affected ($P < 0.05$) days to 50% seedling emergence in a dose-dependent fashion in pigeon pea while there was no significant effect ($P > 0.05$) of the exposure on cowpea. Percentage germination was not significantly affected by the gamma rays exposure. Seedlings raised from pigeon pea seeds exposed to 400, 600 and 800 Gy died after the 2nd month while those of cowpea had reduced survival percentage in a dose-dependent manner (Figures 1-3).

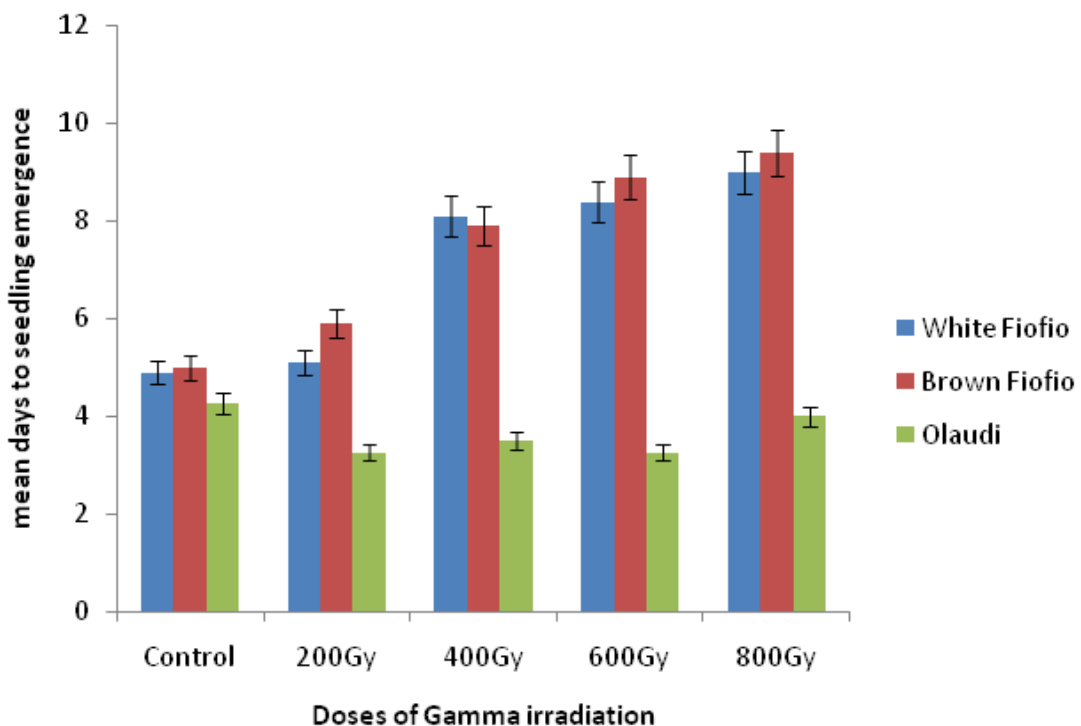


Figure 1. Effect of gamma irradiation on seed germination percentage of different *Cajanus cajan*(L.) Huth landraces.

