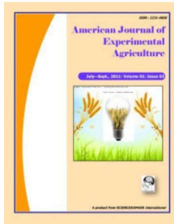

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Radio-Sensitivity of Some Selected Landraces of Pulses to Gamma Irradiation: Indices for Use as Improvement and Preservation Techniques

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Research Article

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ABSTRACT

Aim: Effects of gamma irradiation on amino acid profile, proximate and anti-nutritional compositions of selected landraces of pulses were investigated.
Methods: Seeds of Brown “Fiofio”, White “Fiofio” (*Cajanus cajan*) and “Olaudi” (*Vigna unguiculata*) were exposed to gamma irradiation. Each variety was divided into five groups of fifty grams weight and was exposed to 200, 400, 600, 800Gray (Gy) doses of gamma irradiation from $Cobalt^{60}$ source while the fifth group served as control.
Results: Our results revealed that the proximate, anti-nutritive factors and amino acid profile were significantly ($P<0.05$) reduced with increasing gamma irradiation dose. Though percentage germination was not significantly ($P>0.05$) affected, there was delayed germination and survival percentage became zero percent on exposure to 400Gy and above. “Olaudi” variety was less affected by the gamma rays exposure going by the result of the days to seedling emergence and percentage survival, making pigeon pea species more sensitive to the irradiation.
Conclusion: Taking the results together, it does importantly suggest, however, that lower doses of gamma irradiation might be preferred for achieving the dual purpose of improvement and preservation but high enough to reduce the anti-nutritional factors.

Keywords: Amino acid profile; anti-nutritive composition; gamma irradiation; improvement; proximate; preservation.

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1. INTRODUCTION

Owing to the inadequate supply, coupled with the high cost of protein rich foods of animal origin in the sub-Saharan African countries, exploration and exploitation of inexpensive and highly adapted alternative sources of protein of plant origin (Rajeev and Sridhar, 2008; Udensi et al., 2012a) becomes a sine qua non for increased life expectancy. According to Nielsen, (1991), Tharanathan and Mahadevamma (2003); Udensi et al. (2011b), legumes are valuable sources of complex carbohydrates, protein and dietary fibers. They also contribute significant amount of vitamins and minerals and high energy value. Interestingly, protein contents in legume grains range from 17-40% contrasting with 7-13% of cereals and being equal to the protein content of meat (18-25%) (de Almeida Costa, et al., 2006; Udensi et al., 2011b). Crop development, improvement and preservation are very pivotal in the sustainability and food security of the Sub-Saharan African countries especially, Nigeria (Udensi et al., 2012b). Though there have been so many advances in the use of molecular and biotechnological approaches to this regards, there are still big chasm created between the developed and under-developed world as pertains to the availability of these improved crops, which undoubtedly can be bridged through mutation breeding (Mahandjiev et al., 2001).

It has been observed that genetic variability is very cardinal to successful breeding programme (Udensi et al., 2011a). This variability can be naturally or artificially induced using chemicals or through the exposure to ionizing irradiations such as gamma rays, x-rays, producing mutants with improved characteristics (Ciftci et al., 2006; Boureima et al., 2009). These mutants so produced could then facilitate the isolation, identification and cloning of genes used in designing crops with yield and quality traits (Ahloowalia and Maluszynski, 2001). According to Mahandjiev et al. (2001), induced mutation has great potentials and serves as a complementary approach in genetic improvement of crops. Khan and Al-Qurainy, (2009) reported the use of induced mutation in the improvement of major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated.

Interestingly also, exposing seeds to irradiations have been found to be a potential tool in food preservation against insect infestation and microbial contamination during storage (Gupta, 2009; Hallman, 2001). Dishearteningly, contamination of agricultural produce incurs major economic loss in the tropics and subtropics. Undoubtedly, the conventional method of collection, storage and marketing usually promote the association with several toxigenic moulds. Additionally, most of the common chemical preservatives such as ethylene bromide, methyl bromide, ethylene oxide, aluminum phosphide and malathion (UNEP, 2000) have been banned worldwide and worst still they leave residues, which are hazardous to human health and the environment (Loaharanu, 1994; Hallman, 2001). According to Peraica et al. (1999), Pitt, (2000) legume seeds infested with toxigenic moulds leads to quality deterioration as well as loss in shelf life. Most reports had it that low dose of gamma irradiation induces no substantial changes in the different physico-chemical quality of dried food and grains (Dogbevi et al., 2000; Wu et al., 2002; Al-bashir, 2004; Sung, 2005; Rady et al., 2002; Siddhuraju et al., 2002a; Seda et al., 2001).

According to Ciftci et al. (2006), the availability of seed germination system after irradiation is very crucial in achieving successful mutagenesis. However, physiological and biochemical processes such as germination, seedling emergence and seedling survival have been reported to be affected by gamma irradiation stress. Irradiation of seeds with high dose of gamma rays disturbs protein synthesis Xiuzher (1994), hormonal balance (Rabie et al., 1996), leaf gas-exchange (Stoeva and Bineva, 2001), and enzyme activity (Stoeva and

Bineva, 2001). This research becomes imperative owing to the urgent need to improve these landraces through mutation breeding and also to optimize the technique for seeds preservation. The paper tends to evaluate the effects of gamma irradiation on amino acids profile, proximate and anti-nutritive compositions of the seeds after irradiation. The effect of seed irradiation on percentage germination, days to seedling emergence and percentage survival were assessed.

