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Original Article

MUTAGENIC EFFECTS OF FAST NEUTRON IRRADIATION ON SOME GROWTH AND YIELD PARAMETERS AT M_1 and M_2 OFLIMA BEAN (*Phaseolus lunatus* Linn.)

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ABSTRACT

The studies of induced mutation in *Phaseolus lunatus* L. was conducted at the botanical garden, Department of Botany, Ahmadu Bello University, Zaria. Dry healthy seeds of *P. lunatus* obtained from local farmers with in Zaria were exposed to Fast Neutron irradiation at different doses (0.00Sv, 0.16Sv, 0.32Sv, 0.49Sv and 0.65Sv). The irradiated seeds were sown in 150 polythene bags which were half filled with soil and arranged using a Randomized Complete Block design. The result of this studies showed both positive and negative shift in agronomic characters with plants exposed to lower doses (0.16Sv and 0.32Sv) showing better performance in most agronomic trait in both mutagenic generations while higher dose (0.65Sv) recorded the least performance in most agronomic traits. Chlorophyll mutants such as Zonata, Xantha and Maculata as well as Morphological mutants which includes invaginated leaf margins, bifurcated leaf apex and bifoilate leaves were observed. However, lower doses (0.16Sv and 0.32Sv) of the mutagen were more favourable in inducing beneficial mutation on the growth and yield trait of *P. lunatus.* The effect of the mutagen was significant at $p \leq 0.05$ in plant height 4 weeks after sowing, days to first flowering, days to 50% flowering, pod length, pod diameter, pod weight and 100 seeds weight.

Keywords: Fast neutron*, P. lunatus*, Seeds, Agronomic traits, Chlorophyll mutants and Morphological mutants.

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INTRODUCTION

Phaseolus lunatus is commonly called lima bean, butter bean, sieva bean, sugar bean, Madagascar bean and java bean in English, Wake Rumpa in Hausa, Ukpa in Igbo (Ikechukwu, 2010), Papala in Yoruba (Adegbehingbe, 2013). It belongs to the family Fabaceae (USDA, 2012). Like other grain legumes, lima beans are relatively rich in protein. They contain about 25% protein in the dry matter, a value comparable to that of peas (*Pisum sativum*) and cowpeas (*Vigna unguiculata*), dried vines may be used as animal fodder (Azeke *et al*., 2011). Immature sprouts, leaves, and pods are consumed in Asia with young leaves and pods steamed and green shelled beans cooked as a vegetable. Lima bean helps restore soil fertility by shedding its many leaves that decay and enrich the soil. It is also used as a green manure and cover crop to prevent soil erosion from heavy rainfall in rubber plantations Oliveira *et al*. (2004).

Mutation breeding is a proven supplement and an effective substitute of conventional breeding so as to confer specific improvement in a variety without significantly affecting its acceptable phenotype (Micke, 1998). Physical mutagens like x-rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations have been frequently used to induce mutation (Yakoob and Rashid, 2001). Fast neutrons are produced by cyclotrons or atomic reactors as a result of radioactive decay of heavier elements. Neutrons are uncharged particles, and are highly penetrating in biological tissues; fast and thermal neutrons are densely ionizing radiations (Singh, 2013). The state of nutrition in Nigeria is still characterized by inadequate calorie and protein supplies (FAO, 2004). Despite the great potential of lima bean, it is highly underutilized in Nigeria and it has not received much attention in terms of crop improvement; local cultivars are still being grown by the farmer and so yield is low (Akande and Balogun, 2007). In an attempt to widen the narrow genetic base, food and agricultural scientists are screening lesser known and under-exploited native plants for possible potential sources of food (Ezeagu and Ologhobo, 1995; Vietmeyer and Janick, 1996; Murray *et al*., 2001). One of such crop identified in this context in Nigeria is lima bean, though a minor crop, has been an important source of plant protein to millions of Nigerians (Ikechukwu *et al*., 2010). Thus, this research was targeted towards determining the mutagenic effects of fast neutrons on some growth and yield parameters of *P. lunatus*

