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Effects of Fast Neutron Irradiation on Agronomic Traits of Nigerian Pepper (*Capsicum annuum* L.)

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Summary

The effects of fast neutron irradiation using an Americium-Beryllium source with a flux of 1.5×10^4 n cm^{-2} s^{-1} on the agronomic traits of three varieties of Nigerian pepper – *Capsicum annuum* var. *accuminatum* (MN/SH/001), *C. annuum* var. *abbreviatum* (MN/AR/002), and *C. annuum* var. *grossum* (MN/AT/003) – were studied. Seeds of each variety were irradiated for 0, 30, 60, 90 and 120 min before they were sown – with their respective controls – in order to assess the effects of the different irradiation treatments on the plants. All irradiation periods caused leaf abnormalities such as leaves with reduced size, leaves with invaginated or inverted margins, or with a blunt or bifurcated apex, when compared with control plants. Germination percentage tended to become reduce while there was a pronounced positive trend in plant height, number of leaves/plant, fruits/plant, seeds/fruit, weight, length and width of fruits, by increasing the irradiation period. However, 120 min was the most effective irradiation period to induce viable and useful mutations for yield parameters in pepper.

Key words. Americium-Beryllium – *Capsicum annuum* var. *accuminatum* – *Capsicum annuum* var. *abbreviatum* – *Capsicum annuum* var. *grossum* – germination percentage – leaf abnormalities – mutation

Introduction

Capsicum spp. belong to the nightshade family, *Solanaceae* (GRIN 2009). The genus consists of over 100 species and even more botanical varieties (Ado 1999; Falusi 2007). These include five domesticated species, namely *C. annuum*, *C. frutescens*, *C. baccatum*, *C. chinense* and *C. pubescens*, all believed to have originated from the New World (McLeod et al. 1982; Bosland 1994). *C. annuum* and *C. frutescens* are the most recognized species grown in commercial quantities all over Nigeria (Falusi and Morakinyo 2001; Mady et al. 2005). These two species form an important ingredient in people's diet the world over (GRIN 2009), due to the pungency of the fruits, resulting from the high concentration of capsaicinoid alkaloids (Bosland and Vostava 2000). In addition, *Capsicum* spp. are a rich source of vitamins A and C (ascorbic acid) (Gill 1992; Ado 1999). *Capsicum* fruits are also popular as food spices, colouring agent, as well as pharmaceutical ingredients (Bosland 1996). In African medicine, *Capsicum* spp. are used in treating sore throat (Abdullahi et al. 2003). Capsaicin is used mainly in topical medications in modern medicine as a circulatory stimulant and analgesic. These popular uses of *Capsicum* peppers have fuelled an increasing demand for the crop, and a search for simple but viable ways of increasing supply of the product, independent of man-power and the adequacy of farming conditions. Thus, attention has gradually shifted towards improving the genetic quality of the species through plant breeding and selection. One possible means is through radiation-induced genetic variability. The FAO (2009) reported that 2008 marked the 80th anniversary of mutation induction in plants. The application of gamma rays and other physical mutagens such as fast neutrons has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies (David 2010). The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2700 plant mutant varieties (FAO 2009). The present work aimed to investigate the response of three botanical varieties of *C. annuum* to different doses of fast neutron irradiation (FNI) for different time periods on growth, yield, fruit and leaf characteristics.

Materials and Methods

Fresh fruit of three pepper botanical varieties (50 fruits each) were bought from a local farmer in Minna, Niger

State, Nigeria. The fruits were maintained in separate polythene bags. The varieties were identified as *C. annuum* var. *accuminatum* Fingerh (MN/SH/001), *C. annuum* var. *abbreviatum* Fingerh (MN/AR/002), and *C. annuum* var. *grossum* Sendt (MN/AT/003) (Table 1) using a taxonomic aid provided by Simmond (1976), as well as morphological descriptions of Hutchinson and Dalziel (1963), Schippers (2000) and Abdullahi et al. (2003). Each fruit of three *Capsicum* varieties were cut open and their seeds were removed, kept separately in three trays and sun-dried for 8 h. The dry seeds were irradiated with FNI at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria, using an Americium-Beryllium source with a flux of 1.5×10^4 n cm^{-2} s^{-1} for 0, 30, 60, 90, and 120 min. The equipment used was a Miniature Neutron Source Reactor (MNSR) designed by the China Institute of Atomic Energy (CIAE) and licensed to operate at a maximum power of 31 kW (Sar 2005).