2. MATERIALS AND METHODS

Seeds of three landraces of pulses brown “Fiofio”, white “Fiofio” [*Cajanus cajan* (L) Millsp] and “Olaudi” [*Vigna unguiculata* (L.) Walp] were obtained from the germplasm collection of Dr. Udensi, Ugorji. The seeds were exposed to gamma irradiation at the National Atomic Energy Commission (NAEC), Abuja, Nigeria. Each variety was divided into five groups of fifty grams weight and four groups were exposed to different doses of gamma irradiation (200, 400, 600, 800Gray) from $Cobalt^{60}$ source while the fifth group served as control. The application was done according to the FAO/IAEA Agricultural and Biotechnology Laboratory in Seibersdorf, Austria in February 2008. Some of the irradiated seeds were taken for amino acids, proximate and anti-nutritive composition analyses while the remaining were kept at 4ºC in a refrigerator and were subsequently used for field studies.

2.1 Determination of Proximate Composition of Gamma Irradiated Seeds of Selected Pulses

The protein content was determined using micro Kjeldhal method (N x 6.25) and the carbohydrate content was determined by the difference obtained after subtracting the total organic nitrogen, protein, lipid, ash, fibre, from the total dry matter and expressing as percentage AOAC (2005). The gross food energy (caloric value) was estimated by multiplying the crude protein, crude fat and total carbohydrate at water factors 4, 9 (Okwu, 2006).

2.2 Anti-nutritive Estimation of Gamma Irradiated Seeds of Selected Pulses

Hydrocyanic acid (HCN) was estimated by the alkaline titration method. For HCN determination, alkaline sample solution was titrated with standard 0.02N $AgNO_3$ to a permanent turbid KI indicator end point (1 ml of 0.02N $AgNO_3$ = 1.08 mg HCN) [AOAC, 2000] Phytic acid was determined as Iron precipitate with the assumption that, Iron: Phosphorus molecular ratio is 4:6 according to McCancee and Widdowson, (1953) (The molecular formula of Phytic acid is $C_6H_{18}O_{24}P_6$ with molecular mass of 660g/mol). While oxalate was determined according to Dye, (1956), Oxalate determination involved three steps- digestion, oxalate precipitation and permanganate titration.

2.3 Determination of Amino Acid Profile

Amino acids profile in the samples was determined using methods as described by Speckman et al. (1958). The samples were dried to constant weight, defatted according the methods of AOAC (2006), hydrolyzed, evaporated in a rotary evaporator and then loaded into the Technicon Sequential Multi- Sampol Amino Acid Analyzer (TSM, USA), which lasted for 76 minutes in each loading. Concentration of amino acids (g/100g) was computed with the formula:

$$NH \times W@NH \div 2 \times Sstd \times C$$

Where; NH = Net height; W = Width at half height; Nleu = Norleucine

$$\text{C} = \frac{Dilution \times 16}{Sample\ weight\ (g) \times N\% \times Vol.loaded} + NH \times W(nleu)$$

2.4 Field Experiment

For the field experiment, a plot of land measuring 10x10 meters was manually cleared in the University of Calabar Experimental Farm. Five beds were made with the spacing of 2 meters between beds. 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) using randomized complete block design (RCBD) with 6 replications. Spacing of 50 x 75cm (cowpea) and 20x75cm (pigeon pea) were maintained. After one month of planting, percentage germination and days to seedling emergence while percentage seedling survival was calculated after 2 months.

2.5 Data Collection and Analysis

Data on the amino acids, proximate, anti-nutritive components, days to seedling emergence, percentage germination, days to seedling survival, flowering and maturity were subject to two-way analysis of variance (ANOVA) using a 3 x 5 factorial layout. Significant means were separated with the Least Significance Difference (LSD) (Obi, 2002).

3. RESULTS

3.1 Effect of Gamma Irradiation on Amino Acids Profile

It was observed that there were significant effects (P<0.05) of gamma irradiation on the amino acids profile of the varieties screened (Figures 1-9). The dose of exposure also had significant (P<0.05) effect on the amino acids profile in the seeds. Comparatively, aspartic acid, serine, alanine and valine were significantly enhanced in the seeds of brown “Fiofio” after irradiation at different doses of exposure (Figures 1a, 3a, 5a&b). Generally, however, it was observed that the exposure of these seeds to gamma irradiation caused significant reduction in the level of amino acids in a dose-dependent fashion.

3.2 Effect on Gamma Irradiation on Proximate and Anti-nutritive Composition

Exposing seeds of the selected pulses to gamma irradiation at different doses caused significant effects (P<0.05) on the proximate and anti-nutritive compositions, which were species and dose-specific (Table 1). For crude protein content, irradiating seeds above 200Gy resulted to a significant reduction, the species notwithstanding. The moisture content of both brown and white “Fiofio” decreased with increasing radiation doses right from 200Gy. “Olaudi” moisture content started to decrease only at 400Gy dose. The crude fiber was significantly increased in the seeds of brown “Fiofio” after irradiation, but showed no significant differences in both white “Fiofio” and “Olaudi”. There was no significant difference (P>0.05) on the fat content of brown “Fiofio” and “Olaudi” after radiation but however differed from those of white “Fiofio”. Tannins, oxalate and phytate (anti-nutritive factors) were significantly (P<0.05) reduced in a dose-dependent fashion, the variety notwithstanding.

