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Evaluation of Agro-morphological and Pollen Parameters of M_2 Generation of *Capsicum annuum* Exposed to Fast Neutron Irradiation (FNI)

Kolo Josephine Teni, Falusi Olamide Ahmed, Daudu Oladipupo Abdulazeez Yusuf, Adebola Matthew Omoniyi, Abubakar Abdulhakeem, Gado Aishatu Adamu

Department of Plant Biology, Federal University of Technology, Minna, Nigeria

Email address:
teni.kolo@futminna.edu.ng (K. J. Teni), falusi.olamide@futminna.edu.ng (F. O. Ahmed), dauduoladipupoyusuf@yahoo.com (D. O. A. Yusuf), mo.adebola@futminna.edu.ng (A. M. Omoniyi), abuakim2007@gmail.com (A. Abdulhakeem), ayishatmoh@yahoo.com (G. A. Adamu)

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Abstract: Morphological and pollen parameter evaluation of M_2 generation of fast neutron irradiated capsicum annuum was carried out to assess the heritability of the useful traits observed in the M1 generation. Irradiated M_1 generation seeds at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria using Fast Neutron Irradiation (FNI) from Americium-Beryllium source with flux $1.5x10^4 n.cm^{-2}s^{-1}$ at 0, 30, 60, 90 and 120 minutes exposure periods were collected from the Department of Biological Sciences, Federal University of Technology Minna. The seeds were raise in the experimental garden of the Department in Randomised Complete Block Design (RCBD) and assess for morphological and pollen parameter. The results of the plant height indicated significant difference ($P<0.05$) across the treated plants at 2, 4, 6 and 8 weeks. The minimum height and number of leaf per plant at week 2 (3.53 cm and 6.25) and 4 weeks (11.80 cm and 13.85) was due to plant exposed to 30 minutes irradiated mutant. Maximum number of fruit and weight of fruit per plant was due to 60 minutes IEP with the value of 3.13 and 22.22 g respectively, while the minimum weight was due to 30 minutes exposure periods (13.89 g). Plants with 90 minutes exposure period recorded the highest percentage of pollen viability (96.78) and number of pollen production (79600.00).

Keywords: Fast Neutron Irradiation (FNI), Capsicum Annuum, Irradiation Exposure Period (IEP)

1. Introduction

Capsicuum annuum is the most cultivated species of the genus *Capsicum* in Nigeria. They are grown in many part of the country where fruits are harvested and taken to the market for sales [14]. The fruit is rich in steam-volatile oil, fatty oils, Capsaiciniods, Carotenoid, proteins, fibre and mineral elements [9]. In addition fruit is also known for high content of vitamin A and C especially in the fresh state [4]. Capsicum fruits are well known for its uses; as food spices, colouring agents, as well as pharmaceutical ingredient [21]. This importance has led to increase in demand for the crop, without corresponding increase in supply due to poor and inadequate improved varieties. Though attempts have been made to achieve increase in supply of the crop through increased cultivation of the species, but no adequate success is achieved. Hence, attention is now shifted to improving the genetic composition of the crop through mutation breeding and selection allied mutants with high desirable genetic variability and qualities [20].

Mutation breeding has been successfully used in generating genetic variability in various crops and breeding programmes using ionizing radiations [18]. Ionizing radiations such as fast neutron have been proven to be effective creating vast number of genetic variability which has played a significant role in plant breeding and genetic studies [10]. Ionizing radiation has also been shown to

preserve food, improve microbiological safety or shelf life when used alone or with other methods [5]. Food and Agriculture Organisation [17] reported that mutants generated through induced breeding worldwide have led to the realization of about 2,700 useful varieties. Also, a fast neutron irradiation has been reported by [1] has a valuable tool for developing useful mutants with high agricultural potentials and economic values. Therefore this research aimed at evaluating the sustainability of these useful traits in the M_2 generation mutant pepper.

2. Materials and Methods

2.1. Collection of Seeds and Experimental Site

Mutant seeds of second generation (M_2) of *Capsicum annum* irradiated at the centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria using Fast Neutron Irradiation (FNI) from Americium-Beryllium source with flux $1.5x10^4 n.cm^{-2}s^{-1}$ at 0, 30, 60, 90 and 120 minutes exposure periods were collected from the Department of Biological Sciences, Federal University of Technology Minna and raise in the experimental garden of the Department.

2.2. Planting and Experimental Design

A total of 50 seeds of each mutant of different time of exposure (dose) were nursed on 1 × 1 m nursery bed for a period of thirty days. After the period of nursing, two seedlings of each treatment were transplanted into 10 litres experimental pot filled with sandy-loamy soil to a graduation mark of 7 litres. Each treatment were replicated three time and arranged in a randomised completely block design (RCBD). All agronomic practices were done when necessary and data were collected from three plants per treatment.

2.3. Data Collection

Data on agronomic parameters were assessed using standard procedures of [7, 18]. Days to first emergence was considered as number of days at which a seedling emerges above the soil level after sowing. The number of leaves per plant was determined by direct counting and the plant height was measured in centimeters (cm) using a ruler from the soil level to the plant apex at two weeks interval. For each of the morphological parameters mentioned above, the mean values per plant were determined for the *Capsicum* species. The yield parameters determined were Number of fruits per plant (NF), Length of fruits (NS), and Fresh weight of the fruit (WF).