The sun-dried seeds were tested for viability using the floatation method (Ponnuswamy et al. 1991) before FNI treatment. Treated seeds (100 from each treatment) were then sowed in nursery trays to obtain seedlings, and then transplanted into 3.5-L plastic pots containing garden soil, at a rate of three seedlings per pot after 4 weeks in the nursery. No fertilizer was applied although, when the crop began to flower, an insecticide (Pyrethroids cypermethrin at the rate of 10–15 l ha^{-1} with controlled droplet application using spinning disc sprayers) was applied to prevent insect-borne diseases. The planted seeds were watered once daily between 5.00 and 6.30 pm using borehole water. Each treatment was replicated four times using a completely randomized design (CRD).

Data were collected from100 seeds for each variety to assess germination (survival) percentage, and from 15 plants for each variety to determine number of leaves/plant at maturity, height of plant at maturity (number of days to 50 % flowering) and yield/plant in each M_1 (M = first filial mutant) generation.

Data was analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to separate the means with significant differences detected at P = 0.05. Pearson's correlation analysis was used to find the relationship between treatments and selected parameters.

Results and Discussion

There was a negative correlation between irradiation period and seed survival percentage in MN/SH/001 and MN/AR/002 (Table 2), implying that as irradiation time increased, percentage survival decreased. In both cases the correlation coefficient was strong. Oddly, the correlation was weakly positive for MN/AT/003, implying that an increase in irradiation time did not necessarily lead to a decrease in the survival percentage.

Wide variation was observed in plant height, number of leaves/plant, fruits/plant, and seeds/fruit, and weight, length and width of fruits for all three varieties (Table 3). FNI was capable of producing significant changes in the agronomic traits and yield parameters of pepper plants. Similar effects of ionizing radiation on reproductive and other yield parameters have been reported for tomato exposed to 1–4 mM sodium azide (Adamu and Aliyu 2007). Shin et al. (2011) reported that gamma irradiation-mediated mutation breeding produced useful varieties of sweet potato having a high yield and high starch content. Bhosale and Hallale (2011), on the other hand, observed a wide range of chlorophyll and viable morphological

Table 2. Correlation (r) between the treatments and percentage of seeds that germinated per 100 seeds sown for three pepper (*Capsicum* spp.) varieties.

Treatments	MN/SH/001 (%)	MN/AR/002 (%)	MN/AT/003 (%)
Control	100	100	100
30 min	50	30	52
60 min	51	21	30
90 min	51	16	67
120 min	37	16	66
R	−0.8156	−0.8736	0.2367

Table 1. Description of the pepper (*Capsicum* spp.) varieties used in this study.

Code number	Source	Local name	Botanical name	Description
MN/SH/001	Minna	Ata Shombo	*C. annuum* var. *accuminatum* Fingerh	Medium-sized annual plant, long pointed and pendant fruits with hot taste, one pedicel per node.
MN/AR/002	Minna	Ata Rodo	*C. annuum* var. *abbreviatum* Fingerh	Medium-sized annual plant, small oblong and wrinkled fruits with hot taste, one pedicel per node.
MN/AT/003	Minna	Ata Tatase	*C. annuum* var. *grossum* Sendt	Short annual plant, medium size, bell shaped fruits with mild taste, one pedicel per node.

Table 3. Effects of fast neutron irradiation on agronomic traits of three pepper (*Capsicum* spp.) varieties.