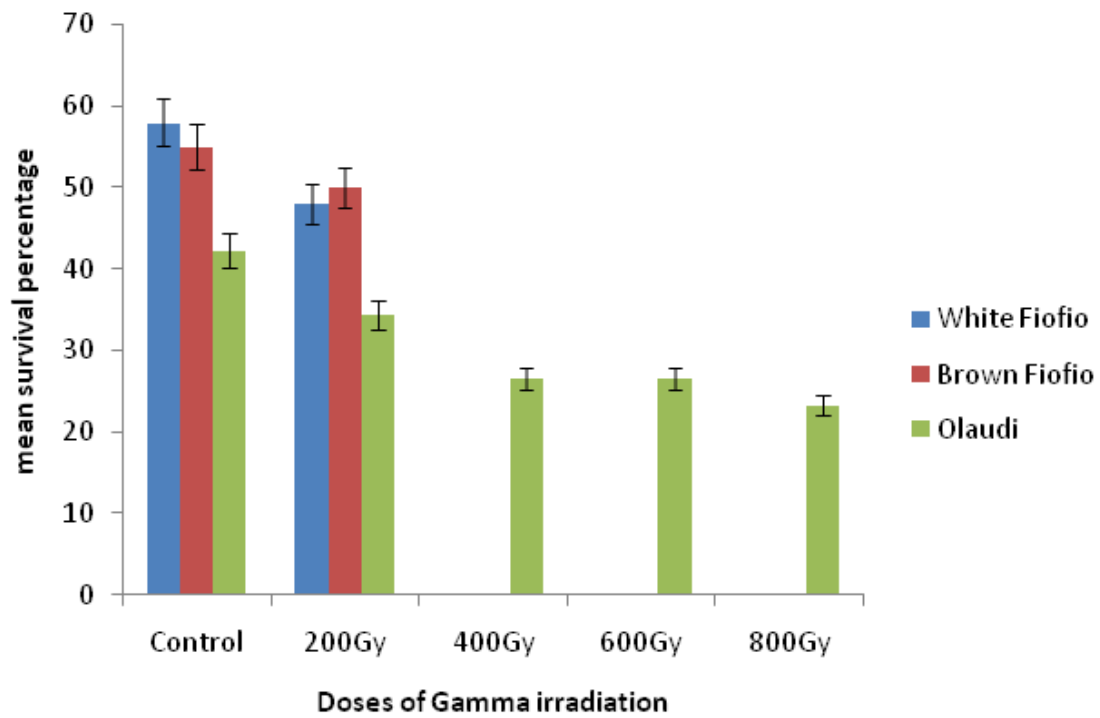


Figure 2. Effect of gamma irradiation on days to 50% seedling emergence of different *Cajanus cajan*(L.) Huth landraces.

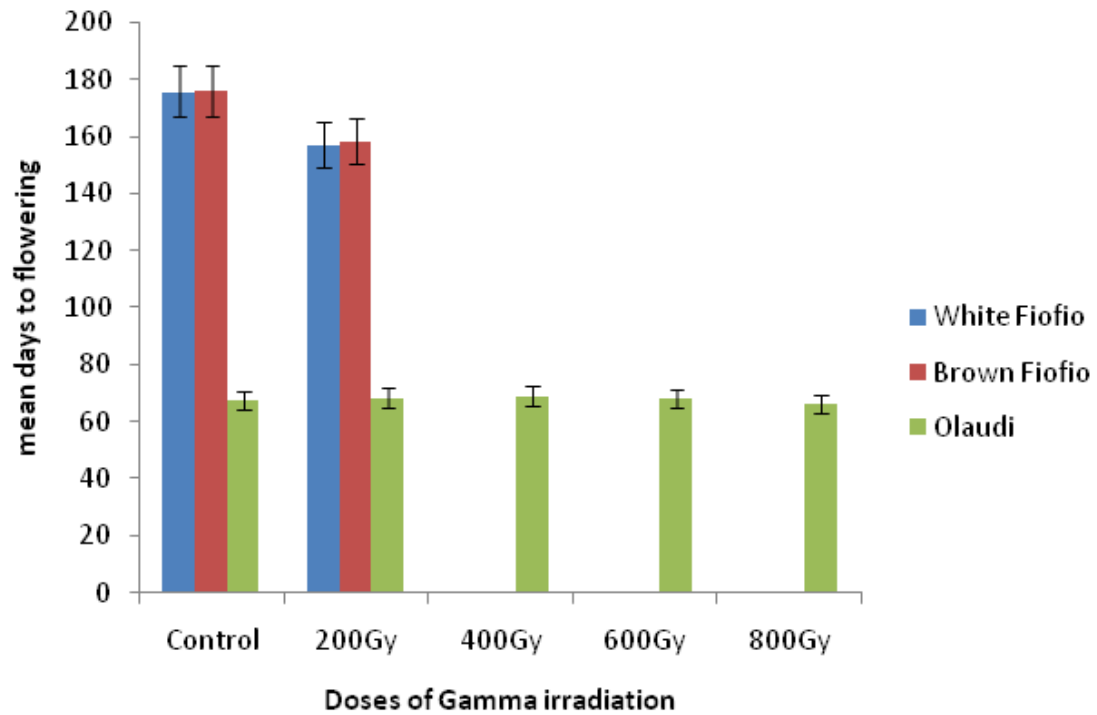


Figure 3. Effect of gamma irradiation on survival percentage of different *Cajanus cajan*(L.) Huth landraces.

