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Full Length Research Paper

Effect of Fast Neutron Irradiation (FNI) on pollen germinability of three Nigerian sesame varieties

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The effects of Fast Neutron Irradiation (FNI) from an Americium Beryllium source with a flux of 1.5 × 10^4 n cm^{-2} s^{-1} on the pollen germinability of three Nigerian sesame (*Sesamum indicum* L.) varieties were investigated. Seeds of Kenana-4, Ex-Sudan and E-8 were irradiated with FNI doses for 30, 60, 90 and 120 min, representing 4, 8, 12 and 16 µs, respectively. The seeds were grown to maturity with their respective controls (0 µsv of FNI) in order to assess the effects of FNI treatments on the pollen germinability of adult plants. Pollen germinability was assessed in 1% (w/v) agar-based solutions containing 1, 2 and 3% (w/v) sucrose. There were highly significant differences between pollen germination percentages and FNI treatments for all three varieties. Higher pollen germination was observed from FNI-irradiated seeds while 2% sucrose showed the highest percentage germination among all concentrations tested. FNI could be used to improve pollen germination and fruit yield in sesame at 8-16 µsv.

Key words: Sesame, Americium Beryllium, sucrose, percentage germination.

INTRODUCTION

Sesame (*Sesamum indicum* L.; Pedaliaceae) is one of the world's most important oilseed crops (Ashri and Van Zantten, 1994) grown principally in countries such as India, Sudan, Ethiopia, Uganda and Nigeria for its oil-rich seeds. In Nigeria, sesame production probably began in the middle belt region of the country and later spread out to other parts of the country (Falusi et al., 2001). Some of the local Nigerian names are *ridi-hausa*, *ishwa-tiv*, *gorigo-igbira*, *eeku-yoruba* and *doo-jukun*. Sesame production is low compared with other oil-rich crops (IAEA, 2001). According to FAO (2011), while sesame production was about 18 million t/year, soybean was 223 million t/year, rape seed was 58.4 million t/year, cotton seed was 40 million t/year and palm oil was 30 million t/year. However, sesame production area was declining in traditional areas (FAO, 2005). Despite the potential for increasing production and productivity of sesame, there are a number of challenges and constraints inhibiting improvement, including the lack of improved cultivars and a poor seed supply system.

Efforts have been made to genetically enhance the crop through induced mutations (Ashri, 1982). Gamma rays at doses ranging from 150-800 Gy could successfully induce useful mutations while fast neutron irradiation (FNI) doses of 30 and 80 Gy were effective for the induction of useful mutations in sesame (Ashri and van Zanten, 1994). Falusi et al. (2012a) used 12 µs of FNI to increase fruit yield, and fruit width and length in pepper.

In most flowering plants, fertilization is required to form fruit and seed in the absence of parthenocarpy. Successful fertilization greatly depends on the germination capacity of pollen (Beyhan and Odabas, 1995). The extent of pollen production, viability and germination percentage in a plant will indicate how effective it would be as a male parent or pollinizer. Research efforts to improve pollen germination in sesame are very scarce. The effect of FNI on pollen has received little attention even though FNI damage to vegetative organs in pepper has been investigated

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Table 1. Some physical and chemical properties of the soil used in this study.

pH	OC	OM	N	Exchangeable cations (Cmol/Kg)				EA (Cmol/Kg)	CEC (Cmol/Kg)	Sand (%)	Silt (%)
				Na	K	Ca	Mg				
6.72	1.63	2.84	0.1	0.133	0.153	5.936	4.117	0.18	10.52	82.52	10.28

OC = organic carbon, OM = organic matter, TN = total nitrogen, EA = exchange acidity, CEC = cation exchange capacity.

(Falusi et al., 2012b). Therefore, this study aims to study the effect of FNI on pollen germinability of three varieties of sesame as a means of improving crop production.