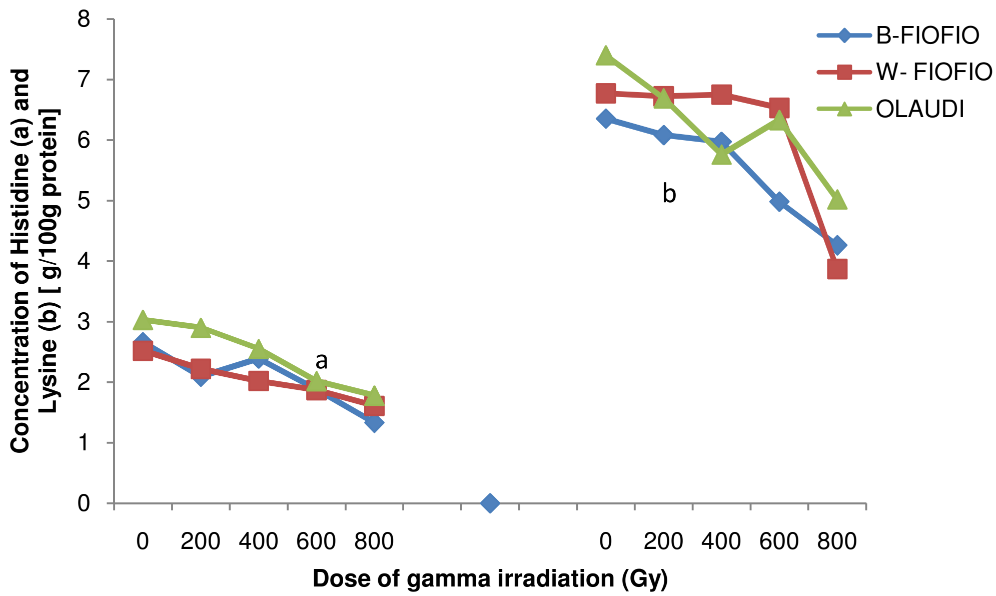

Fig. 1. Effect of gamma irradiation on Aspartic acid (a) and Arginine (b) concentrations in seeds of selected legumes

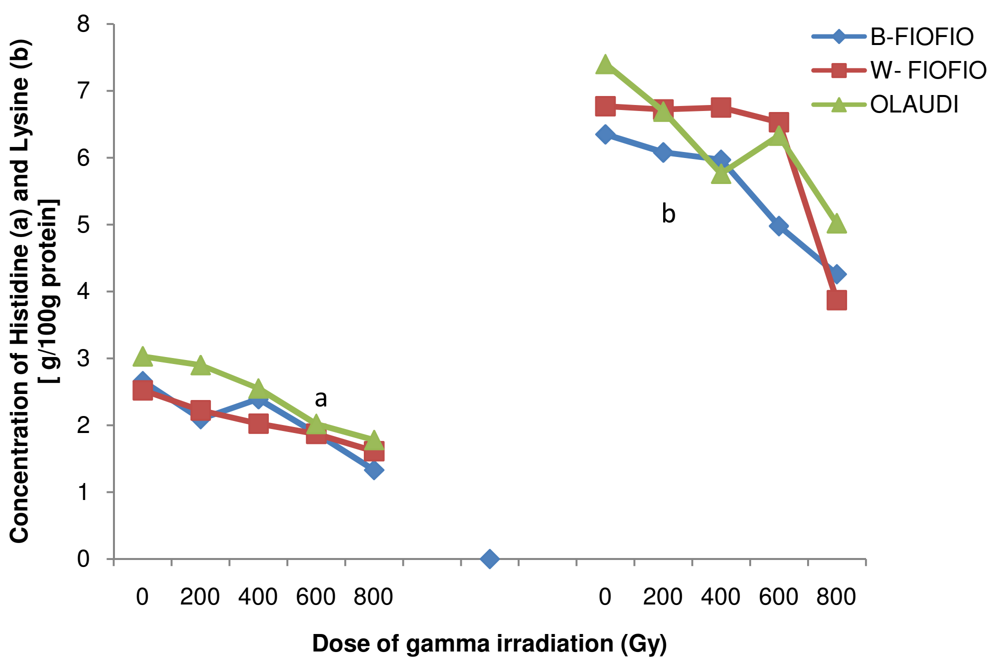

Fig. 2. Effect of gamma irradiation on Histidine (a) and Lysine (b) concentrations in seeds of selected legumes

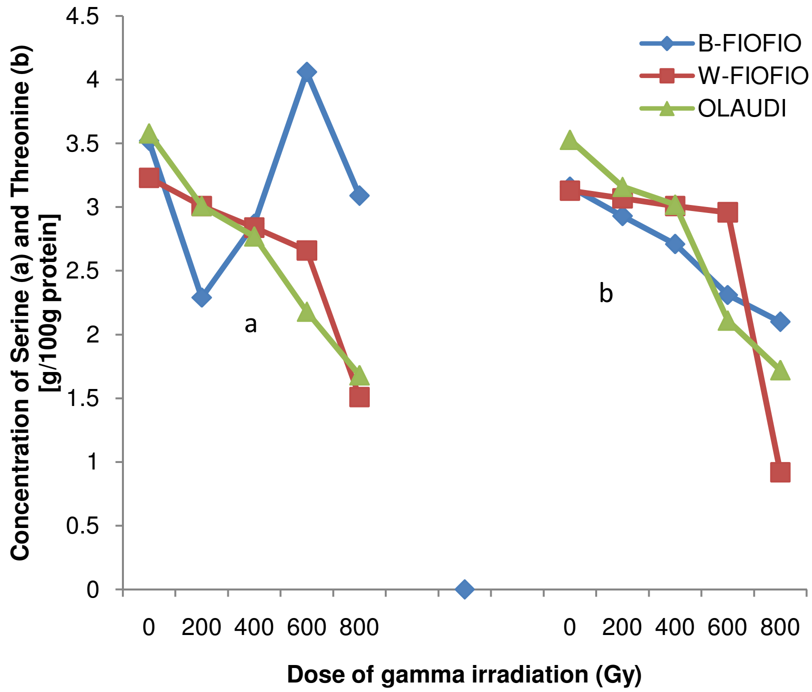

Fig. 3. Effect of gamma irradiation on Serine (a) and Threonine (b) concentrations in seeds of selected legumes

