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EFFECT OF EXPOSURE TIME OF FAST NEUTRON IRRADIATION ON GROWTH AND YIELD PARAMETERS OF TOMATO (*Solanum lycopersicum*)

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ABSTRACT

Fast neutron irradiation (FNI) is a useful tool for developing plant varieties with great economic values and agricultural potentials. Therefore, this research was carried out to evaluate effects of fast neutron irradiation on the growth and yield parameters of tomato (*Solanum lycopersicum*); with the aim of obtaining useful and desirable agronomical traits that could

Introduction

Fruits and vegetables play a critical role in human life as they remain the only alternative sources of nutrition and medicine [1]. For ages, humans have been dependent on plants and edible vegetables for survival. Fruits and vegetables are rich in fibre, vitamins, phytochemicals and micronutrients that are essential for human wellbeing. Postharvest factors such as inappropriate storage conditions, mechanical injuries and harsh environmental conditions favourable to the development of micro-organisms and insects have contributed to their decay (about 16%), thereby causing considerable reduction in fresh produce every

be used for large scale production. This was done using an Americium-Beryllium source with a flux of 1.5×10^4 $cm^{-2}s^{-1}$. The tomato seeds were irradiated for 0, 30, 60, 90 and 120 minutes equivalent to 0 rad, 4 rad, 8 rad, 16 rad and 24 rad respectively before they were sown, with their respective controls. The effects of the different irradiation treatments were accessed on percentage germination and survival, plant height and number of leaves per plant in three accessions of the plant. The seeds were plated in the laboratory for germination study and in experimental pots arranged in randomized completely block design (RCBD) in the garden. Highly significant differences ($p\leq 0.05$) were observed in percentage germination and survival, plant height and number of leaves/plant in all the accessions. However, the study revealed that 90 min (16 rad) was an effective irradiation period to induce viable and useful mutations for yield parameters in tomato and that the accession NG/MR/5/9/006 performed better than other plants in most of the parameters studied. These results demonstrated that FNI is a prime tool for enhancing the efficiency of breeding *S. lycopersicum* and evolving higher yield variants through proper selection.

Keywords: Fast neutron; irradiation exposure period; morphological variation; mutation; *Solanum lycopersicum L.*

year [2, 3]. Globally, 1,667.737 metric tonnes (Mt) of fruits and vegetables are produced every year [4]. Report from Food and Agricultural Organization (FAO) showed that 25-35 % of fruits and vegetables are lost through natural causes by microbes, pests and insects [3, 5]. Therefore, preservation of produce is a prerequisite for improving both food safety and food quality.

Tomato (*Solanum lycopersicum L.*) is one of the most important and widely cultivated vegetable crops in the world occupying the second place in the fresh vegetable market and the food processing industry. It has an extensive worldwide distribution which accounts for more than 15% of world vegetable production (over 177 million metric tons in 2016; www.fao.org/faostat). *Solanum lycopersicum L.* belongs to the night shade family *Solanaceae,* originated in the South America. Tomato bears a fleshy berry fruit and has been used extensively as a model plant for fruit ripening studies [6], research in genetics, fruit development and disease resistance [7]. It has huge economic value due to its short life cycle and high yield.

Solanum lycopersicum is a rich source of micronutrients and contribute greatly to a healthy, well-balanced diet [8], as they are rich in minerals, vitamins, essential amino acids, sugars, dietary fibres, vitamin B and C, iron and phosphorus. They can be eaten directly as raw vegetable or processed into different products such as ketchup, sauce, chutney, juice, soup and puree. In developing countries like Nigeria, efficient storage, packaging, transport and handling techniques are virtually non-existent with perishable crops [9], leading to considerable loss of produce. Hence, there is a need to develop alternative methods for shelf life enhancement without having adverse quality effects (as imposed by other methods). Mutation technology has successfully been used to induce favorable mutations and to generate genetic variability for breeding and genetic studies. This could be achieved using fast neutron irradiation (FNI) doses and the traits of the resulting mutants are inheritable.

Fast neutrons irradiation is a valuable tool for developing varieties with high agricultural qualities and huge economic values [10, 11]. Authors have exploited FNI to improve yield components of considerable varieties of crops like Nigerian pepper (*Capsicum* sp.) [11-15], Lagos spinash (*Celosia argentea*) [10, 16], Okra (*Abelmoschus esculentus*) [17], Chicken pea (*Cicer arientinum*) [18, 19], and a host of other crops. These reports showed that FNI is an effective tool for crop improvement. Therefore, the research was carried out to evaluate the effects of fast neutron irradiation on the growth and yield parameters of *Solanum lycopersicum* to induce changes in the genotype for selection of desirable traits that could be used for large scale production.