MATERIALS AND METHODS

Seed Source and Mutagenic Treatment

Healthy landraces of *P. lunatus* were obtained from local farmers in Zaria, Kaduna State, Nigeria and landrace identity was confirmed at International Centre for Tropical Agriculture (CIAT), Cali, Colombia. About 0.25kg of healthy dry seeds of *P. lunatus* was exposed to Fast neutrons from Americium Beryllium source (^{241}Am/Be), with a flux of 1.5 x $10^4 ncm^{-2}s^{-1}$ at Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria for 0hrs, 2hrs, 4hrs, 6hrs and 8hrs equivalent to 0.00Sv, 0.16Sv, 0.32Sv, 0.49Sv and 0.65Sv doses respectively

Experimental design and sowing of seeds

Top soil was collected from uncultivated land within the botanical garden in Ahmadu Bello University, Zaria and after treatment it was filled into one hundred and fifty (150) polythene bags (51.5 X 38.3cm) arranged in a randomized complete block design (RCBD) with three replications for each treatment to raise M_1 and M_2 generation. The polythene bags were half filled each weighing 8.5kg and placed at a spacing of 10 x 90 cm. A total of 1.35 x 10^{-3} hectares (13.5msq) of land were used for this research. Three seeds were sown in each bag which was later thinned to one per bag.

Sampling Collection

Agro-morphological data such as Percentage germination, plant height 4 weeks after sowing (WAS), 8WAS, 12WAS, number of days to first flowering, number of days to 50% flowering, number of pods per plant, pod length, pod weight, pod diameter, number of seeds per pod and 100 seeds weight.

Data Analyses

Agro-morphological data obtained was subjected to one way analysis of variance (ANOVA) using SAS (2002) version 9.1 to determine the significant effect of different concentrations of the mutagens ($p \leq 0.05$) and Duncan's Multiple Range test (DMRT) was used to separate the means obtained, where significant.

RESULTS

Effects of Fast Neutron on some growth and yield parameters of *P. lunatus* at M_1Generation

The result of the effect of different doses of fast neutron irradiation on the agronomic traits of *P. lunatus* is shown in Table 1. Plant height at 8 and 12 WAS, days to first flowering, days to 50% flowering, number of pods per plant, pod length and pod diameter were significantly different ($P \leq 0.05$). The highest plant height (157.32cm) and least plant height (76.25cm) at 8WAS were observed at 0.32Sv and the control respectively. Plant height 12WAS increased with an increase in dose, 0.32Sv recorded the highest plant height (179.79cm) while the control recorded the least height (105cm). However, the highest number of days to first (86.00) and 50% (93.00) flowering was recorded at 0.49Sv dose while the fewest (73.33 and 80.67) respectively was at 0.16Sv. The number of pods per plant decreased with an increase in dose of the mutagen, where the control produced the highest (17.80) and the least (10.40) was produced at 0.65Sv. Pod length also varied with dose although not linearly, the highest pod length (6.59cm) was recorded at 0.49Sv and the least by the control (5.36cm). On the other hand 0.49Sv and the control recorded the highest (1.77cm) and least (1.43cm) pod diameter respectively. Pod weight was observed to have increased with an increase in dose of fast neutrons; 0.49Sv recorded the highest mean value 7.20g) while the least mean value (5.50) was recorded by the control treatment.

Effects of Fast Neutron on some growth and yield parameters of *P. lunatus* at M_2Generation

Number of days to first flowering, days to 50% flowering, pod length, pod diameter, pod weight and 100 seed weight showed significant difference ($P \leq 0.05$) (Table 2) The control treatment showed the highest number of days to first (54.33) and 50% flowering (63.67) while the fewest number of days to first flowering (50.00) was recorded at 0.16Sv and 0.65Sv and 0.49Sv recorded the fewest number of days to 50% flowering (59.00). Although, not linearly the pod length increased with an increase in dosage; o.32Sv produced longest pod (5.79cm) and the shortest pod (4.73) was produced by the control treatment. Similarly, 0.32Sv produced the pods with the widest diameter (1.74) while the control had the narrowest diameter (1.58cm), this was followed closely by 0.16Sv with a diameter of 1.60cm. The highest pod weight (6.47g) was observed at 0.33Sv and the least (4.27g) in the control treatment, 100 seed weight also varied amongst the different doses of fast neutrons with 0.16Sv weighing the highest (32.33g) and 0.65Sv weighing the least (28.33g)

Table 1: Effects Fast Neutrons on Agronomic Traits of *Phaseolus lunatus* at M_1 Generation