Morphological and yield traits

For pigeon pea variety, there were significant effects ($P < 0.05$) of gamma irradiation on all the growth traits investigated. Additionally, plants raised from seeds exposed to 200 Gy of gamma irradiation performed better than the plants in control. For instance, at 24 weeks, plant height, number of leaves and leaf area of white "Fiofio," brown "Fiofio" and their controls were 237.3cm, 208.5cm; 223.7cm, 189.6cm; 343.0, 315.9; 279.1, 256.7; and 704.5cm², 124.7cm²; 279.1cm², 256.7cm², respectively (Table 1). Though the percentage survival for cowpea variety was quite high when compared to those of pigeon pea, the effects of the exposure caused significant reduction ($P < 0.05$) on growth traits in a dose-dependent fashion. Plants raised from 200 Gy irradiated seeds had 146.6cm as vine length while the plants raised from 800 Gy irradiated seeds had 60.8cm after 10 weeks. Leaf area and number of leaves were 233.58cm²; 116.5cm² and 64.6; 40.8, respectively (Tables 3).

Comparing pigeon pea plants raised from seeds exposed to 200 Gy gamma irradiation and those in control, days to 50% flowering was reduced, but it did not significantly affect the time, since it took 50% of the plants to reach maturity. Conversely, the treatment did not show obvious effect on days to 50% flowering and maturity in cowpea (Figures 4 & 5).

There was significant positive effect ($P < 0.05$) of gamma irradiation on yield traits in both varieties, especially when exposed to 200 Gy of gamma rays, the variety notwithstanding. Plants raised from white "Fiofio" seeds exposed to 200 Gy of gamma irradiation produced more flowers, pods, seeds, increased pod length and higher seed yield than the brown "Fiofio". This effect was also similar when the seeds of cowpea were exposed to 200 Gy of gamma irradiation. For instance, white "Fiofio" and brown "Fiofio" produced 440.9 and 149.7 pods with seed yield of 2645.1 and 583.5 per plant while "Olaudi" produced 41.8 pods and seed yield of 593.6, respectively (Table 2 & 4).

Discussion

Though mutation breeding has found great relevance in the improvement of desired

traits in agricultural crops (Ciftci et al., 2006; Boureima et al., 2009); there are still fears in several quarters regarding the safety of this technique (Xiuzher, 1994; Rabie et al., 1996; Stoeva & Bineva, 2001; Boureima et al., 2009; Udensi et al., 2012c). Obviously, if the technique is found to be detrimental to crops or environment, the aim would have been compromised.

Exposing seeds of these legumes to gamma irradiation did not significantly affect germination percentage rather it delayed days to seedling emergence in pigeon pea but did not affect the seedling emergence of cowpea seeds. Additionally, the treatment also decreased survival percentage of the two crops though it affected pigeon pea seedlings more as seedlings raised from their seeds that were exposed to 400, 600 and 800 Gy did not survive after two months post-germination. Undoubtedly, the sensitivity of crops to gamma irradiation is species-specific Pathirana & Subasingbe, 1993; Udensi et al., 2012c). This could be the underlying cause of the varietal differences observed in the result. Though Khan & Al-Qurainy (2009) reported that seed germination is either delayed or inhibited due to mutagenic treatment, our present result is not in tandem with this position, since germination percentage was not significantly affected. A striking possibility is that these gamma irradiated seeds might have developed tolerance to the inhibitory effect of the treatments and had therefore improved their physiological conditions resulting to delayed seed germination without necessarily affecting germination percentage.

It was observed that it took a longer period for seeds exposed to high doses of gamma irradiation to emerge, especially for pigeon pea varieties. It is therefore not surprising to observe that such seeds or even the seedlings derived there, especially as for pigeon pea, had a lower survival percentage as they died some weeks after seedling establishment. It is, however, reasonable to affirm that the general lower survival percentage reported in this current study could be attributed partly to physiological perturbations and partly to the chromosomal damages and disarrangements (Tosca et al., 1995; Mensah & Akomah, 1997; Mensah et al., 2005; Mensah et al., 2007). Though

Table 1. Effect of gamma irradiation on morphological traits in *Cajanus cajan*(L.) Huth.