2.4. Determination of Pollen Production

The pollen production test was carried out using the method described by [22]. Ten flower buds for each treatment were randomly selected for the study. The flower buds were divided into two groups, each group containing anthers from five flower buds. The anthers were gently crushed with a glass rod in a vial of 1ml of distilled water. A drop of the prepared sample was added to two counting area of haemocytometric slide (0.1mm in depth) and covered with a cover slip. Pollen counting was done on four randomly selected squares areas. The procedure was replicated twice for each treatment and the average pollen grain amount per flower (P/F) was calculated using the formula below

$$P/F = \text{Pollen count} \times 1000mm^3 / 0.1mm^3 / 5 \text{ flowers.}$$

2.5. Determination of Pollen Viability

The pollen viability test was carried out using the method described by [23]. 0.01g of methylene blue was dissolved in 10ml distilled water. 2g of sodium citrate dihydrate was added, stirred until dissolved and filtered using whatman No 1 filter paper. The mixture volume was makeup to 100ml with distilled water. Pollensof each treatment were placed prepared solutions with the aid of brush and the number of viable pollen were counted after few minutes under light microscope (X10 magnification). Pollen grains that were stained dark blue in colour were counted as viable while those with a light blue colour or not stained at all were considered non viable. Approximately 300 pollens were counted in each field. Pollen viability percentages were calculated for each variety using the formula below.

$$\text{Percentage pollen viability} = \frac{Number\ of\ viable\ pollens}{Total\ number\ of\ pollens\ counted} \text{ X } 100$$

2.6. Data Analysis

Data obtained were subjected to analysis of Variance (ANOVA) to test for significance among the treatment andDuncan's multiple range test was used to separate the means where there were differences.

3. Results

3.1. Plant Height

The results of the plant height indicated significant difference ($P<0.05$) across the treated plants at 2, 4, 6 and 8 weeks. The minimum height (3.53 cm) was recorded on plant exposed to 30 minutes irradiation mutant. This value was significantly lower than the value of the control plant (7.38 and18.63 cm) for week two and four respectively. Plants with 120 minutes IEP recorded the maximum height (18.98 cm) for the first four weeks (Table 1).

3.2. Number of Leaves

Significant differences ($P< 0.05$) were observed with respect to the total number of leaves per plant. The total

number of leaves per plant increased with increase in IEP in the first four weeks, with 30 minutes IEP recorded the least number of leaves per plant in week 2,4,6 and 8 (6.25, 13.85, 25.35 and 46.95) respectively and were statistically different from the plants with 90 and 120 minutes IEP (Table 2).

Table 1. *Effect of fast neutron irradiation on plant height of MN/TA/001.*

SAMPLE	WEEK 2	WEEK 4	WEEK 6	WEEK 8
CONTROL	7.38±0.26^{b}	18.63±0.87^{b}	26.78±1.44^{c}	33.05±1.53^{a}
30 MINS	3.53±0.21^{a}	11.80±0.97^{a}	20.05±1.49^{ab}	29.50±1.46^{a}
60 MINS	6.43±0.49^{b}	15.98±1.16^{b}	24.95±1.19^{bc}	30.98±1.02^{a}
90 MINS	8.13±0.80^{b}	16.88±1.46^{b}	19.43±1.69^{a}	27.70±2.20^{a}
120 MINS	10.13±0.83^{c}	18.98±1.97^{b}	22.66±2.32^{abc}	29.40±2.72^{a}

Table 2. *Effect of fast neutron irradiation on number of leaves for MN/TA/001.*

SAMPLE	Week2	Week4	Week6	Week8
Control	7.30±.26^{ab}	20.35±1.38^{b}	29.65±2.24^{a}	47.05±5.64^{a}
30 mins	6.25±.20^{a}	13.85±1.49^{a}	25.35±2.51^{a}	46.95±6.68^{a}
60 mins	7.10±.35^{ab}	17.75±1.90^{ab}	33.95±2.86^{a}	49.95±2.77^{a}
90 mins	7.70±.45^{b}	21.05±2.34^{b}	32.80±3.17^{a}	59.00±7.05^{a}
120 mins	8.05±.54^{b}	22.40±2.84^{b}	32.50±4.06^{a}	53.80±7.13^{a}

Values with same letter, in a column, are not significantly different at (P> 0.05).

3.3. Number of Fruits Per Plant (NOF)

Significant differences (P< 0.05) were observed in the number of fruits per plant in this species of capsicum. Plants with 60 and 120 minutes exposure periods recorded the maximum number of fruits per plant (3.13 and 3.00 respectively) (Table 3). This value is statistically different from the control which recorded the minimum number of fruits per plant (1.79).

3.4. Fresh Weight of Fruits (FWF)

In MN/TA/001 the maximum weight was recorded due to the control (23.47g) this value was significantly different from all the other IEPs except 60 minutes IEP (22.22g); while the minimum weight was recorded due to 30 minutes exposure periods (13.89g) (Table 3).