MATERIALS AND METHODS

Collection of seeds

The seeds of three accessions of tomato were obtained from the National Gene bank at the National Center for Genetic Resources and Biotechnology (NACGRAB), Moor plantation, Ibadan, Oyo State, Nigeria. They were given the codes: NH/MR/5/9/006, NG/AA/9/9/037 and NHGB/09/114.

Certification of seed viability

The seed viability was done before and after irradiation using germination test method as described by Songsri *et al.* [20]. Germination of seeds was tested in covered plastic petri dishes (5 cm diameter) on moistened filter paper. The experiment was monitored on daily basis. The germination percentage was calculated as follows and was used to ascertain the viability of the seeds.

$$\text{Germination} = \frac{\text{Total number germinated}}{\text{Total number of seeds used}} \times 100\%$$

Experimental procedures

The seeds were irradiated with fast neutron at the Centre for Energy and Research Training (CERT), Ahmadu Bello University Zaria, Kaduna State, Nigeria, using an Americium-Beryllium source with a flux of 1.5×10^4 $cm^{-2}s^{-1}$ for five different irradiation exposure periods (IEPs): 0, 30, 60, 90, and 120 min equivalent to 0rad, 4 rad, 8 rad, 16 rad and 24 rad respectively. This equipment 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 [21]. Three accessions namely (NG/MR/5/9/006, NG/AA/9/9/037 and NHGB/09/114) were selected for this study. The non-irradiated seeds served as controls.

The seed planting and management was done according to the method of Falusi and Daudu (2014). A total of 100 seeds of each accession of different time of exposure (dose) were nursed on 1 x 1 m nursery bed for 4 weeks to obtain seedlings. After the period of nursing, it was transplanted into 3.5L plastic pots containing sandy-loamy soil, at a rate of three seedlings/pot. Although, when the crop began to flower, an insecticide (pyrethroid cypermethrin) at a rate of 10 to 15 Lha^{-1} with controlled droplet application using spinning disc sprayers) was applied to prevent insect attack and diseases, but no fertilizer was applied. The planted seeds were watered once daily between 5.00 and 6.30 pm using bore-hole water. Each treatment was replicated three times and arranged in a completely randomized design (CRD) and all agronomic practices were carried out when necessary and data were collected from three plants per treatment. Plant grown parameters (germination percentage, survival percentage, plant height (cm) and number of leaves/plant at maturity) were measured at 2, 4 weeks and at maturity.

Statistical Procedures

Data collected were subjected to analysis of variance (ANOVA) using SPSS version 20 to compare the means and Duncan Multiple Range Test (DMRT) was used to separate the means with significant differences detected at $p = 0.05$.

Figure 1. Cross section of experimental pots showing various treatments

Figure 2. Tomato fruits after germination

RESULTS AND DISCUSSION

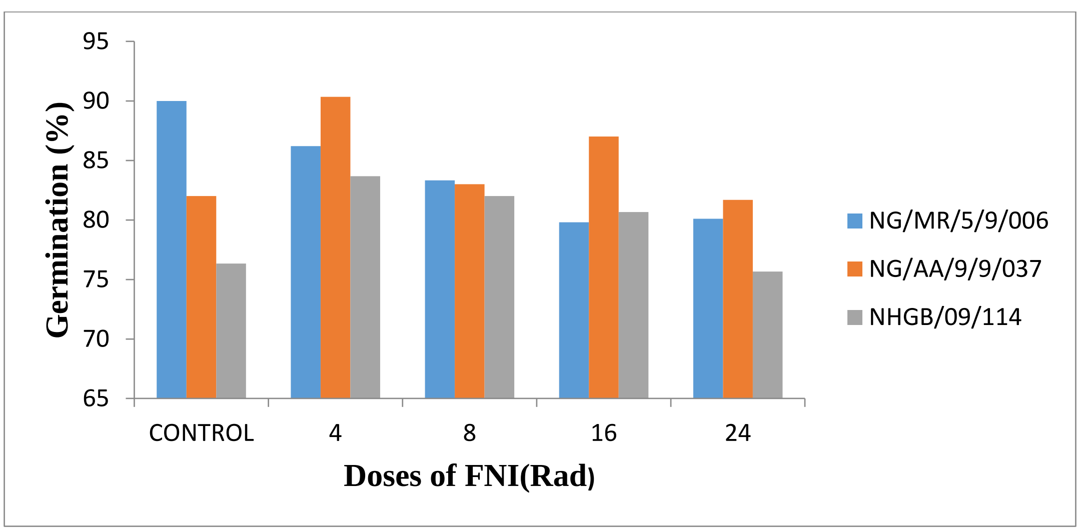

Figure 3. Effect of fast neutron irradiation on germination percentage of *S. lycopersicum*