Parameters	White "Fioflo"				Brown "Fioflo"					
	Control	200	400	600	800	Control	200	400	600	800
Plant height @ 8wks (cm)	46.1±1.51d	56.1±4.08e	9.7±0.57b	4.7±0.38a	2.5±0.42a	56.8±1.96e	37.1±2.10c	6.7±0.42ab	4.2±0.27a	2.9±0.21a
Plant height @ 16wks (cm)	155.3±2.09cd	157.4±1.80d	ND	ND	ND	153.0±1.46c	130.0±2.10b	ND	ND	ND
Plant height @ 24wks (cm)	208.5±1.21c	237.3±2.12e	ND	ND	ND	189.6±2.01b	223.7±1.12d	ND	ND	ND
No of leaves @ 8wks	21.6±0.69g	18.4±0.75f	9.7±0.29d	7.9±0.51bc	6.3±0.61ab	36.1±1.10h	13.9±0.51e	8.6±0.37cd	6.4±0.57ab	5.1±0.46a
No of leaves @ 16wks	169.6±1.60d	232.4±1.04e	ND	ND	ND	123.3±1.06b	129.3±0.92c	ND	ND	ND
No of leaves @ 24wks	315.9±0.51d	343.0±0.82e	ND	ND	ND	256.7±1.34b	279.1±0.60c	ND	ND	ND
Leaf area @ 8wks (cm ²)	208.5±1.21c	114.7±1.80e	30.3±1.11bc	28.7±0.42bc	23.7±0.42a	116.7±1.34ef	118.1±1.65f	34.0±0.93d	31.0±0.82cd	27.6±0.48b
Leaf area @ 16wks (cm ²)	93.4±1.45d	124.7±1.13b	ND	ND	ND	123.3±1.06b	129.±0.92c	ND	ND	ND
Leaf area @ 24wks (cm ²)	124.7±1.44b	704.5±0.48d	ND	ND	ND	256.7±1.34b	279.1±0.60c	ND	ND	ND

*Means followed with the same case letter along each horizontal array, indicate no significant difference (p>0.05). ND= No data

Table 2. Effect of gamma irradiation on yield attributes of *Cajanus cajan* (L.) Huth.

Parameters	White "Fiofo"			Brown "Fiofo"						
	Control	200	400	600	800	Control	200	400	600	800
No. of flowers plant ⁻¹	205.1±1.68d	746.3±1.38e	ND	ND	ND	80.1±0.51b	187.1±0.83c	ND	ND	ND
No of pods plant ⁻¹	119.3±0.52c	440.9±10.58e	ND	ND	ND	137.9±1.60d	146.70.5b	ND	ND	ND
Pod length (cm)	6.7±2.09b	7.3±0.29c	ND	ND	ND	7.0±0.22b	6.7±0.29b	ND	ND	ND
No of seeds pod ⁻¹	4.7±0.29b	5.7±0.18c	ND	ND	ND	4.3±0.29b	5.4±0.20c	ND	ND	ND
Seed yield	596.6±2.60ab	2645.1±63.50c	ND	ND	ND	689.3±7.98b	583.5±7.98a	ND	ND	ND
100 seed weight (g)	9.8±0.85b	12.5±0.84c	ND	ND	ND	11.9±0.49c	10.0±0.85b	ND	ND	ND

*Means followed with the same case letter along each horizontal array indicate no significant difference (p>0.05), ND= No data

Table 3. Effect of gamma irradiation on morphological traits in cowpea (*Vigna unguiculata* (L.) Walp).

Parameters	Dose of gamma irradiation(Gy)			
	Control	200	400	800
Vine length @5wks (cm)	19.0±0.32 ^b	21.8±0.8 ^d	18.5±0.8 ^b	10.46±0.55 ^a
Vine length @10wks (cm)	120.8±1.16 ^c	146.6±1.2 ^d	66.8 ± 0.8 ^b	62.2 ± 1.02 ^a
Leaf area @ 5 wks (cm ²)	215.56±1.78 ^e	141.43±1.07 ^d	78.01±0.94 ^c	73.7 ±1.15 ^b
Leaf area @ 10 wks (cm ²)	198.97±1.30 ^d	233.58±1.59 ^a	174.46±1.19 ^c	123.78±1.02 ^b
No. of leaves @5wks	19.0±0.4 ^b	22.6±0.40 ^c	17.4±0.75 ^b	13.2±0.49 ^a
No. of leaves @ 10 wks	57.2±1.07 ^c	64.6±0.68 ^d	48.2± 0.97 ^b	39.6±0.68 ^a
No. of primary branches @10wks	3.4±0.25 ^{ab}	4.0±0.55 ^b	3.6±0.25 ^{ab}	2.0±0.90 ^a
No. of secondary branches @10wks	0.4±0.4 ^a	0.2±0.2 ^a	0.4±0.24 ^a	0.6±0.01 ^a
Internode length @5wks	5.38±0.20 ^b	5.7±0.48 ^b	4.9±0.33 ^{ab}	4.4±0.19 ^a
Internode length @10wk	6.4±0.25 ^a	7.8±1.10 ^a	7.4±0.81 ^a	6.1±0.86 ^a