TRT	PGERM (%)	PH4 (cm)	PH8 (cm)	PH12 (cm)	DFF	D50%F	NPPP	PDLT (cm)	PDDIA (cm)	PDWT (g)	NSPP	100SWT (g)
0.0Sv	86.67^{a}	32.23^{a}	76.25^{b}	105.00^{b}	77.67^{b}	85.00^{b}	17.80^{a}	5.36^{c}	1.43^{d}	5.50^{b}	2.60^{a}	30.00^{a}
0.16Sv	83.33^{a}	40.80^{a}	85.18^{b}	151.85^{a}	73.33^{c}	80.67^{c}	14.40^{b}	5.97^{b}	1.60^{bc}	6.20^{ab}	2.87^{a}	30.67^{a}
0.32Sv	80.00^{a}	28.47^{a}	157.32^{a}	179.97^{a}	79.67^{b}	86.67^{b}	13.13^{bc}	6.08^{b}	1.69^{ab}	6.77^{a}	2.80^{a}	31.00^{a}
0.49Sv	80.00^{a}	31.97^{a}	113.25^{ab}	169.74^{a}	86.00^{a}	93.00^{a}	10.53^{c}	6.59^{a}	1.77^{a}	7.20^{a}	2.80^{a}	32.33^{a}
0.65Sv	80.00^{a}	29.50^{a}	103.80^{b}	158.13^{a}	79.67^{b}	87.00^{b}	10.40^{c}	5.69^{bc}	1.55^{cd}	5.60^{b}	2.67^{a}	32.33^{a}
SEM±	7.89	4.73	15.18	13.38	1.01	1.10	0.92	0.13	0.04	0.34	0.25	1.19

NOTE: Means with the same letter within a column are not significantly different at $P \leq 0.05$
TRT- treatment, PGERM- percentage germination, PH4-plant height at 4 weeks, PH8-plant height at 8 weeks, PH12-plant height at 12weeks, DFF-days to first flowering, D50%F-days to 50% flowering, NPPP-number of pods per plant ,PDLGTH-pod length, PDDIA- pod diameter, PDWT-pod weight, NSPP-number of seeds per pod 100SWT-one hundred seeds weight

Table 2: Effects Fast ``Neutrons on Agronomic Traits of *Phaseolus lunatus* at M_2 Generation

TRT	PGERM (%)	PH4 (cm)	PH8 (cm)	PH12 (cm)	DFF	D50%F	NPPP	PDLT (cm)	PDDIA (cm)	PDWT (g)	NSPP	100SWT (g)
0.0Sv	93.33^{a}	37.93^{b}	98.88^{a}	112.63^{a}	54.33^{a}	63.67^{a}	6.87^{a}	4.73^{c}	1.58^{b}	4.27^{c}	2.20^{a}	29.90^{b}
0.16Sv	100.00^{a}	60.66^{a}	100.35^{a}	118.26^{a}	50.00^{c}	61.33^{b}	6.60^{a}	5.34^{b}	1.60^{b}	4.60^{b}	2.53^{a}	32.33^{a}
0.32Sv	93.33^{a}	50.01^{ab}	103.74^{a}	117.30^{a}	50.33^{c}	62.33^{ab}	7.47^{a}	5.79^{a}	1.74^{a}	6.47^{a}	2.67^{a}	30.33^{ab}
0.49Sv	90.00^{a}	57.32^{ab}	94.94^{a}	111.87^{a}	51.67^{b}	59.00^{c}	8.13^{a}	5.40^{b}	1.68^{a}	6.20^{ab}	2.67^{a}	29.67^{b}
0.65Sv	96.67^{a}	51.45^{ab}	99.80^{a}	109.93^{a}	50.00^{c}	59.67^{c}	8.07^{a}	5.61^{ab}	1.68^{a}	6.23^{ab}	2.60^{a}	28.33^{b}
SEM±	3.80	6.33	4.84	7.15	0.38	0.46	1.09	0.08	0.02	0.24	0.17	0.68

NOTE: Means with the same letter within a column are not significantly different at $P \leq 0.05$
TRT- treatment, PGERM- percentage germination, PH4-plant height at 4 weeks, PH8-plant height at 8 weeks, PH12-plant height at 12weeks, DFF-days to first flowering, D50%F-days to 50% flowering, NPPP-number of pods per plant ,PDLGTH-pod length, PDDIA- pod diameter, PDWT-pod weight, NSPP-number of seeds per pod 100SWT-one hundred seeds weight

Chlorophyll mutant such as Zonata showed dark light coloured bands like distinct zones spread over the leaf, Xantha showed light yellow colour indicating that the carotenoids prevailed over the chlorophyll, Maculata mutant showed chlorophyll or carotene destruction in the form of dots on the surface of the leaves.