MATERIALS AND METHODS

The study was carried out during the rainy season, between May and August 2012, at the Department of Biological Science, Federal University of Technology, Minna, Niger State, Nigeria. Seeds of three varieties of sesame (Kenana-4, Ex-Sudan and E-8) were received from the National Cereal Research Institute, Badeggi, Niger State, Nigeria. They were irradiated with FNI for 0, 30, 60, 90 and 120 min (resulting in 0, 4, 8, 12 and 16 µs) from an Americium Beryllium source with a flux of 1.5×10^4 n cm^{-2} s^{-1} at the Centre for Energy Research Training Institute, Ahmadu Bello University, Zaria, Nigeria. The irradiated seeds were planted alongside their respective control (0 µs FNI) in the Experimental Garden of the Centre for Preliminary and Extra Mural Studies, Federal University of Technology, Minna, Nigeria. A factorial design was adopted using a randomized block design with 30 pots per block. The experiment was replicated four times, with a total of 120 pots. Ten seeds were planted per pot (that is, 5 per hole in each pot). Three weeks after planting, each pot was thinned to two plants per pot. A total of 8 pots for each treatment were used. The physical and chemical properties of the soil used are shown in Table 1. No fertilizer was applied, although an insecticide (pyrethroid cypermethrin at a rate of 10 to 15 L/ha with controlled droplet application using a spinning disc sprayer) was applied to prevent insect-borne diseases. Forty weeks after planting, matured flower buds for the pollen germination test were randomly collected from 8 plants from each treatment at the full bloom stage in July. Flower buds were collected in the morning between 8 and 9 am. Anthers were removed from flower buds and pollen grains were allowed to dehisce before commencing the germination test.

Pollen germination was assessed using the hanging drop method (Beyhan and Odabas, 1995). Sucrose (BDH Chemicals Ltd., Poole, UK) at different concentrations (1, 2 and 3%, w/v) was added to water containing 1 g/L agar (BDH Chemicals Ltd.), after which 10 ml of the medium was added to each Petri dish (two for each treatment). The dishes were subdivided into quadrants, each quadrant representing a replication totalling 8 replications for each treatment. About 300-350 pollen grains were sprinkled on each Petri dish with the aid of a brush. Petri dishes were closed and sealed with Parafilm® to prevent water loss from pollen and were incubated at 30°C for 24 h. After germination, pollen was placed in a fridge at 2°C for a maximum of 24 h until it was counted. Pollen germination was viewed with an AGARY microscope (Lagos, Nigeria) at X100 magnification. Pollen was considered to have germinated if the pollen tube length was at least equal to or greater than the pollen grain diameter. Percentage germination was calculated using the formula:

$$\text{Percentage germination} = \frac{\text{Number of pollen germinated}}{\text{Total number of pollen counted}} \times 100$$

Data were subjected to analysis of variance (ANOVA) using the TARIST computer package. Duncan's multiple range test was used to separate the means when significant. Pearson's linear correlation was used to find the relationship between percentage germinability and FNI.

RESULTS AND DISCUSSION

Highly significant differences in pollen germination between Kenana-4, Ex-Sudan and E-8 were observed after exposure to FNI and culture at different concentrations of sucrose (Table 2). Higher percentages of pollen germination were observed following FNI irradiation relative to the unradiated control. For Kenana-4 and E-8, the highest percentage germination was observed with 8 µs (60 and 78%, respectively) while for Ex-sudan, the highest percentage germination was observed with 12 and 16 µs (53%). This indicates that the pollen grains of all three sesame varieties are not resistant to FNI. Previous studies on pollen showed that ionizing radiation could have a positive effect on pollen germination (Koti et al., 2004). Wang et al. (2006) observed that pollen germination of *Poa annua* (31.7%) and *Polygonum aviculare* (30.7%) increased by exposure to UV-B radiation for 60 min compared to their respective unexposed controls (11.6 and 15.2%, respectively). FNI has a stimulatory effect on sesame pollen germination (Figure 1A-C) and consequently may increase fruit yield. Germanà et al. (2003) hypothesized that an electromagnetic field can increase the transport of calcium across the cell membrane and alter pollen

Table 2. Effect of FNI on the percentage pollen germinability of three sesame cultivars (Kenana-4, Ex-Sudan and E-8) at different sucrose concentrations.