*Means followed with the same case letter along each horizontal array indicate no significant difference (p>0.05)

Table 4. Effect of gamma irradiation on yield traits in cowpea (*Vigna unguiculata* (L.) Walp).

Parameters	Dose of gamma irradiation (Gy)				
	Control	200	400	600	800
No. of flowers plant ⁻¹	42.8±1.02 ^c	69.8±0.51 ^d	42.6±0.68 ^c	40.0±0.32 ^b	37.2±0.92 ^a
No. of pods plant ⁻¹	19.6±0.51 ^b	41.8±0.97 ^e	29.2±0.37 ^d	23.0±0.90 ^c	16.2±0.86 ^a
No. of seeds pod ⁻¹	13.2±0.37 ^c	14.2±0.86 ^c	9.4±1.80 ^b	9.6±0.68 ^b	5.8±0.58 ^a
Pod length (cm)	13.02±0.71 ^b	15.0±0.82 ^c	13.3±0.24 ^b	12.64±0.54 ^b	10.1±0.88 ^a
Seed yield	277.8±16.67 ^b	593.6±50.8 ^c	274.4±31.78 ^b	219.2±11.36 ^b	95.2±13.4 ^a
100 seed weight (g)	8.8±0.2 ^{ab}	10.6±0.68 ^c	10.4±0.75 ^{bc}	10.6±0.25 ^c	8.4±0.75 ^a

*Means followed with the same case letter along each horizontal array indicate no significant difference (p>0.05)

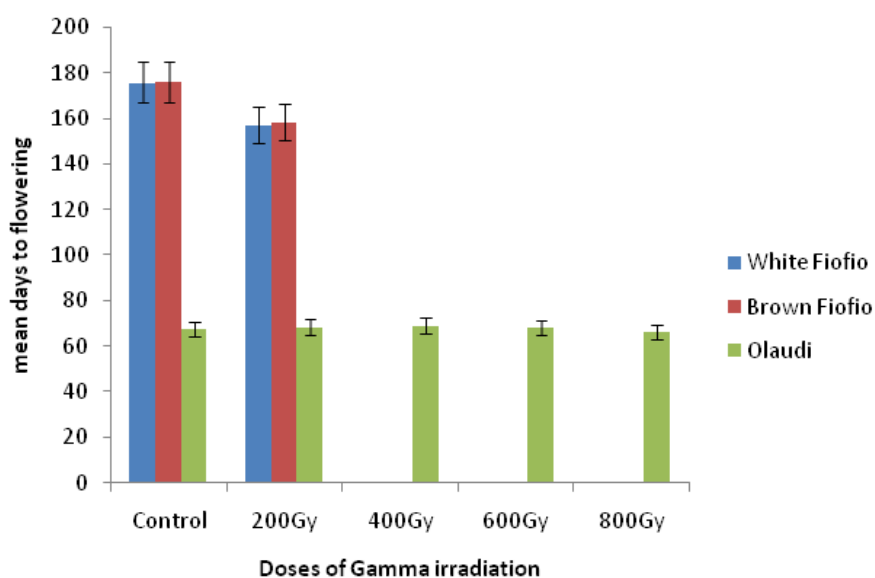


Figure 4. Effect of gamma irradiation on days to 50% flowering of different *Cajanus cajan*(L.) Huth landraces.