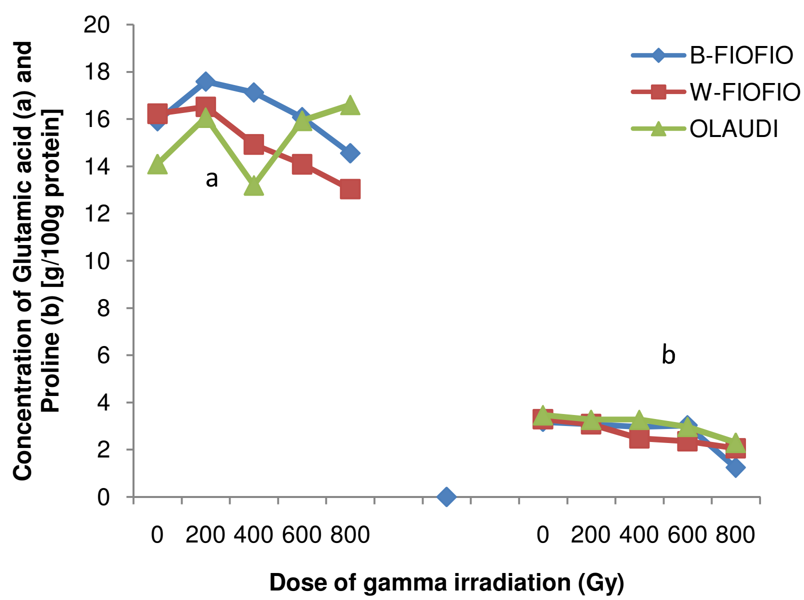

Fig. 4. Effect of gamma irradiation on Glutamic acid (a) and Proline (b) concentrations in seeds of selected legumes

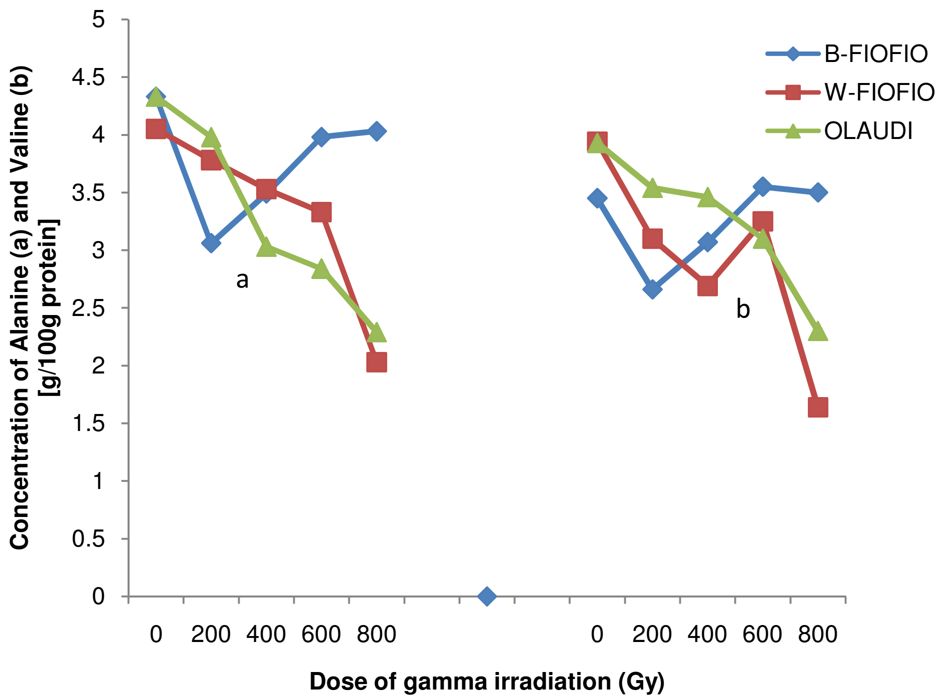

Fig. 5. Effect of gamma irradiation of pulses' seeds on Alanine (a) and Valine (b) concentrations

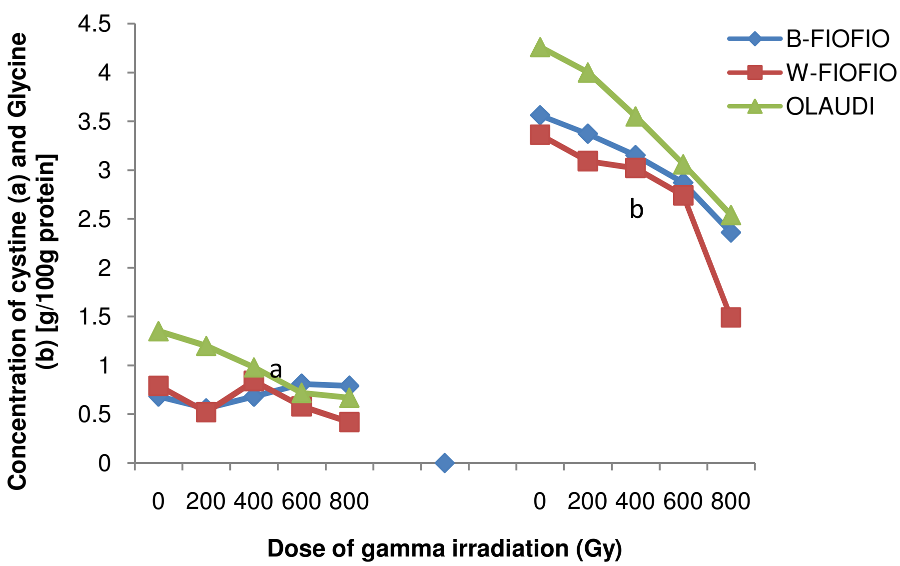

Fig. 6. Effect of gamma irradiation on Cystine (a) and Glycine (b) concentrations in seeds of selected legumes