However, morphological mutants such as bifoilate leaves, invaginated leaf margin, double invaginated leaf margin and bifurcated leaf apex were observed.

Plate 1: Control

Plate 2: Zonata

Plate 3: Xantha

Plate 4: Maculata

Plate 5: Double invaginated margin

Plate 6: Bi-furcated apex

Plate 7: invaginated margin

Plate 8: Bifoilate leaf

DISCUSSION

The increase in plant height at 8WAS, 12WAS, pod length, pod diameter and pod weight with increase in exposure to fast neutrons irradiation at M_1 generation, may be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al.* (2001); probably by increasing the rate of cellular division and expansion at their meristematic regions. This is also in agreement with the findings of Hoballah (1999) who reported increase in plant height of Sesame due to radiation mutagenesis, Lysenkov (1989) who induced a wide range of viable mutants and observed increase in wheat height, Adamu *et al.* (2002) when they irradiated the seeds of five varieties of groundnut (*Arachis hypogaea*) with gamma rays Cobalt 60 (^{60}Co) source, Falusi *et al.* (2012) when they irradiated the seeds of *Capsicum annum* with fast neutrons but disagrees with the findings of Adamu (2004) who exposed (pop-corn maize) to thermal neutron and gamma irradiation.

Days to first flowering and 50% flowering which also increased with an increase in exposure to irradiation might be due to inhibitory effects of the mutants and physiological changes in the plant as a result of delayed cellular division prior to flowering and reproductive stage especially at higher doses, this conforms to the findings of Sadiq (2015) and contradicts the findings of Mensah *et al.* (2007), in sesame and Mathew (2014), in pigeon pea. The number of pods per plant when compared to the control decreased with an increase in dosage of the radiation, this might be as a result of increased injuriousness on exposure to higher doses of fast neutrons irradiation, this is similar to the findings of Muhammad *et al*. (2013) when they treated *Sesamum indicum* with fast neutrons irradiation and observed a decrease in the number of capsules per plant with an increase in dose and disagrees with the findings of Mathew (2014), in pigeon pea and Sadiq (2015), in soya bean.

Percentage germination, Plant height 8WAS, 12WAS, Number of pods per plant and number of seeds per pod at M_2 generation were not significantly different ($P > 0.05$) this might be as a result of the fact that the dosage of fast neutrons was not adequate enough to cause reasonable level of variability in this character. This is similar to the findings of Abdullahi *et al*. (2015) who treated *Phaseolus vulgaris* with gamma rays. Days to first flowering and 50% flowering were significantly different when compared to the control and this maybe as a result of the heritable physiological changes that must have occurred at the M_1 generation. Pod length, Pod weight, Pod diameter and 100 seed weight were significantly different from the control and this study agrees with the findings of Elangovan and Pavadai (2015).

Chlorophyll and morphological mutants were classified in accordance with the system of Gustafsson (1940 and 1941). Macro mutations are generally used to evaluate the genetic effects of various mutagens. Gaul (1964) reported that chlorophyll mutations are employed as markers for the evaluation of gene action of mutagenic factors in inducing mutation. Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatment and have been reported in various pulse crops by several workers including Gautam *et al*. (1992).They do not have any major implication in mutation breeding but is an indication of the effectiveness and efficiency of the mutagen in inducing mutation.

CONCLUSION

This research concluded that the effects of fast neutrons irradiation varied with the various doses. Lower doses (0.16Sv and 0.32Sv) of the mutagen were more favourable in inducing beneficial mutation on the growth and yield trait of *P. lunatus.* The effect of the mutagen was significant at $p \leq 0.05$ in plant height at 4 weeks, days to first flowering, days to 50% flowering, pod length, pod diameter, pod weight and 100 seeds weight at the M_2 generation.

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