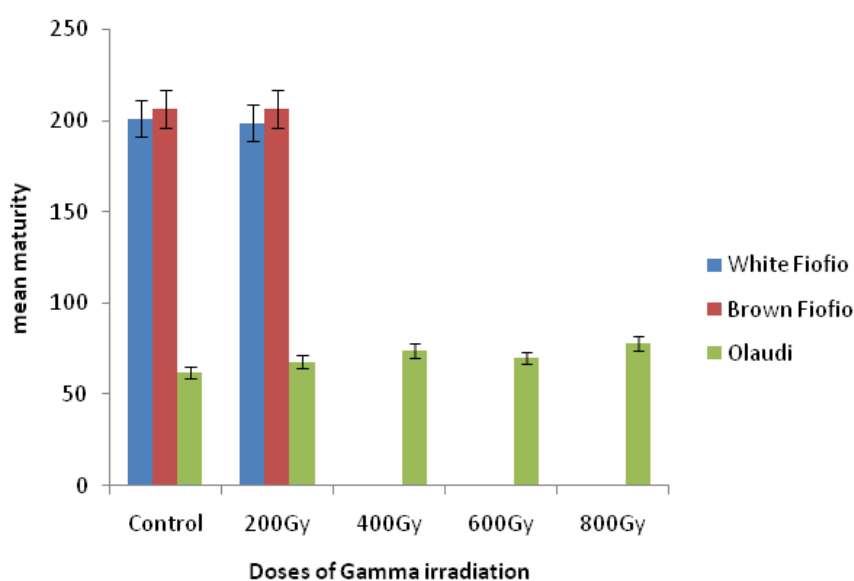


Figure 5. Effect of gamma irradiation on days to 50% maturity of different *Cajanus cajan*(L.) Huth landraces.

the survival percentage for cowpea seedlings was quite high when compared to those of pigeon pea, there was significant reduction on the general performance in growth traits in a dose-dependent fashion. This was the position of Boureima et al. (2009) on differential varietal sensitivity to nuclear irradiation. Cheema & Atta (2003) and Pons et al. (2001) have reported significant height reduction in basmati rice and rice, respectively, following gamma irradiation with increasing doses. This was, however, attributed to mutagenic-induced chromosomal damage during cell division (Boureima et al., 2009). This corroborates our present results.

There was significant retardation in seedling growth, especially for pigeon pea plants raised from seeds exposed to 400, 600 and 800 Gy of gamma irradiation. Probably, this growth instability might have led to their death. However, plants raised from seeds exposed to 200 Gy performed better than plants in the control for all the morphological traits evaluated. This was the same for plants raised from seeds of cowpea exposed to gamma irradiation but differed from the former, since plants raised from seeds treated with higher doses of gamma rays have survived and could grow. What it suggests implicitly is that cowpea seeds have better capacity to withstand the toxic effects associated with gamma irradiation than pigeon pea ones.

Expectedly, the more the number of leaves per plant, the greater will be the photosynthetic activities and the broader the leaf, the more surface area that will be available for photosynthesis (Fagwalawa, 2000; Akinyele & Osekita, 2006; Udensi et al., 2011a, b, 2012a, b). These relationships were strictly obeyed, especially in plants raised from seeds exposed to 200 Gy gamma irradiation, the variety notwithstanding. It will be correct to assert that exposing legumes, especially pigeon pea and cowpea, to appropriate dose of gamma rays could cause holistic improvement of morphological and yield traits. Polyploidy has been associated with high morphological and yield performances of crops (Brisibe et al., 2011; Udensi et al., 2011c). It is probable that gamma irradiation may have induced polyploidy in these crops, especially at the dose of 200 Gy.

There was significant reduction in the days to flowering when pigeon pea plants raised from seeds exposed to 200 Gy were compared to plants in control. This reduction in the days to flowering did not affect significantly the time of maturity. It might imply that the time to flowering may not necessarily correlate with the maturity time. It is noteworthy to state that the goals of genetic manipulation of crops include reducing the time of flowering, maturity, etc. It thus means that any breeding methods that fail in this respect, giving the state of food insecurity, especially in Africa, should be reconsidered.

It was observed from our present results that the increase in the flower production for plant raised from seeds treated with 200 Gy gamma irradiation might have led to the increase in the number of pods produced. Obviously, when pod length increases, the number of seeds therein should also increase proportionally. Our results revealed synergy among the yield-contributing traits, which might have led to the increase in the seed yield obtained for plants raised from seeds exposed to 200 Gy of gamma irradiation, the variety notwithstanding. The implication of these results is that gamma irradiation seems to have enhanced seed productivity in these legumes by improving yield-contributing traits. According to earlier reports of Udensi et al. (2011a, b; 2012a, b), there are positive relationships between number of flowers, number of pods, pod length, number of seeds per pod and seed yield in these legumes studied.