3.5. Length of Fruits (LOF)

No significant difference (P< 0.05) was observed in the length of fruits in MN/TA/001 though the highest length was recorded in plants with 120 minutes IEP (8.75cm) while the least length of fruits was recorded in plants with 60 minutes IEP (7.65cm) (Table 3).

Table 3. *Effect of fast neutron irradiation on yield parameter of MN/TA/001*

SAMPLE	NOF	FWF	LOF
CONTROL	1.79±.26a	23.47±2.13c	8.30±.70a
30 MINS	2.79±.28b	13.89±2.99a	7.85±.60a
60 MINS	3.13±.45b	22.22±3.64c	7.65±.56a
90 MINS	2.60±.31ab	18.06±4.28b	8.65±.73a
120 MINS	3.00±.28b	16.68±2.17ab	8.75±.52a

Values with same letter, in a column, are not significantly different at (P> 0.05).

NOF-Number of Fruits per plant; FWF-Fresh Weight of Fruits LOF-Length of Fruits

3.6. Pollen Production

Significant differences (P< 0.05) were observed in the pollen production and viability of the *Capsicum annuum*. In MN/TA/001 the plants with 90 minutes exposure periods had the highest number of pollen production (79600.00), this value is statistically different from the control with the least number of pollen production (Table 4).

Table 4. *Effect of fast neutron irradiation on the pollen production and viability of MN/TA/001.*

	Anther/Flower	Pollen/Anther	Pollen/Flower	%Viable	%Nonviable
Control	10.00±0.00^{a}	2568.00±483.88^{a}	25680.00±4838.84^{a}	94.63±1.11^{ab}	5.37±1.11ab
30 mins	10.00±0.00^{a}	3040.00±205.13^{a}	30400.00±2051.34^{a}	95.77±0.63^{ab}	4.23±0.63ab
60 mins	10.40±0.40^{ab}	8324.00±6234.54^{a}	24800.00±5768.88^{a}	92.68±2.24^{a}	7.32±2.24b
90 mins	11.60±0.40^{c}	7074.60±2200.22^{a}	79600.00±23005.74^{b}	96.78±0.77^{b}	3.22±0.77a
120 mins	11.20±0.49^{bc}	3235.80±293.40^{a}	36640.00±4466.05^{a}	94.19±0.39^{ab}	5.81±0.39ab

Values followed by the same superscript alone a column, are not significantly different at (P>0.05)

3.7. Pollen Viability

In *Capsium annnuum* (MN/TA/001), Plants with 90 minutes exposure period recorded the highest percentage of pollen viability (96.78), is statistically different from 60 minutes exposure periods (92.68±2.24a) (Table 4).

4. Discussion

Plant height/Number of leaves per plant

Significant differences observed in the plant height and numbers of leaves per plant in *Capsicum annum* in this study were inconformity with the work of [16] who reported a similar result in M1 and M_2 of *Capsicum annuum* var abbreviatum Fingerh exposed to different time of fast neutron irradiation. [8, 13] reported that an increase in irradiation exposure period tend to increase certain morphological traits such as plant height. The Higher plant height recorded in 120 minutes IEP, may probably facilitate mechanical harvesting when large scale commercial pepper plantations are involved [24].

Contrary to these results [2, 3] who work on pop-corn maize and tomato reported a decrease in the height of plant as the exposure period of both thermal neutron and gamma irradiation increases. They reported a decrease in the number of leaves per plant due to exposure to different irradiation respectively.

Yield morphological parameters.

Similar to the result obtained in this study where different irradiation exposure periods favoured different yield parameters in *Capsicum annuum,* [16] reported that yield parameters were significantly increased as IEP increase, with 60 IEP been the most effective IEP to induce viable and useful mutants for yield improvements. A similar result was also reported by [2, 15]. It was also reported by [19] that Irradiation at different dose levels can stimulate the biosynthesis of some phenolic compounds in plants.

Pollen production/viability

Pollination and fertilization among crop plants are compulsory to obtain fruits and seeds [12]. The increase in pollen production with increase in IEP recorded in *Capsicum annuum* is in agreement with work of [6] who reported that increase in pollen production also increases the chances of genetic variation. However, the changes produced by Fast Neutron Irradiation could play a significant role in the improvement of the crop. [11] reported that Fast Neutron Irradiation could be used to induce genetic variability and improve the agronomic traits in pepper plants.

5. Conclusion

It is therefore concluded that distinct variations exist among the Fast Neutron Irradiated M_2 Capsicum annuum. Certain traits life Length of Fruits, Weight of fruits and Number of fruits per plant are enhanced by 60 and 120 minutes IEP. However, 60 minutes IEP tends to be the optimum dose for inducing beneficial mutant in the M1 pepper plant. Significant useful traits selected from M1 generation were inheritable as recorded in this present study. Therefore, induced mutation by Fast Neutron Irradiation (FNI) has indicated that heritable useful traits can be generated that could be used in further breeding and improvement of C. annuum.

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