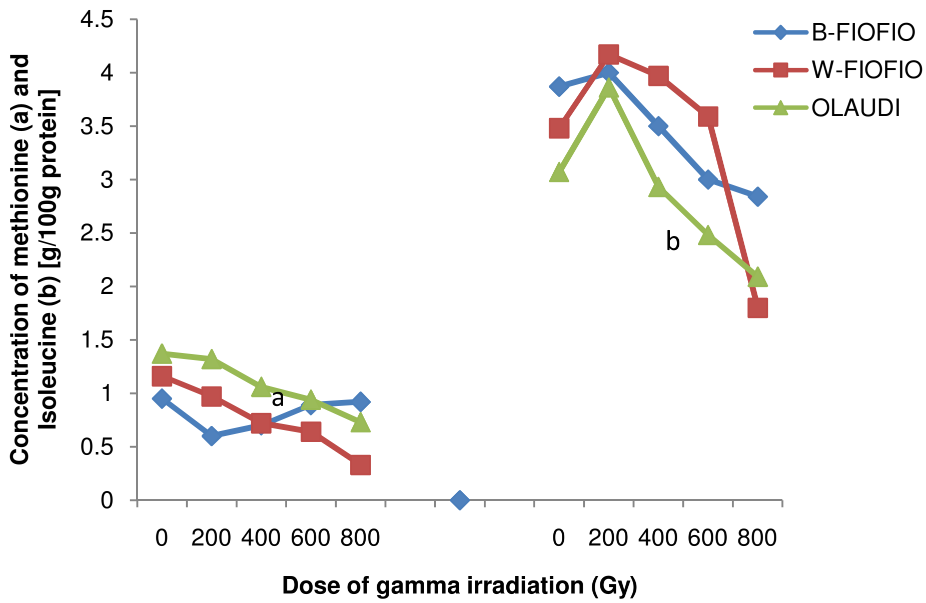

Fig. 7. Effect of gamma irradiation on Methionine (a) and Isoleucine (b) concentrations in seeds of selected legumes

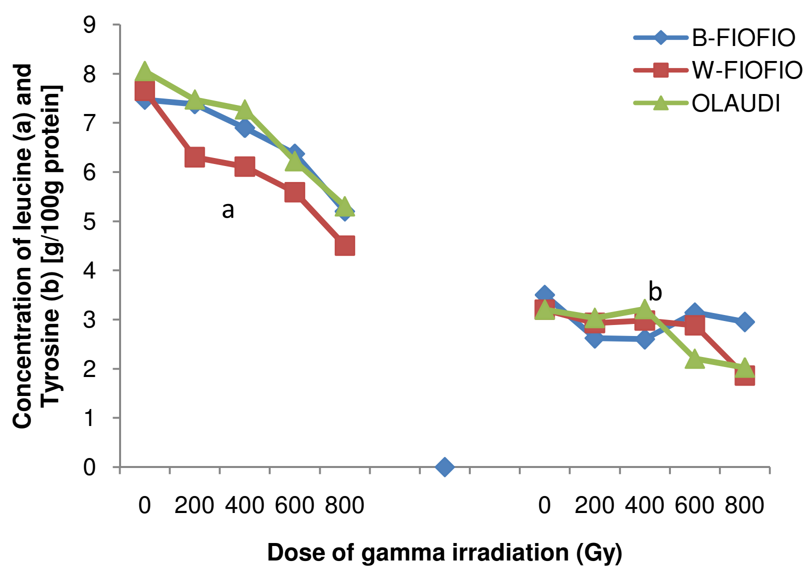

Fig. 8. Effect of gamma irradiation on Leucine (a) and Tyrosine (b) concentrations in seeds of selected legumes

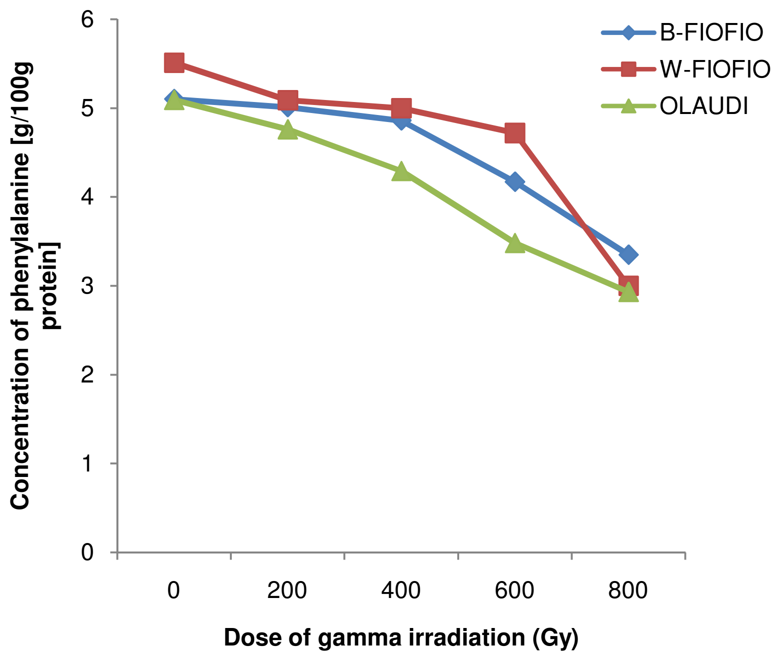

Fig. 9. Effect of gamma irradiation on Phenylalanine concentrations in seeds of selected legumes