Conclusions

It should be understood that the aim of this research basically was not to compare the performances of both legumes rather to ascertain their sensitivity to gamma irradiation. From this present study, it is obvious that irradiating seeds of pigeon pea and cowpea at the dose of 200 Gy will cause lots of improvements on both morphological and yield traits. It therefore implies that if this process is directed appropriately, it could serve as alternative for improving yield performances of these landraces of legumes.

References

- Ahloowalia, B.S., Maluszynski, M. 2001. Induced mutation: a new paradigm in plant breeding. *Euphytica* 118(2): 167-173.
- Akinyele, B.O., Osekita, O.S. 2006. Correlation and path coefficient analyses of seed yield attributes in okra (*Abelmoschus esculentus* (L.) Moench). *African Journal of Biotechnology* 5: 1330-1336.
- Boureima, S., Diouf, M., Silme, R.S., Diop, T., Vandamme, P., Cagirgan, M.I. 2009. Radiosensitivity of African sesame cultivars to gamma-rays. *Turkish Journal of Field Crops* 14(2): 181-190.
- Brisibe, E.A., Udensi, O., Ntui, V.O., Otu, P.A., Chukwurah, P.N. 2011. Sensitivity of some quantitative and yield characters of 'Egusi' melon (*Colocynthis citrullus* L.) to treatment with microtubule inhibitors. *African Journal of Plant Science* 5(13): 759-766.
- Center for New Crops and Plants Products. 2002. *Cajanus cajan*(L.) Huth. http://www.hort.purdue.edu/newcrop/duke_energy/Cajanus_cajan_us_cajun.html<Access in 10 Jan. 2009>
- Chandra, A., Pental, D. 2003. Regeneration and genetic transformation of grain legumes: An overview. *Current Science* 84:381-387.
- Chaudhury, D., Madanpotra, S., Jaiwal, R., Saini, R., Kumar, P.A., Jaiwal, P.K. 2007. *Agrobacterium tumefaciens*-mediated high frequency genetic transformation of an Indian cowpea (*Vigna unguiculata* L. Walp) cultivar and transmission of transgenes into progeny. *Plant Science* 172:672-700.
- Cheema, A.A., Atta, B.M. 2003. Radio sensitivity studies in basmati rice. *Pakistan Journal of Botany* 35(2):197-207.
- Ciftci, C.Y., TurkanDivanli, A., Khawar, K.M., Atak, M., Ozcan, S. 2006. Use of gamma rays to induce mutations in four pea (*Pisum sativum*L.) cultivars. *Turkish Journal of Biology* 30: 29-37.
- Costa, G.E.A., Queiroz-Monici, K.S., Reis, S.M.P., Oliveira, A.C. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry* 94: 327-330.
- Fagwalawa, L. D. 2000. *Agro-physiological characterization of some early, medium and late maturing varieties of cowpea under sole and inter-cropping systems*. (PhD Thesis) - Bayero University, Kano, Nigeria.
- Geetha, N., Venkatachalam, P., Prakash, V., Laxmiritr, G. 1998. High frequency induction of multiple shoots and plant regeneration from seedling explants of pigeon pea [*Cajanus cajan* (L.) Huth]. *Current Science* 17:1036-1041.
- Ivo, N.L., Nascimento, C.P., Vieira, L.S., Campos, F.A., Avegao, F.J. 2008. Biolistic-mediated genetic transformation of cowpea (*Vigna unguiculata*) and stable Mendelian inheritance of transgenes. *Plant Cell Reports* 27:1475-1483.
- Khan, S., Al-Qurainy, F. 2009. Mutagenic effects of sodium azide and its application in crop improvement. *World Applied Science Journal* 6(12):1589-1601.
- Kumar, P.S., Subrahmanyam, N.C., Farris, D.G. 1985. Plantlet regeneration from immature embryos of pigeonpea. *International Pigeon pea News Letter* 3:12-15.
- Mahandjiev, A., Kosturkova, G., Mihov, M. 2001. Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens. *Israeli Journal of Plant Science* 49(4): 279-284.
- Menda, N., Semel, Y., Peled, D., Eshed, Y., Zamir, D. 2004. *In silico* screening of a saturated mutation library of tomato. *Plant Journal* 38:861-872.
- Mensah, J.K., Obadoni, B.O., Akomeah, P.A., Ikhajagbe, B., Ajibolu, J. 2007. The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). *African Journal of Biotechnology* 6: 534-538.
- Mensah, J.K., Akomeah, P.A. 1997. Mutagenic effects of hydroxylamine and streptomycin on the growth and seed yield of the cowpea, *Vigna unguiculata* L. Walp. *Legume Research* 15: 39-44.
- Mensah, J.K., Akomeah, P.A., Ekpekurede, E.O. 2005. Gamma irradiation induced variation of yield parameters in cowpea (*Vigna unguiculata*(L.) Walp.). *Global Journal of Pure Applied Science* 11: 327-330.
- Pathirana, R., Subasinghe, S. 1993. Response of two sesame cultivars to seed irradiation with gamma rays. *Journal of the National Science Council of Sri Lanka* 21(2):183-188.
- Pons, L. M., Milan, W. A., Cepero, G. M. C. 2001. Radio sensitivity of rice variety Perla. *Centro-Agricola* 28(2):5-8.
- Popelka, J.C., Gollasch, S., Moore, A., Molvig, L., Higgins, T.J.V. 2006. Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transformation of the transgenes to progeny. *Plant Cell Reports* 25: 304-312.
- Rabie, K., Shenata, S., Bondok, M. 1996. *Analysis of Agricultural Science*. University of Egypt, Cairo. Egypt. 566 p.