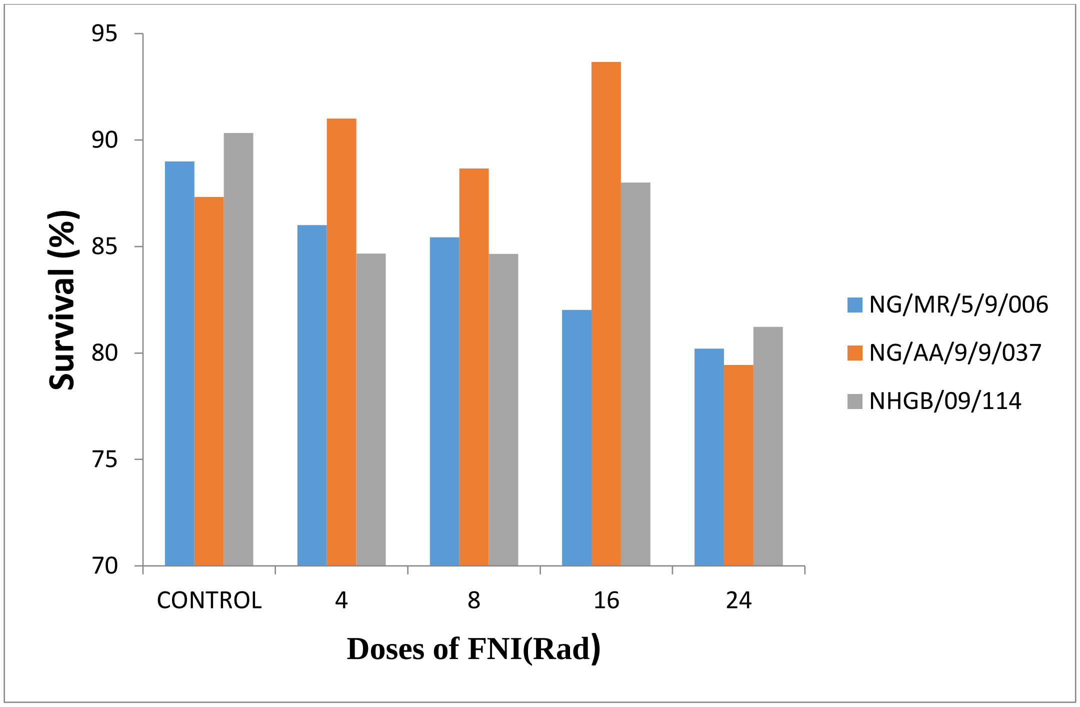

Figure 4. Effect of fast neutron irradiation on survival percentage of *S. lycopersicum*

Table 1. Effects of fast neutron irradiation on plant height at different periods after germination of *S. lycopersicum*

Treatment Combination	PH (cm) 2wks	PH (cm) 4wks	PH (cm) @MAT
NG/MR/5/9/006			
Control	11.41±1.76c	22.49 ± 3.36b	35.07 ± 5.86b
4rad	10.42±1.88a	21.44 ±2.34a	31.95 ± 5.32a
8rad	11.04 ± 1.28b	23.01 ± 2.96b	26.48 ± 6.42c
16rad	10.58 ± 1.50a	21.39 ± 2.66a	22.71 ± 4.72a
24rad	10.53 ± 1.40a	22.98 ± 3.08b	23.84 ± 5.92b
NG/AA/9/9/037			
Control	7.49±1.24c	11.95 ± 2.32b	15.07 ± 4.40b
4rad	3.38±0.88a	11.65 ± 2.14b	11.56 ±4.00a
8rad	3.89 ± 0.92b	10.79 ± 1.78a	15.75 ± 4.60b

16rad	2.51 ± 0.62a	11.65 ± 2.30b	14.81 ± 4.40b
24rad	7.17 ± 0.04c	12.28 ± 2.96b	19.94 ± 5.40c
NHGB/09/114			
Control	11.53 ± 5.84c	12.78 ± 3.20b	14.32 ± 4.40b
4rad	10.96 ± 5.62b	13.06 ± 3.88c	17.56 ± 5.00c
8rad	10.52 ± 5.14a	11.14 ± 2.80a	12.68 ± 4.60a
16rad	10.99± 5.46b	12.30 ± 3.58b	13.01 ± 4.20b
24rad	11.52 ± 5.70a	11.01 ± 2.60a	12.73 ± 5.40a