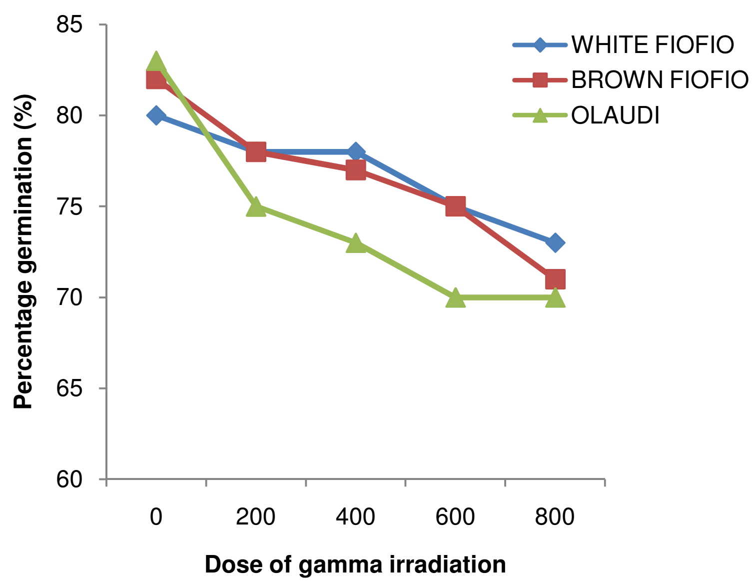

Fig. 10. Effect of seed irradiation on percentage germination on selected pulses

3.3 Effect of Irradiation on Percentage Germination, Days to Seedling Emergence and Percentage Seedling Survival

Result showed that the dose of gamma irradiation did not significantly affect the percentage germination of the irradiated seeds (P>0.05) when compared with the controls. We observed that days to seedling emergence was significantly delayed (P>0.05) in a dose-dependent fashion, which however, was species-specific. Brown "Fiofio" variety was worse affected by the exposure when compared with other varieties, especially at higher doses. There was no significant difference between the days to seedling emergence of the seeds in the control group and the irradiated ones for "Olaudi" variety (Figure 11).

Percentage survival of the seeds after irradiation was significantly affected, especially the pigeon pea varieties. Result revealed that irradiating seeds of pigeon pea at doses of 400, 600 and 800Gy, respectively caused total death of the plant after 2 months. Though the cowpea variety ("Olaudi") survived, though sparingly, at these doses; comparatively, there was significant (P<0.05) reduction in the survival percentage as the exposure dose increased (Figure 12).

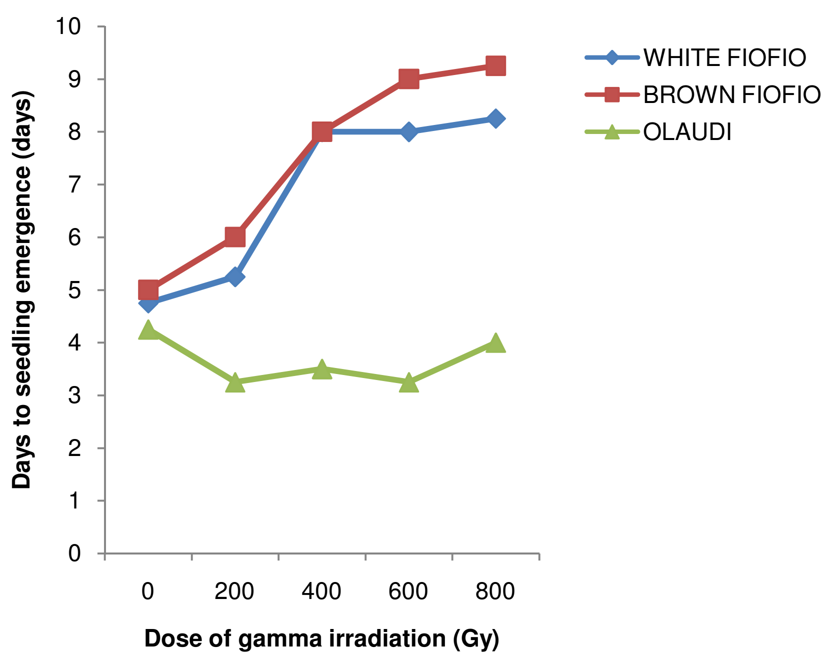

Fig. 11. Effect of seed irradiation on days to seedling emergence of selected pulses

Table 1. Proximate and anti-nutritive compositions of seeds of selected landraces of pulses exposed to gamma irradiation at varying doses