Characters	Irradiation period (min)				
	0	30	60	90	120
MN/SH/001					
Plant height (cm)	44.2 ± 5.2 a	45.3 ± 11.5 a	52.9 ± 4.9 ab	50.2 ± 14.1 ab	59.9 ± 6.0 b
Leaves (n plant^{-1})	129.9 ± 10.4 a	132.7 ± 44.6 a	173.0 ± 36.5 a	161.8 ± 62.5 a	230.8 ± 32.8 b
Fruits (n plant^{-1})	8.0 ± 2.5 a	15.3 ± 4.9 b	14.8 ± 3.8 b	14.3 ± 5.0 b	15.8 ± 4.9 b
Seeds (n fruit^{-1})	102.8 ± 18.2 a	105.0 ± 21.7 ab	108.1 ± 22.2 ab	110.3 ± 23.8 ab	125.8 ± 0.5 b
Length of fruit (cm)	7.3 ± 1.4 a	7.5 ± 1.7 a	6.9 ± 1.5 a	7.7 ± 1.5 a	8.2 ± 1.7 a
Width of fruit (cm)	1.9 ± 0.3 a	2.0 ± 0.2 abc	1.9 ± 0.3 ab	2.2 ± 0.2 bc	2.3 ± 0.5 c
Weight of fruit (g)	7.7 ± 0.7 a	8.4 ± 2.0 a	8.7 ± 1.8 a	9.1 ± 1.1 ab	10.2 ± 1.5 b
MN/AR/002					
Plant height (cm)	21.7 ± 3.7 a	29.9 ± 7.7 b	32.8 ± 5.1 b	29.3 ± 7.1 b	33.7 ± 10.6 b
Leaves (n plant^{-1})	67.1 ± 19.7 a	99.2 ± 32.8 b	113.0 ± 2.1 b	99.1 ± 32.3 b	120.1 ± 53.0 b
Fruits (n plant^{-1})	15.9 ± 7.6 a	17.8 ± 12.9 a	19.0 ± 7.6 a	19.7 ± 8.6 a	17.5 ± 9.1 a
Seeds (n fruit^{-1})	54.9 ± 21.2 a	66.1 ± 11.1 ab	71.9 ± 16.6 b	73.5 ± 19.8 b	72.0 ± 23.9 ab
Length of fruit (cm)	4.0 ± 0.9 a	4.3 ± 0.7 ab	5.3 ± 0.7 c	4.3 ± 1.1 ab	4.9 ± 1.2 bc
Width of fruit (cm)	3.0 ± 0.8 a	3.6 ± 0.4 b	3.9 ± 0.3 b	3.7 ± 0.5 b	3.7 ± 0.3 b
Weight of fruit (g)	10.1 ± 3.8 ab	9.6 ± 3.2 a	11.0 ± 1.7 ab	12.2 ± 3.6 ab	12.9 ± 2.4 b
MN/AT/003					
Plant height (cm)	36.22 ± 10.06 a	34.14 ± 6.16 a	31.5 ± 3.4 a	36.3 ± 4.9 a	37.7 ± 7.0 a
Leaves (n plant^{-1})	82.67 ± 29.67 a	77.33 ± 17.70 a	68.7 ± 10.0 a	86.6 ± 17.8 a	90.6 ± 28.5 a
Fruits (n plant^{-1})	3.20 ± 2.44 a	1.90 ± 2.17 a	2.0 ± 1.2 a	1.7 ± 1.6 a	4.1 ± 8.0 a
Seeds (n fruit^{-1})	185.00 ± 67.48 a	174.00 ± 108.73 a	172.8 ± 77.5 a	138.1 ± 63.7 a	145.9 ± 49.2 a
Length of fruit (cm)	9.57 ± 1.69 a	8.44 ± 2.24 a	9.3 ± 1.0 a	8.6 ± 2.1 a	8.0 ± 3.0 a
Width of fruit (cm)	4.23 ± 0.91 a	4.03 ± 0.63 a	3.8 ± 0.8 a	3.4 ± 1.0	3.7 ± 1.1 a
Weight of fruit (g)	54.60 ± 14.51 b	48.80 ± 16.40 b	48.8 ± 16.4	28.3 ± 17.2 a	41.8 ± 18.3 ab

Values are mean ± SD. Values followed by the same letter(s) within the same row do not statistically differ at the 5 % level according to Duncan's multiple range test, analyzed separately for each variety.

mutations with gamma irradiation in M2 generation of black gram (*Vigna mungo*).