- Solleti, S.K., Bakshi, S., Purkayastha, J., Panda, S.K., Sahoo, L. 2008. Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean α -amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. *Plant Cell Reports* 27:1841-1850.
- Somers, D.A., Samac, D.A., Olhoff, P.M. 2003. Recent advances in legume transformation. *Plant Physiology* 131: 892-899.
- Stoeva, N., Bineva, Z. 2001. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-radiation contamination I. Growth, photosynthesis rate and contents of plastid pigments. *Journal of Environmental Protection and Ecology* 2: 299-303.
- Tharanathan, R.N., Mahadevamma, S. 2003. Grain legumes: A boon to human nutrition. *Trends in Food Science and Technology* 14: 507-518.
- Tosca, A., Pandolfi, R., Citterio, S., Fansoli, A., Sgorbati, S. 1995. Determination of flow-cytometry of the chromosome doubling capacity of colchicine and oryzalin in gynogenetic haploid of gerbera. *Plant Cell Report* 14: 455-458.
- Udensi, O., Edu, E.A., Umana, U.J., Ikpeme, E.V. 2011a. Estimate of genetic variability in locally grown pulses [*Cajanus cajan* (L.) Huth and *Vigna unguiculata* (L.) Walp]: A pancea for sourcing superior genotypes. *Pakistan Journal Biological Science* 14(6): 404-407.
- Udensi, O., Umana, U.J., Edu, E.A., Ikpeme, E.V. 2011b. Screening locally grown pulses for proximate, anti-nutritive and mineral compositions: Indices for conservation and improvement. *International Journal Agricultural Research* 6(6): 504-510.
- Udensi, O., Edu, E.A., Ntui, V.O., Otu, P.A., Urua, I.S., Akpan, G.A. 2011c. Cyto-sensitivity of some locally grown pigeon pea [*Cajanus cajan*(L.) Huth] to amiprofos methyl treatment as a guide for improvement. *International Journal Current Research* 3(11): 176-180.
- Udensi, O., Edu, E.A., Ikpeme, E.V., Ebigwai, J.K., Ekpe, D.E. 2012a. Biometrical evaluation and yield performance assessment of cowpea [*Vigna unguiculata* (L.) Walp] landraces grown under lowland tropical condition. *International Journal Plant Breeding and Genetics* 6(1): 47-53.
- Udensi O., Ikpeme, E.V., Edu, E.A., Ekpe, D.E. 2012b. Relationship studies in cowpea [*Vigna unguiculata* (L.) Walp] landraces grown under humid lowland condition. *International Journal Agricultural Research* 7(1): 33-45.
- Udensi, O., Arong, G.A., Obu, J.A., Ikpeme, E.V., Ojobe, T.O. 2012c. Radio-Sensitivity of Some Selected Landraces of Pulses to Gamma Irradiation: Indices for Use as Improvement and Preservation Techniques. *American Journal of*

Experimental Agriculture 2(3):320-335.

Xiuzher, L. 1994. Effect of irradiation on protein content of wheat crop. *Journal of Nuclear Agricultural Science* 15: 53-55.