Dose	Brown "Fiofio"					"White Fiofio"					"Olaudi"				
(Gy)	0	200	400	600	800	0	200	400	600	800	0	200	400	600	800
Proximate Comp.															
Crude protein	23.74^{h} ±0.33	23.2^{h} ±0.09	22.71^{g} ±0.1	21.51^{f} ±0.47	13.79^{a} ±0.12	23.27^{h} ± 0.16	20.46^{e} ± 0.06	20.83^{e} ±0.08	18.81^{c} ±0.07	17.15^{b} ±-0.15	25.33^{i} ±0.25	21.67^{f} ±0.07	19.55^{d} ±0.25	19.68^{d} ±0.2	17.37^{b} ±0.14
Moisture	6.03^{b} ± 0.03	5.41^{a} ±0.06	5.21^{a} ± 0.1	4.89^{a} ± 0.09	4.54^{a} ± 0.22	7.18b^{c} ± 0.08	6.75^{b} ± 0.05	6.63^{b} ±0.14	6.19^{b} ±0.09	5.85^{ab} ± 0.08	6.08^{b} ±0.05	5.93^{b} ±0.09	5.55^{a} ±0.17	5.41^{a} ±0.13	4.64^{a} ±0.06
Crude fibre	1.88^{a} ± 0.05	2.04^{b} ±0.03	1.96^{b} ± 0.06	2.04^{b} ± 0.07	2.23^{b} ± 0.05	1.53^{a} ± 0.07	1.55^{a} ± 0.03	1.54^{a} ±0.07	1.59^{a} ±0.06	1.59^{a} ± 0.05	1.99^{b} ±0.05	1.97^{b} ±0.08	2.05^{b} ±0.04	2.02^{b} ±0.09	2.15^{b} ±0.07
Ash	4.16^{f} ±0.01	4.04^{d} ±0.01	3.96^{c} ± 0.02	3.93^{c} ± 0.0 3	3.15^{a} ± 0.01	4.53^{ij} ±0.01	4.50^{i} ± 0.02	4.55^{i} ±0.02	4.51^{h} ±0.01	4.43^{g} ± 0.03	4.08^{e} ±0.02	4.02^{d} ±0.01	3.93^{c} ±0.01	3.82^{b} ±0.02	3.80^{b} ±0.01
Fat	3.80^{b} ±0.09	3.33^{b} ±0.08	3.39^{b} ±0.04	3.57^{b} ±0.11	3.45^{b} ± 0.17	3.04^{a} ±0.13	3.10^{a} ± 0.14	3.21^{a} ±0.25	2.90^{a} ±0.33	2.79^{a} ± 0.26	3.50^{b} ±0.22	3.82^{b} ±0.1	3.77^{b} ±0.13	3.79^{b} ±0.12	3.54^{b} ±0.14
Anti-nutritive comp.															
Tannins	597.14^{h} ± 0.45	398.15^{f} ±0.6	367.05^{e} ± 0.37	197.70^{c} ±0.22	132.14^{a} ±0.65	566.55^{g} ±0.26	132.7^{b} ±0.15	132.68^{b} ± 0.28	132.48^{b} ± 0.18	132.2^{a}8± 0.26	928.35^{j} ±0.58	730.09^{i} ±0.87	597.12^{h} ±0.42	265.39^{d} ±0.3	198.45^{c} ±0.25
Oxalate	1276.5^{l} ±4.37	908.02^{k} ±0.39	809.32^{i} ±1.53	765.34^{h} ±3.97	608.68^{c} ±0.24	1745.85^{n} ±2.21	1285.78^{m} ±1.07	850.3^{j} ±0.84	649.32^{d} ±0.83	583.05^{b} ±0.53	2107.6^{o} ±0.69	747.39^{g} ±1.1	693.31^{f} ±3.41	658.82^{e} ±0.68	442.83^{a} ±0.79
Phytate	110.78^{f} ±0.3	102.1^{d} ±1.35	101.2^{d} ±1.2	97.16^{c} ±0.48	94.38^{b} ±0.26	104.21^{de} ±0.13	104.4d^{e} ± 0.24	100.26^{d} ± 0.35	109.68^{f} ± 0.22	93.79^{b} ±0.98	120.75^{h} ±0.46	113.18^{g} ±0.09	103.0^{de} ±2.28	97.48^{c} ±0.29	87.22^{a} ±0.21

**Means with the same superscript along each horizontal array indicates no significant difference (P < 0.05) from each other*

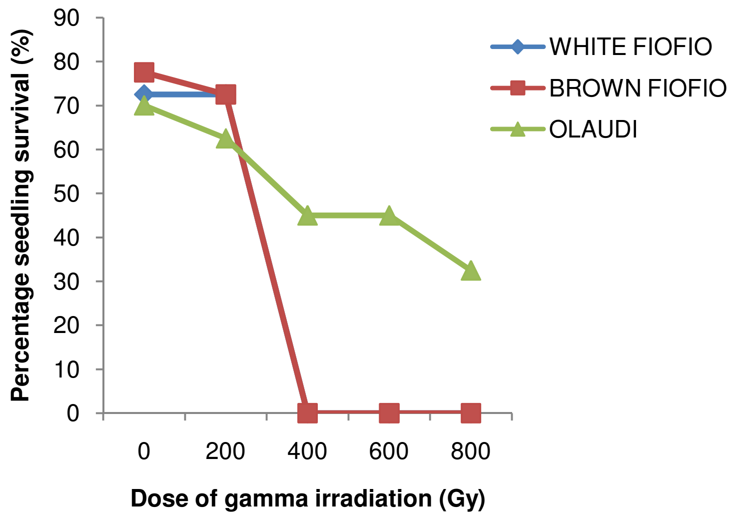

Fig. 12. Effect of seed irradiation on percentage seedling survival of selected pulses

4. DISCUSSION

Mutation breeding, which is based on the creation of variation, selection, evaluation and subsequent multiplication of desired genotypes, has been used as an alternative technique for the improvement of desired traits in agricultural crops (Ciftci et al., 2006; Boureima et al., 2009). This notwithstanding, there are reports to the fact that the exposure of seeds to gamma irradiation either for improvement or seed preservation has negative effects (Xiuzher, 1994; Rabie et al., 1996; Stoeva and Bineva, 2001; Boureima et al., 2009), which might compromise with the aim of the improvement as well as the quality of the seeds.