Treatment	Sucrose concentration (%, w/v)		
	1%	2%	3%
Kenana-4			
0 µs	32.33 ± 0.58^{ab}	42.33 ± 2.52^{defg}	28.17 ± 0.21^{bcde}
4 µs	27.67 ± 1.16^{bc}	32.57 ± 2.41^{g}	14.32 ± 0.08^{ef}
8 µs	34.76 ± 0.05^{ab}	60.10 ± 2.25^{b}	32.20 ± 0.20^{abc}
12 µs	40.40 ± 0.53^{a}	50.50 ± 0.40^{bcde}	20.32 ± 0.02^{cdef}
16 µs	36.00 ± 1.00^{ab}	45.45 ± 0.64^{cdef}	30.10 ± 1.00^{bcd}
Ex-Sudan			
0 µs	28.33 ± 0.58^{bcd}	46.00 ± 3.14^{cdef}	42.55 ± 0.05^{ab}
4 µs	25.10 ± 1.00^{bcde}	37.60 ± 0.72^{efg}	20.18 ± 0.28^{cdef}
8 µs	15.41 ± 0.36^{efg}	48.47 ± 3.54^{bcde}	45.03 ± 0.55^{a}
12 µs	22.63 ± 2.41^{cde}	53.70 ± 1.84^{bcd}	43.83 ± 0.76^{ab}
16 µs	25.30 ± 0.26^{bcde}	53.76 ± 3.01^{bcd}	45.17 ± 0.21^{a}
E-			
0 µs	5.87 ± 0.15^{gh}	57.63 ± 1.66^{bc}	30.37 ± 0.32^{abc}
4 µs	16.73 ± 0.58^{def}	28.03 ± 0.32^{f}	22.14 ± 0.02^{cdef}
8 µs	8.13 ± 0.15^{fgh}	77.90 ± 1.00^{a}	10.22 ± 0.01^{f}
12 µs	20.20 ± 2.00^{cde}	49.00 ± 2.91^{bcde}	15.54 ± 0.36^{def}
16 µs	0.00 ± 0.00^{h}	60.10 ± 1.10^{b}	40.14 ± 0.053^{ab}

Means ± Standard deviation (SD) followed by the same letter(s) within the same column do not statistically differ at 5% level tested by Duncan's multiple range test.

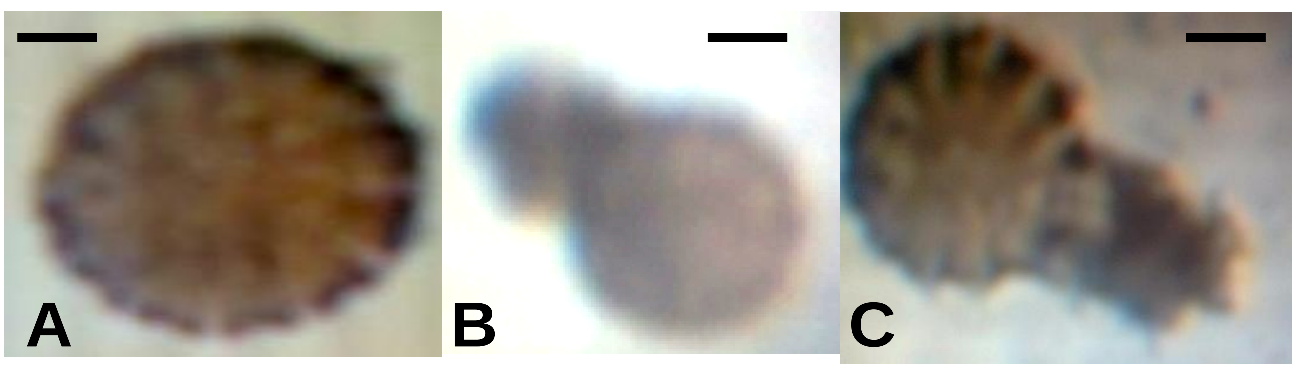

Figure 1. Micrographs of pollen grains of: (A) E8 before germination, (B) untreated (control plants) of Ex-Sudan 24 h after germination, and (C) Kenana-4 24 h after germination. Bar = 1 µm.

germination. Falusi et al. (2012a) observed an increase in number of fruits in pepper after exposure to 12 µs of FNI.

FNI was correlated to percentage pollen germination at various levels in the three sesame varieties at different sucrose levels (Table 3). For positive correlations, as the dose of FNI increased, there was an increase in percentage pollen germination. The opposite was true for negative correlations. The differences among the sesame varieties in terms of sensitivity to FNI could be used in breeding programs. Ertan (2009) reported that with increasing dose of gamma irradiation, pollen germinability decreased significantly in pumpkin (*Cucurbita maxima*) and winter squash (*Cucurbita pepo*). Girjesh and Priyanka (2007) reported that the percentage of pollen

Table 3. Correlation between FNI and percentage germinabilty in three sesame cultivars (Kenana-4, Ex-Sudan and E-8) at different sucrose concentrations.

Treatment	Sucrose concentration (%, w/v)		
	1%	2%	3%
Kenana-4	0.632243	0.425145	0.206161
Ex-Sudan	-0.16907	0.225524	0.168082
E-8	-0.29333	0.828764	0.410400

Very weak correlation: 0.100 to 0.190; Weak correlation: 0.200 to 0.390; Moderate correlation: 0.400 to 0.690; Strong correlation: 0.700 to 0.890; Very strong correlation: 0.900 to 1.00.

germination decreased significantly as the dose of gamma ray increased in soybean (*Glycine max*).

FNI can be used to induce variability in pollen germination in sesame and can be of importance to plant breeders.

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