Values are presented in mean ± standard error of mean. Values followed by different superscript alphabet within the same column are significantly different at $p<0.05$ level of significant according to Duncan's multiple range test, analyzed separately for each accession.
PH - Plant Height, MAT – Maturity, @ - At

Table 2. Effects of fast neutron irradiation on number of leaves/plant at different periods after germination of *S. lycopersicum*

Treatment Combination	NOL @TP	NOL @FL	NOL @FT
NG/MR/5/9/006			
Control	$23.83{\pm}4.45^{b}$	33 ± 5.6^{b}	$77.00{\pm}7.11^{d}$
4rad	$20.50{\pm}2.14^{a}$	32 ± 2.7^{a}	$65.00{\pm}5.47^{c}$
8rad	$26.37{\pm}22.16^{c}$	31 ± 1.7^{a}	$40.66{\pm}3.94^{a}$
16rad	$27.00{\pm}3.23^{c}$	40 ± 2.9^{b}	$56.83{\pm}7.84^{b}$
24rad	$22.16{\pm}1.92^{b}$	30 ± 2.3^{a}	$68.16{\pm}4.58^{c}$
NG/AA/9/9/037			

Control	12.83±1.88[a]	27 ± 2.5[b]	61.16±8.54[c]
4rad	20.50±1.58[c]	30 ±3.5[b]	55.66±1.81[b]
8rad	12.16±1.55[a]	22 ± 2.5[a]	35.16±5.49[a]
16rad	16.83±2.35[b]	23 ± 2.1[a]	41.66±4.09[b]
24rad	21.33±3.25[c]	36± 4.4[c]	83.33±15.46[d]
NHGB/09/114			
Control	12.83±1.88[a]	27 ± 2.5[b]	61.16±8.54[c]
4rad	12.16±1.55[a]	22± 2.5[a]	35.16±5.39[a]
8rad	18.66±1.46[b]	26± 2.2[a]	48.66±3.00[b]
16rad	21.33±3.25[c]	36 ± 4.4[c]	83.33±15.4[b]
24rad	16.73±1.15[b]	27 ± 1.6[b]	55.40±4.71[b]

Values are presented in mean ± standard error of mean. Values followed by different superscript alphabet within the same column are significantly different at $p<0.05$ level of significant according to Duncan's multiple range test, analyzed separately for each accession.

TP - transplant, FL - flowering, FT - fruiting, NOL - number of leaves, @ - At

The effect of FNI on germination of seed plant to maturity stage was shown in figure 3. Germination percentages were significantly different ($p<0.05$) at different doses of FNI as the highest germination percentage of (90.33) was achieved with NG/AA/9/9/037 at dose 4 rad FNI while the least percentage of (75.00) was recorded in NHGB/09/114 at dose 24 rad FNI treatment. However, these values were less than the control values. Slight reductions in germination percentage with increased irradiation time of exposure were observed from doses 4 rad to 24 rad. The variations observed in the germination percentage of *S. lycopersicum* at different periods were significant due to the effect of FNI. The results revealed that as irradiation time increased, percentage germination decreased. This is in agreement with the work of Domingo *et al.* [22], Daudu *et* *al.* [23], Falusi *et al.* [12], Muhamune and Kothekar [24] and Abubakar *et al.* [10]. They reported decrease trend in germination percentage with increased mutagenic concentrations/doses.

However, Poornananda and Hosakatte [25] reported 100% germination with niger (*Guizzotia abyssinica*) seeds treated with gamma irradiation. The decrease in germination observed in this study may be attributed to disturbances or changes at cellular level which could be physiological or physical or the combination of both effects [10]. Muhamune and Kothekar [24] have envisaged that disturbance in the formation of enzymes involved in germination process could be a physiological effect. Furthermore, this reduction might also be due to chromosomal damage and lethal effects caused by the mutagen on meristematic tissues of the seeds thus, causing considerable reduction in germination [10, 26].