Our results revealed that there were significant effects ($P<0.05$) of gamma irradiation on the proximate, anti-nutritive and amino acid profile, which were dose-dependent. Expectedly, there are varying reports on the effects of gamma irradiation on the seeds contents and growth of different crops. For instance, while Dario and Salgado (1994) and Rajeev and Sridhar (2008) reported significant increase in proteins of irradiated cowpea and *Mucuna pruriens*, El-Niely(2007) observed that moisture, crude protein, crude fat, crude fiber and ash were unchanged by irradiation but significantly reduced phytic acids and tannins of legumes investigated. Additionally, Rajeev and Sridhar (2008) observed that radiation preserves the overall quality and improves shelf life of legume seeds. Obviously, it should be understood that the effects of gamma irradiation are species, time and dose-specific. This however, will explain the discrepancies observed in our results. The morphological, structural and functional changes due to the exposure of seeds to gamma irradiation depends on the strength and the duration of the stress (Hameed et al., 2008) and this might also account for different effects observed in our present report. The species of crops used notwithstanding, our results corroborate the reports of Dogbevi et al. (2000), Al-bashir (2004), Hassan et al. (2009) that lower doses of gamma irradiation did not have adverse effects on the nutritive composition.

Siddhuraju et al. (2000; 2002) reported poor nutritional values of food legumes due to the presence anti-nutritional factors. Our result showed that there was significant reduction of tannins, oxalate and phytate. It does therefore means that exposing seeds of cowpea and pigeon pea landraces to gamma irradiation could be a veritable tool in reducing these anti-nutritive factors. Their reduction or total removal becomes imperative owing to their detrimental effects such as inhibiting the digestibility of protein and reduction of the bioavailability of some essential minerals (Van der Poel, 1990; Rehman and Shah, 2001). Gupta (2009) reportedly used gamma irradiation as a means of food preservation against insect infestation and microbial contamination which has however caused several economic losses during storage. From our result, high doses of gamma irradiation (400Gy, 600Gy and 800Gy) caused adverse effects to the nutritional quality of the seeds. This does not rule out completely its use as a preservation technique. It does importantly suggest that lower doses of gamma irradiation might be employed to achieve the dual purpose of improvement and preservation.

Protein breakdown and recycling, depends on the proteolytic enzymes levels. These enzymes play important part in the plant's response to environmental stress (Hieng et al., 2004). Our result revealed that seed irradiation did not significantly affect percentage germination but rather adversely affected days to seedling emergence and percentage survival. Though these enzymes were not assayed in this report, it is most likely that the levels of these proteolytic enzymes as opined by Hieng et al. (2004) were affected which, may have weakened their potency and efficacy at shock-absorbing the stress occasioned by the exposure, especially at higher doses. Contrary to our result on percentage germination, Xiuzher (1994), Rabie et al. (1996), Stoeva and Bineva (2001) reported adverse affects of gamma irradiation on protein synthesis, hormone balance, enzyme activity, water exchange, which understandably should have affected germination and other physiological processes. Reduction in seed germination in mutagenic treatments has been reported to be due to delayed or inhibition in physiological and biological processes necessary for seed germination, which include enzyme activity, hormonal imbalance and inhibition of mitotic process (Khan and Al-Qurainy, 2009). This additionally, might affect ATP biosynthesis resulting in decreased availability of ATP molecule (energy deprivation, as a result of general inhibitions of cellular respiration and oxidation phosphorylation processes), which may slow down the germination rate. As true as this submission could be, our result suggests that the exposure may not have affected the ATP biosynthetic pathways. Arguably, another striking possibility is the fact that the irradiated seeds might have probably developed tolerance to the inhibitory effect of gamma irradiation on germination and had therefore improved their physiological conditions resulting to delayed seed germination without necessarily affecting percentage germination.

Our result also showed that percentage survival; especially of the pigeon pea varieties was reduced to zero percent when the seeds were exposed to 400, 600 and 800Gy, respectively. Speculatively, however, this could be attributed probably to physiological disturbance, which may have affected some biochemical pathways in the seedling. Expectedly, the exposure may have resulted to chromosomal damages due to the toxicity associated with direct interaction between gamma rays and DNA molecules (Brisibe et al., 2011). Additionally, this poor survivability may not be far from the fact that in response to environmental stress such as gamma irradiation, proteins that should have been rebuilt were damaged, or/and misfolded leading to the lack of production of free amino acids that will be required for the synthesis of new proteins, which could have sustained the plants (Schaller 2004; Grudkowska and Zagdanska, 2004).

Going by the confines of our present results, especially on germination and percentage survival of the plants, it suggests that "Olaudi" variety (cowpea) was less affected by the gamma rays exposure. What this might mean is that “Olaudi” had greater capacity to withstand the stress, which probably might be genetic, structural or texture of the seeds.

5. CONCLUSION

Implicitly, our results revealed that the proximate, anti-nutritive factors and amino acid profile were significantly ($P<0.05$) reduced with increasing dose, indicating that the dose of gamma irradiation employed were high to cause the desired improvement in the nutritive components. Interestingly, reduction in the anti-nutritive factors might have positive effect on seed quality. Though percentage germination was not significantly affected, there was delayed germination and survival percentage became zero percent on exposure to 400, 600 and 800Gy, respectively. Taking these results together, lower doses of gamma irradiation might be preferred for better results. Understandably, it will be very wise to strike a balance between its use for preservation and improvement as to have positive impacts on the nutritional, growth and development of the crops while still reducing the anti-nutritive compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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