For MN/SH/001, FNI for 120 min could effectively induce viable and useful mutations in pepper yield parameters. A similar result was reported by Reddy and Smith (1978) and Adamu et al. (2004), who reported that obtaining mutations in the crops they studied (*Sorghum bicolor* and *Lycopersicon esculentum*, respectively) using ionizing radiation was dependent on dose, as shown in our pepper varieties (Table 3).

All the plants produced from the non-irradiated seeds – which served as the control – produced normal leaves (Fig. 1A–C). However, this was not the case among plants whose seeds were irradiated. Leaf irregularities such as small leaves, chlorophyll-deficient leaves, or leaves with invaginated margins, with inverted margins, with blunt apices and with bifurcated apices (Fig. 1D–I) were observed. These leaf morphological abnormalities that were observed are indications that FNI affected the plants negatively. Shah et al. (2008), Khan and Aslam (1982) and Islam et al. (1994) reported similar leaf morphological abnormalities in *Crotalaria saltiana* and *C. juncea* treated with ionizing radiation. The MN/AT/003 chlorophyll-deficient mutants might be the main reason why yield parameters (e.g. number of fruits/plant) decreased (Table 3). A similar result was obtained by Adamu (2004) in maize (*Zea mays* var. *praccox* Sturt) plants irradiated with thermal neutron and gamma rays (^{60}Co) in which chlorophyll-deficient mutants were induced.

Low genetic variability for major characters is a limiting factor for the improvement of a crop but induced mutations can provide an additional source of variability for quantitative and qualitatively inherited traits in a number of crop plants, as shown effectively in this study for pepper through the use of FNI.

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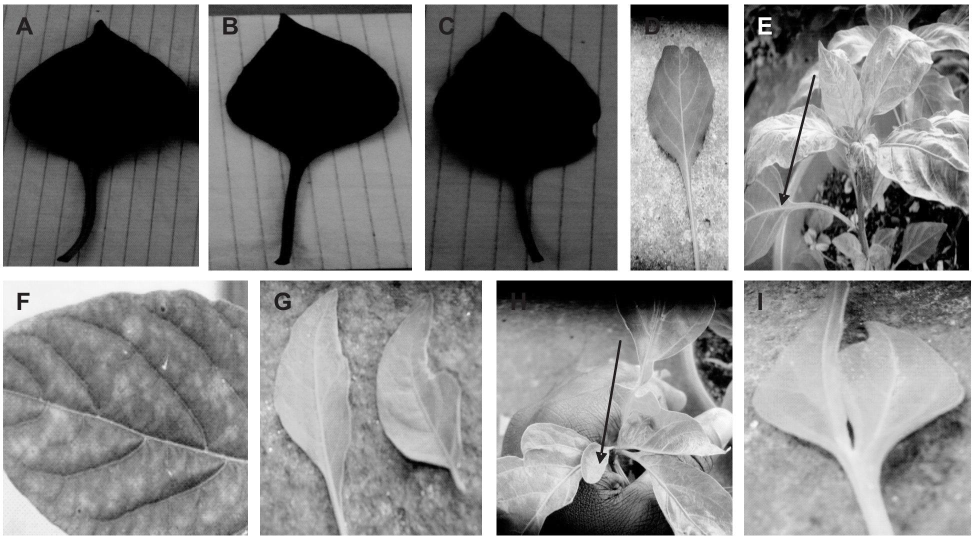

Fig. 1. A, B, C. Normal leaves of *Capsicum annuum* var. *accuminatum* (MN/SH/001), *C. annuum* var. *abbreviatum* (MN/AR/002), and *C. annuum* var. *grossum* (MN/AT/003), respectively. D. Leaf with bifurcated apex in MN/SH/001 (60 min irradiation exposure). E. Fully mature MN/AT/003 plant with chlorophyll mosaic leaves. Arrow indicates leaf in F. F. Leaf showing chlorophyll mosaic points in MN/AT/003 (90 min irradiation exposure). G. Leaves showing invaginated margins in MN/SH/001 (120 min irradiation exposure). H. Crinkled leaves with dented margins in MN/AT/003. The arrow also shows a small leaf with a blunt apex (30 min irradiation exposure). I. A leaf that became bi-foliate in MN/SH/001 (30 min irradiation exposure).

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