The survival percentages were significantly different ($p<0.05$) at different doses of FNI (Figure 4). In NH/MR/5/9/006 and NHGB/09/114 accessions, significant differences ($p<0.05$) were observed as the highest survival percentage of (89.00 and 90.33) was seen in the control while the least (80.21 and 81.23) was seen in 24 rad FNI respectively. However, in NG/AA/9/9/037 accession, highest survival percentage of (93.67) was seen in dose 16 rad FNI and the least (79.43) in 24 rad FNI. The FNI treated seeds showed a negative correlation between irradiation period and seedling survival percentage, implying that as irradiation period increased, percentage seedling survival decreased (Figure 4). Similar results were observed in chemical mutagen treated *Sesamum indicum* [27], *Lycopersicon esculentus* [28] and gamma irradiation-treated *Guizzotia abyssinica* seeds [25]. However, inconsistent reduction in survival percentage recorded in this study could be due to the fact that survival rate depend on different factors, ranging from the type of cell, frequency of chromosomal damage and mutagenic disturbances. In support of this, a study by Kiong *et al.* [29] revealed that increase in frequency of chromosomal damage with increasing radiation dose may be responsible for less germination and reduction in plant growth and survival.

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Plant height is an essential trait in breeding practices of crop improvement. In this study, highly significant differences were observed in plant height of all the three accessions (Table 1). The result showed highly significant differences ($p \leq 0.05$) in plant height of the three accessions at different doses. For all accessions, irradiated plants had reduced plant height at 2 weeks. As the week progresses, significant variation ($p \leq 0.05$) in plant height were observed with the highest mean of plant height consistently obtained in 4 rad irradiated plants except for NG/AA/9/9/037 accession, which was achieved at 24 rad dose. In NG/MR/5/9/006 accession, the highest plant height (35.07) was seen in the control and a relatively high (31.95) in 4 rad FNI. In NG/AA/9/9/037 accession, the highest plant height (19.94) was found at maturity and was significantly different ($p<0.05$) from the control (15.75). This was similar to accession NHGB/09/114 with highest plant height (17.56), which was also significantly different ($p<0.05$) from the control (14.32). This may be attributed to the stimulation in the production of endogenous growth hormones such as auxins to enhance proliferation of the cells [10]. Similar studies showed that an increase in irradiation exposure period tends to increase certain morphological traits such as plant height as reported by Hegazi and Hamideldin [17], Daudu *et al.* [23], Falusi *et al.* [12], Abubakar *et al.* [10] and Kolo *et al.* [11]. However, Poornananda and Hosakatee [25] and Adamu [30] reported a decrease in the height of plants with increased exposure period of irradiation.
Quantitative estimation of number of leaves at transplant (TP), flowering (FL) and fruiting (FT) were presented in Table 2. Statistical analysis showed that there were significant differences ($p \leq 0.05$) in mean number of leaves among all the plants irradiated. For accessions NG/AA/9/9/037 and NHGB/09/114, the highest mean numbers of leaves were achieved at 24 rad and 16 rad, while the lowest mean was recorded at 8 rad and 4 rad respectively. The highest values recorded were 21.33, 36.00 and 83.33 at transplant, flowering and fruiting respectively. These values were significantly higher from the values of control plants. The highest number of leaves at flowering (40.00) was recorded in NG/MR/5/9/006 at 16 rad, however it was not significantly different ($p>0.05$) from the control. FNI generally increased the vegetative growth of the three

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accessions. This result is in agreement with the work of Kolo *et al.* [11]. However, it differs completely from the findings of Poornananda and Hosakatee [25] on niger seeds treated with gamma rays and sodium azide; Adamu and Aliyu [28] on tomato treated with sodium azide and Adamu [30] on pop-corn maize plants treated with thermal neutron and gamma irradiation. They all reported a decrease in the number of leaves/plant due to exposure to different irradiation sources.

CONCLUSION

Genetic diversity is a fundamental tool in breeding programmes as well as taxonomic studies. This study has further established that fast neutron irradiation (FNI) is a potent physical mutagen capable of inducing beneficial mutation in tomato, *Solanum lycopersicum.* The three accessions were responsive to FNI treatment, an indication that either of them could can be used to increase growth, yield characters and induce variability in crops through the isolation of beneficial mutants and thus serve as the parent plant in breeding and improvement programmes, or through mass propagation *in vitro*. This response differs for the accessions and inconsistent with different doses of the mutagen. The best irradiation dose to induce this beneficial mutation is at the 16 rad exposure time. Generally speaking, the accession NG/MR/5/9/006 performed better than other plants in most of the parameters studied. It is therefore recommended that further studies should test for the geno-toxicity of the crop and exploit those exposure periods that produced promising traits for future breeding programme of tomato.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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